GENETIC BACKGROUND ANALYSIS AND RUTIN CONTENT EVALUATION OF GUANGXI *SOPHORA JAPONICA* CV. JINHUAI GERMPLASM RESOURCES

TANG, J. M.[1,2#*] – LIU, M.[1#] – YANG, Y. S.[1] – ZHU, S. Y.[1] – ZOU, R.[1*] – JIANG, H. L.[1]

[1]*Guangxi Key Laboratory of Plant Functional Substances and Resources Sustainable Utilization, Guangxi Institute of Botany, Guangxi Zhuang Autonomous Region and Chinese Academy of Sciences, Guilin 541006, China*

[2]*The Unit Agricultural Research Department, Graduate School, Kagoshima University, Kagoshima 8900065, Japan*

[#]*These authors contributed equally to this work*

**Corresponding authors*
e-mail: tangjianmin899@hotmail.com, 838421387@qq.com

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Abstract. In this study, the *Sophora japonica* cv. jinhuai from different germplasm resources in 6 townships of Quanzhou County, Guilin, were used as materials to investigate and compare the biological traits of different strains. The ISSR analysis marker technology was used to construct a DNA molecular genetic map of *S. japonica* cv. jinhuai to explore Germplasm genetic relationship. High-performance liquid chromatography (HPLC) was used to determine the content of rutin in various qualities of *S. japonica* cv. jinhuai and evaluate the quality of *S. japonica* cv. jinhuai The main findings are as follows: (1) Among the 11 *S. japonica* cv. jinhuai varieties, the early-maturing X-3 has a high rutin content and has the potential to harvest two locust rice a year, and can be used as a line for new cultivation technology development; The medium-ripening strain X-1 has strong vigor and high content of golden locust trees. It has a wide adaptability area and has good promotion prospects. (2) Through ISSR analysis of *S. japonica* cv. jinhuai, the genetic relationship among *S. japonica* cv. jinhuai germplasm is relatively close. (3) The *S. japonica* from "Tehuang" (D-1) has the highest content of rutin than others. Also, this strain has unique traits compared to others'. It is recommended to focus on breeding as new varieties for promotion.
Keywords: *Sophora japonica* cv. Jinhuai, *biological characteristics, ISSR molecular marker, rutin content, HPLC*

Introduction

Sophora japonica is a widespread species distributed in China from Liaoning in the north to Guangdong in the south. It is mainly cultivated in Guangxi, Hunan, Shandong, Shanxi, and other regions. Cultivated varieties (strains) of Sophora japonica trees in various places are mainly bred from wild or semi-wild Sophora japonica trees in their respective regions. *Sophora japonica* cv. jinhuai, which is famous for its golden dried buds (also known as *Sophora japonica*), is an excellent variety cultivated from *Sophora japonica* (Yang et al., 2023; Jiang, 2013). According to the *Dictionary of Chinese Medicine* (Nanjing University of Traditional Chines Medicine, 2006), the main medicinal components of *Sophora japonica* are flavonoids and other active substances. Flavonoids includ rutin and quercetin, which can be used for treating various bleeding symptoms. The stability of capillaries can be increased, they can be improved myocardial circulation. Moreover, they also have anti-inflammatory, antiviral and antioxidant effects (Ma et al., 2019; Liu et al., 2006; Gu et al., 2012). *Sophora japonica*

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can be used not only as medicine, but also as food. People often use *Sophora japonica* as condiment add it to soup or porridge and drink it as tea (Li et al., 1999).

China is a rutin exporter, and foreign countries import a large amount of sophora rice annually for research and development of health products (Li et al., 2008). Most of the cultivated varieties of *Sophora japonica* in different places are selected from wild or semi-wild *Sophora japonica* in their respective areas. At present, China is one of the biggest exporters of rutin. The produced *Sophora japonica* is mainly used to extract rutin, so the content of rutin not only determines the quality of *Sophora japonica*, but also determines the economic benefits of producing *Sophora japonica*. It was found that the rutin content of *Sophora japonica* made in northern and southern China was quite different (Li et al., 2006; Li et al., 2013). By comparison, it was found that the *Sophora japonica* cv. jinhuai grown in Quanzhou county of Guangxi had the best quality. The *Sophora japonica* produced in this place had the highest content of rutin (Li et al., 2009a). Whether *Sophora japonica* cv. jinhuai is suitable for promotion and introduction to other places needs urgent research. The formation of geographical variation in plant functional components is related to various factors, including changes in the environment of the place of origin, changes in ecological factors, and genetic factors in germplasm resources (Wei, 2003). Research on the germplasm resources of *Sophora japonica* cv. jinhuai, understand the genetic variation level and genome evolution classification system of Sophora japonica, explore the correlation between genetic diversity and *Sophora japonica* cv. jinhuai varieties, discover excellent varieties of Sophora japonica, and guide the selection and breeding of improved varieties of *Sophora japonica* cv. jinhuai. Variety identification is of great significance to the development of the locust industry and the local economy.

But the flowering period of Sophora japonica cv. jinhuai is inconsistent, there are few flowering branches, and the yield of Sophora japonica cv. jinhuai is low, which seriously affects the economic benefits of Sophora japonica cv. jinhuai cultivation. There is no investigation and evaluation of the germplasm resources of Sophora japonica cv. jinhuai in China and abroad. Therefore, taking the genetic background of Guangxi Sophora japonica and the main quality indicator rutin as the core evaluation criteria, we carried out a germplasm resource survey and breeding of excellent varieties of Sophora japonica in Guangxi and its application in industrial production and selected wonderful Sophora japonica varieties, will provide a high-quality variety of resources and technical support for the development of the sophora rice industry in Guangxi and even the country.

Materials and methods

Plants materials

The main cultivation areas of *Sophora japonica* cv. jinhuai in Guangxi are Dongshan Town, Baibao Township, Jiantang Township, Lianghe Township, Shaoshui Township and Xianshui Township in Quanzhou county of Guilin city. The main cultivated varieties were early-maturing *Sophora japonica* cv. jinhuai (G-1, mature in late June to early July), medium-maturing *Sophora japonica* cv. jinhuai (G-2, mature in mid-July to late July) and late-maturing *Sophora japonica* cv. jinhuai (G-3, mature in late August to early September). *Sophora japonica* cv. jinhuai planted in Quanzhou county of Guilin city was taken as the main research object in this study. Three to five *Sophora japonica* cv. jinhuai plants of the same germplasm with good local growth

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condition were selected. First, collect the *Sophora japonica* and leaves, then dry them, finally, collect them in sample bags and take them back to the laboratory for use. Sampling locality, variety, abbreviations, geographical coordinates and altitude are shown in *Table 1*.

Table 1. *Sampling locality, variety, abbreviations, geographical coordinates and altitude of the Sophora japonica cv. jinhuai*

Sampling locality		Variety	Abbreviations	Longitude and latitude	Altitude (m)
Xianshui Town		M (Miaotouqing) M (Honghua) E L	X-1 X-2 X-3 X-4	25°50.973′N 110°48.028′E	221
Shaoshui Town		E M	S-1 S-2	25°52.960′N 110°51.829′E	202
Lianghe Town	Tianqian Village	M (Honghua)	L-1	25°51.758′N 111°07.156′E	338
	Shangguan Village	E	L-2	25°47.860′N 111°10.043′E	339
Baibao Town		E	B-1	25°51.758′N 111°07.157′E	376
Jiantang Town		M	J-1	25°55.150′N 110°59.526′E	215
Dongshan Town		M (Tehuang)	D-1	25°52.150′N 110°23.510′E	258

Early-maturing *Sophora japonica* cv. jinhuai = E; Medium-maturing *Sophora japonica* cv. jinhuai = M; Late-maturing *Sophora japonica* cv. jinhuai = L

Test equipment and reagents

Vernier caliper, tape measure, silica gel, micro gas furnace, electronic balance, Agilent-1100 analytical high-performance liquid chromatograph equipped with vacuum online degasser, quaternary gradient pump, diode array multi-wavelength detector DAD (American Agi-lent Company); Ultrasonic extractor (Guangzhou Sinnoco Ultrasonic Equipment Co., Ltd.); constant temperature drying oven (Shanghai Jinghong). PVP, gravel sand, liquid nitrogen, acetone, 2×CTAB extraction buffer, β-thioethanol, chloroform/isoamyl alcohol (24: 1), 3 mol/L sodium acetate solution, isopropyl alcohol, 75% ethanol, Absolute ethanol, 0.1×TE, 10×Buffer, MgCl2, deoxyribonucleotides (dNTPs), TaqDNA polymerase, ISSR primer, ultrapure water, agarose; methanol (chromatographically pure American TEDIA Company); phosphoric acid reagent for analysis Pure et al.

Experiment method

Determination of biological characteristics

Among the morphological characteristics of plants, some are stable and conservative and are less affected by external factors. Some morphological characteristics are

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susceptible to genotype and external factors. Therefore, it is necessary to know which characters are sensitive to environmental influence and which morphological characteristics can affect the rutin content of *Sophora japonica* (Ai, 2007). Select 3-5 *Sophora japonica* cv. jinhuai plants of the same germplasm with good local growth conditions, measure the plant height, leaf length, leaf width, leaf thickness, petiole thickness; calculate the fresh weight, dry weight, and moisture content, rate of Sophora rice.

DNA extraction

The extraction of DNA from plants generally adopts CTAB method. Total genomic DNA of *Sophora japonica* cv. jinhuai was extracted following the CTAB method (Li et al., 2009b). The washed forceps and disposable small steel beads were placed in a washed and uncontaminated mortar, poured into an appropriate amount of anhydrous ethanol, and ignited for sterilization. After the sterilized equipment was brought to room temperature, about 500 mg of the dried leaves of H. dentata were taken in a sterile 2 mL centrifuge tube with forceps, and then the appropriate amount of PVP and 3–4 small steel balls were added. The centrifuge tube was placed in a bead mill to crush the sample. Then the total DNA was extracted from H. dentata by the modified CTAB method. The concentration and quality of the total DNA obtained were detected by 1.2% agarose gel electrophoresis and microUV spectrophotometry, and finally, the extracted DNA was stored at -20°C.

ISSR reaction system and procedure

The samples were verified with reference to the 11 ISSR primers (*Table 2*) selected by Tang (Tang et al., 2014; Wei et al., 2012) and the ISSR-PCR reaction system and procedures. Experimental procedure: Firstly, primer screening was required. The initial ISSR-PCR reaction system and procedures in this experiment were as follows: The total volume was 25 μL. There were 2.5 μL 10×Buffer, 2.0 mmol·L^{-1} $MgCl_2$, 0.4 mol·L^{-1} ISSR primer, 0.5 mmol·L^{-1} dNTP, 1.0 U Taq enzyme, and about 50 ng template DNA, respectively. During operation, a large amount of reagent was added firstly. In order to avoid the inactivation of the enzyme, Taq enzyme needed to be added at last. Finally, make up to 25 μL with Ultrapure water. The amplification procedures were as follows: predenaturation (94℃, 5 min), 45 cycles (denaturation 94℃, 30 s; Annealing at 52℃, 45 s; Extension 72℃, 7 min), extension (72℃, 7 min), preservation (4℃). Detection of PCR amplification products: Firstly, make a 1.5% agarose gel and place it in the electrophoresis tank. Take 10 μL of the DNA amplification product stained with Bromophenol blue and spot it in the spotting well of the agarose gel. The DNAMarker on the first spot of the spotting well was used as the band reference. The electrophoresis time was about 1.5 h. After that, the electrophoresed agarose gel was placed in EB solution (0.5 μg/mL) and stained for 20 min. Finally, take pictures in the Gel Imaging System.

Determination method of rutin content

Chromatographic conditions: ZORBAX Eclipse XDB-Cg column (4.6 mm Detection wavelength: 254 nm; injection volume: 10 μl. Preparation of rutin reference substance: Precisely weigh 10 mg of rutin standard substance, place it in a 50 ml volumetric flask, and dilute to the mark with methanol to prepare a reference substance stock solution

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containing 0.2 mg of rutin per 1 ml. Preparation of the test solution: Precisely weigh about 100 mg of dry sophora rice powder (passed through a 40-mesh sieve), place it in a 50 ml volumetric flask, add methanol to the mark and soak for 1 h. Ultrasonic treatment (power 250 W, frequency 25 kHz) for 40 min. Let cool to room temperature, make up the lost amount to the mark with methanol, shake well, filter, accurately measure 2 ml of the additional filtrate, put it in a 10 ml volumetric flask, add methanol to the mark, shake well, and filter with a microporous membrane (0.45 um) Filter to obtain the test solution. The standard curve and linear range were used to accurately absorb 4, 6, 8, 10, and 12 μl of rutin reference solution respectively. Analyze according to the above chromatographic conditions. Taking the concentration of the reference solution as the abscissa (X) and the peak area as the ordinate (Y), we get the rutin reference solution. Linear equation is: $Y = 865.71X + 2.54$, $R = 0.9992$. The results show that the rutin content measurement has a good linear relationship between 2.0–6.0 μg. Determination of sample content: Take 3 batches of sample powder, about 0.1 g each, prepare according to the test sample preparation method, and accurately absorb 10 μl respectively. Measure according to the above chromatographic conditions, and calculate the rutin content (Li, 2014; Shu et al., 2017).

Table 2. *Primer name, sequence and statistics of amplified bands of 11 materials*

Primer name	Primer sequence (5'-3')	Annealing temperature (℃)	Total number of amplified bands	Number of polymorphic bands	Percentage of polymorphism (%)
UBC-835	(AG)8YC	45.9	10	8	80.00
UBC-840	(GA)8YT	42.0	10	9	90.00
UBC-843	(CT)8RA	55.3	10	9	90.00
UBC-845	(CT)8RG	50.9	6	5	83.33
UBC-846	(CA)8RT	42.0	8	6	75.00
UBC-847	(CA)8RC	50.3	9	7	77.78
UBC-855	(AC)8YT	51.4	9	8	88.89
UBC-856	(AC)8YA	45.9	10	10	100.00
UBC-857	(AC)8YG	50.3	11	10	90.91
UBC-861	(ACC)6	59.0	10	8	80.00
UBC-890	HVH(TG)7	50.3	8	6	75.00
Total			101	86	85.15

Y = (C,T), R = (A,G)

Data analysis

Using the electrophoresis spectrum of the DL 2000 Marker as a reference, the bands were read manually according to the electrophoretic mobility of each band, and the ISSR-PCR amplification bands were recorded separately by Excel 2010. The bands that were clear were recorded as "1", and those that were absent or faint were recorded as "0". The bands that were clear were recorded as "1" and those that were absent and faint were recorded as "0", and a 1/0 matrix was established. The similarity method of NTSYS-pc software calculates the genetic similarity coefficient of all Sophora japonica germplasms, and clusters the genetic similarity data of Sophora japonica germplasm according to the Clustering method. Dominant of Popgene (Francis) software calculates the genetic distance between *Sophora japonica* cv. jinhuai germplasms. GenAleX software was used to conduct bivariate principal coordinate analysis of the genetics of

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Sophora japonica cv. jinhuai germplasm (Yang et al., 2023; Li, 2014; Gu et al., 2011). Based on the results of rutin content determination, the difference in rutin content between different *Sophora japonica* cv. jinhuai germplasms was calculated, and GenAleX-mantel was used to analyze the correlation between *Sophora japonica* cv. jinhuai germplasm and rutin content.

Results and discussion

Biological characteristics of different strains of Sophora japonica cv. jinhuai

The height of *Sophora japonica* cv. jinhuai is about 2.5-3.7 m and the leaflets are opposite (or alternate), oblong, round at the base, tapering at the apex, and shallowly serrate at the edge. The leaf size of *Sophora japonica* cv. jinhuai with different germplasm is different, for example, the base leaf length of early-maturing varieties in Xianshui Town is only 5.2 cm to 6.1 cm, while that of new varieties can reach 8.0 cm to 9.1 cm. The diameter of petiole is 0.21 cm to 0.25 cm. The total number of leaves is odd or even. The number of leaves ranges from 11 to 23. The mature time of *Sophora japonica* cv. jinhuai is from July to August. Verticillaster. The spike with high position is larger, with an average of 70 cm×45 cm. The fresh weight of the branches with flowers, such as X-2, is 310 g. The best time to harvest *Sophora japonica* cv. jinhuai should be when it has been fully expanded but not opened.

Most of the growers in the north of Guangxi took the medium-maturing *Sophora japonica* cv. jinhuai, which had the advantages of high adaptability, strong stress resistance, stable yield, as the main planting variety. “Honghua”, “Miaotouqing” and “Tehuang” strains of medium-maturing *Sophora japonica* cv. jinhuai have their own characteristics compared with the original varieties of medium-maturing *Sophora japonica* cv. jinhuai. “Honghua” is different from other medium-maturing *Sophora japonica* cv. jinhuai due to the red color of its flower. The characteristic of “Miaotouqing” is that its branches are green. The “Tehuang” variety in Dongshan, Guangxi area has the characteristics of extra yellow color of steamed *Sophora japonica* cv. jinhuai. Early-maturing *Sophora japonica* cv. jinhuai, such as X-3, S-1, L-2 and B-1, generally have small leaves, smaller leaf width than other varieties and larger spikes. The specific gravity of flowers and branches was 50.00%-66.67%, which was similar to that of other acacia trees. However, the dry-wet specific gravity of early-maturing *Sophora japonica* cv. jinhuai was higher, 50.42%-74.88%. The water content of S-1 was relatively small in early-maturing variety. It was also found that S-1 was the better germplasm for early-maturing *Sophora japonica* cv. jinhuai due to its longer branches, largest flowers and highest estimated value. L-2 also has good quality and stress resistance. The proportion of flowers and branches was as high as 70.58%, which was the highest among all the germplasm resources of *Sophora japonica* cv. jinhuai, and the water content of the *Sophora japonica* was only 34.17%. Among the medium-maturing *Sophora japonica*, the X-1 had the excellent traits of large leaves and small water content in *Sophora japonica* cv. jinhuai, but the proportion of *Sophora japonica* cv. jinhuai and branches was small, so the yield was slightly lower than others. The traits of “Honghua”, such as X-2 and L-1, and those of medium-mature *Sophora japonica* cv. jinhuai, such as S-2, and “Tehuang” (D-1) were relatively stable, but the water content of J-1 was larger. X-4 as late- maturing variety had little difference in other traits than other germplasm except for smaller leaves, but its yield was not high. The specific data are shown in *Table 3*.

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Table 3. *Comparison of morphological characteristics of Sophora japonica cv. jinhuai*

Morphological character	X-1	X-2	X-3	X-4	S-1	S-2	L-1	L-2	B-1	J-1	D-1
Leaf length and leaf width at base (cm)	8.4/5.4	7.5/3.1	5.8/3.2	5.8/2.8	6.2/2.8	8.0/4.0	6.1/2.8	6.8/3.1	7.1/3.1	5.8/2.3	6.8/3.3
Leaf thickness at base (cm)	0.032	0.021	0.025	0.016	0.021	0.025	0.017	0.020	0.028	0.021	0.021
Length and width of leaves on tender tip (cm)	4.3/1.7	3.9/1.8	4.2/1.8	5.1/2.0	4.2/1.9	3.9/2.0	5.4/2.3	5.1/2.3	3.9/1.6	3.9/1.9	4.4/1.7
Thickness of leaves of tender tip (cm)	0.016	0.018	0.025	0.011	0.019	0.023	0.024	0.023	0.020	0.019	0.029
Total number of leaves	11-17	13-23	15-21	17-23	13-17	13-19	11-17	15-17	11-21	13-15	11-19
The length and width of the leaves (cm^2)	24×12	24×12	21×10	27×11	25×12	26×11	30×10.5	30×13	35×16	25×7.2	32×12
Diameter of petiole (cm)	0.19	0.19	0.19	0.19	0.19	0.20	0.16	0.19	0.28	0.20	0.20

Sophora japonica cv. jinhuai DNA extraction results

DNA in chloroplasts was extracted from mature and healthy leaves of *Sophora japonica* cv. jinhuai of different germplasm. The extraction results are shown in *Table 4*. The quality of extracted DNA depends on the applied method but is not connected to the quality of the genotype. The concentration of DNA extracted by L-2 was the highest, which was 525.4 ng/μl. The DNA concentration of J-1 was the lowest, which was 181.3 ng/L. DNA purity (i.e., 260/280) within the range of 1.80-2.00 indicates moderate purity, while too high or too low indicates more impurities. Among them, the DNA purity of S-2 was the lowest, only 1.36. The DNA purity of S-1, L-2 and B-1 was also low, which were 1.66, 1.61 and 1.52 respectively. The overall purity of DNA was good.

Table 4. *Concentration and purity of DNA extracted from various Sophora japonica cv. jinhuai*

Number	**Concentration (ng/μl)**	**A260**	**A280**	**260/280**	**260/230**
X-1	241.4	4.828	2.632	1.83	0.98
X-2	316.8	6.335	3.22	1.97	1.31
X-3	261.2	5.224	2.62	1.99	1.3
X-4	460.3	9.206	4.752	1.94	1.33
S-1	420.3	8.406	5.073	1.66	0.82
S-2	362	7.241	5.322	1.36	0.51
L-1	258.8	5.176	2.749	1.88	1.03
L-2	525.4	10.508	6.53	1.61	0.8
B-1	299.6	5.991	3.939	1.52	0.75
D-1	433.6	8.671	4.636	1.87	1.1
J-1	181.3	3.626	1.953	1.88	1.16

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Determination of ISSR-PCR reaction system of Sophora japonica cv. jinhuai

By optimizing the design of four levels of the five factors of ISSR-PCR reaction system, and based on orthogonal experiments and screening of each reaction condition, the optimal reaction system for ISSR-PCR was determined, that is: the total volume is 25 μL. It contains 10 × Buffer 2.5 μL, $MgCl_2$ 2.5 mmol·L-1, ISSR primer 1.2 μmol·L-1, dNTP 0.25 mmol·L-1, Taq enzyme 2.0U, template DNA about 70 ng, and add ultrapure water to 25 μL. At the same time, the optimal annealing temperature of each primer and the number of PCR cycles were discussed. 52 cycles amplified more products and the bands were clearer, saving time compared to 55 cycles. Therefore, 52 cycles were determined to be the optimal cycle of the system. times, and verify the ISSR-PCR reaction system (*Fig. 1*).

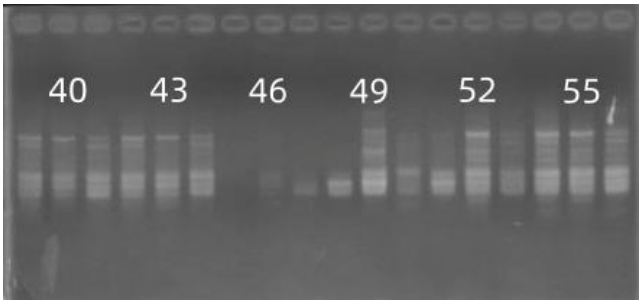

Figure 1. *Filtering of cycle times*

ISSR polymorphism analysis of Sophora japonica cv. jinhuai germplasm

A total of 11 primers were screened among the 100 primers. 11 primers were used to amplify the DNA of 11 *Sophora japonica* cv. jinhuai materials by ISSR-PCR. And the experimental results are shown in *Table 5*. Through the analysis of the amplified product bands, it was found that the total number of amplified bands by the 11 primers was 101, of which 86 were polymorphic, and the percentage of polymorphism was 85.15%. According to calculation, each primer could amplify 9.18 reaction bands on average. The total number of bands amplified by primer UBC-857 was the largest, which was 11. The results of spectral bands showed that there were certain conservative sequences among the germplasm of *Sophora japonica* cv. jinhuai for the test, which further indicated that the germplasm of *Sophora japonica* cv. jinhuai had close genetic relationship. At the same time, the experimental results also showed that B-1, X-2 and X-3 had the most polymorphic bands, indicating that the genetic diversity of these two *Sophora japonica* was higher than that of other *Sophora japonica* cv. jinhuai. In addition, some *Sophora japonica* cv. jinhuai presented specific bands or deletion bands in the experiment. For example, D-1 presented specific bands at about 600 bp and L-2 lacked amplification bands at about 1200 bp. Both specific bands and deletion bands could be used as effective markers for variety identification (*Fig. 2*).

Genetic similarity analysis of different varieties of Sophora japonica cv. jinhuai

The genetic similarity coefficients of 11 species of *Sophora japonica* cv. jinhuai are shown in *Table 7*. It could be seen from *Table 7* that the results of the genetic similarity coefficient and the genetic distance were consistent. Through the great genetic

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similarity coefficient of various *Sophora japonica* cv. jinhuai in the range of 0.7386-0.9034, it showed that the genetic basis of the 11 golden locust germplasms had no obvious difference, and the genetic relationship was relatively close to each other. The genetic similarity coefficient between L-2 and L-1 was the highest, which was 0.9034 (the genetic distance was the smallest, which was 0.1881). But the coefficient of genetic similarity between X-2 and L-1 was the lowest, which was 0.7386. The genetic similarity coefficient of early-maturing *Sophora japonica* cv. jinhuai in different regions ranged from 0.7613 to 0.8352.

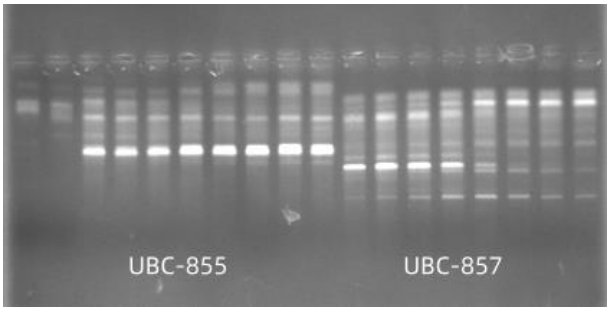

Figure 2. *Amplification map of primer UBC-855 and UBC-857*

Table 5. *Genetic similarity coefficient (bottom left) and genetic distance (top right) of the germplasm resources of Sophora japonica cv. jinhuai*

Number	L-2	B-1	L-1	X-2	S-1	X-4	S-2	D-1	X-3	X-1	J-1
L-2		0.5431	0.1881	0.5268	0.2472	0.3365	0.3773	0.3773	0.5108	0.4345	0.3232
B-1	0.7500		0.5108	0.3232	0.5268	0.3635	0.4055	0.3499	0.2845	0.3499	0.4643
L-1	0.9034	0.7556		0.5596	0.1766	0.3102	0.3773	0.4643	0.4796	0.4643	0.2472
X-2	0.7556	0.8352	0.7386		0.5431	0.4055	0.4199	0.4199	0.3232	0.4199	0.4493
S-1	0.8693	0.7556	0.8977	0.7500		0.3499	0.4199	0.4493	0.4643	0.4199	0.3102
X-4	0.8295	0.8181	0.8465	0.8011	0.8238		0.2231	0.2973	0.3102	0.2973	0.2973
S-2	0.8125	0.8011	0.8181	0.7954	0.7954	0.8806		0.2351	0.4055	0.3365	0.2845
D-1	0.8125	0.8238	0.7840	0.7840	0.7840	0.8465	0.8750		0.4345	0.1653	0.3635
X-3	0.7613	0.8522	0.7670	0.8352	0.7784	0.8409	0.8011	0.7840		0.3232	0.4643
X-1	0.8011	0.8352	0.7954	0.8068	0.7954	0.8579	0.8409	0.8863	0.8465		0.3913
J-1	0.8352	0.7784	0.8750	0.7840	0.8409	0.8465	0.8522	0.8181	0.7784	0.8181	

Cluster analysis of germplasm resources of Sophora japonica cv. jinhuai

In order to further reveal the genetic relationship of *Sophora japonica* cv. jinhuai germplasm resources in northern Guangxi, the genetic similarity coefficients in *Table 5* were made into a clustering dendrogram (*Fig. 3*) by using NTSYSpc. It could be seen from *Figure 1* that L-1 and L-2 firstly clustered into one class, and their genetic similarity coefficients were also the largest. After that, L-2, S-1 and J-1 of early-maturing strains were clustered into one class, which also showed that they had a common genetic origin. In another main class, D-1 and X-1 were clustered into the one class. But the X-2 in “Honghua” was taken as a single branch, indicating that the genetic background of X-2 was special.

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Double principal coordinate analysis

Principal coordinate analysis (PCoA), which was based on genetic distance and genetic similarity coefficient, reflects the genetic similarity through the positional relationship of various qualities in the principal coordinate diagram (Wei and Bai, 2015). Compared with clustering analysis, PCoA could intuitively and accurately reflect the genetic relationship between the germplasm of *Sophora japonica* cv. jinhuai from multiple levels. The experimental material was the leaves of multiple plants of the same germplasm, differences between species had been taken into account. Therefore, the principal coordinate analysis (PCoA) of this experiment could be regarded as a double principal coordinate analysis (DPCoA). The results are shown in *Figure 4*.

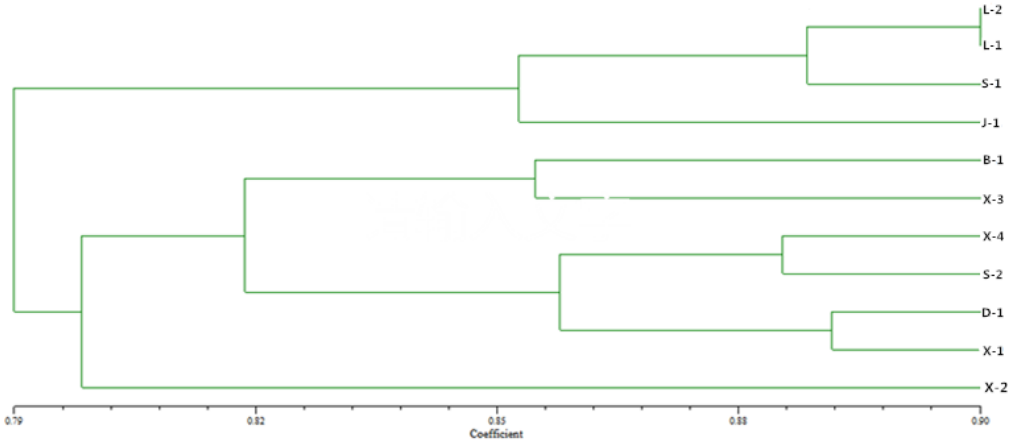

Figure 3. *NTSYS clustering dendrogram of 11 materials constructed according to the similarity coefficient*

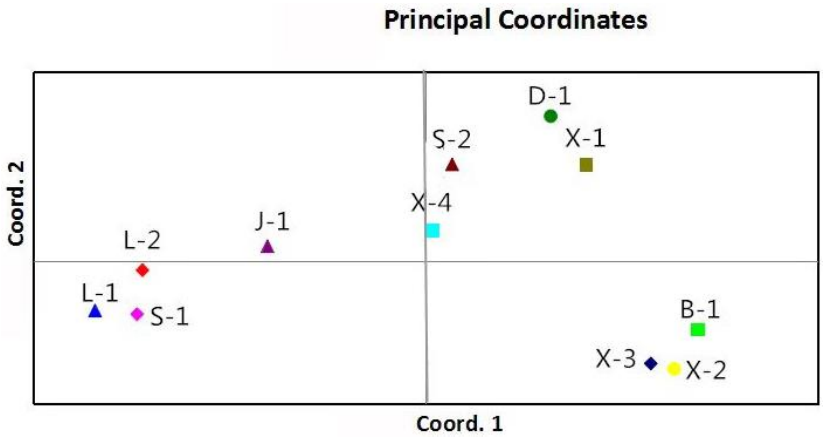

Figure 4. *Two-dimensional point diagram of the first and second principal coordinates of the germplasm material of Jinhua*

DPCoA sequencing of *Sophora japonica* cv. jinhuai germplasm by ISSR markers showed that the contribution rates of variance of the first three principal coordinates were 33.15%, 19.75% and 14.08%, respectively. The corresponding accumulation contribution rates were 33.15%, 52.91% and 66.98%, respectively. The satisfaction

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standard was the cumulative contribution rate of variance of 40%. The variance accumulation contribution rate of this experiment was 66.98%, indicating that the dimensionality reduction effect was good. The distribution of *Sophora japonica* cv. jinhuai germplasm materials was very obvious in the DPCoA ranking map, and there was no overlap between the materials. According to the ranking map, the 11 materials could be divided into three categories.

The first category consisted of four materials in the 1st and 4th intervals, which was L-1, L-2, S-1 and J-1. The second category consisted of three materials in the 2nd intervals, which was X-2, X-3 and B-1. The third category consisted of four materials in the 3rd intervals, which is X-1, X-4, S-2 and D-1. Compared with cluster analysis, principal coordinate analysis could intuitively and accurately reflect the genetic relationship between *Sophora japonica* cv. jinhuai germplasms from multiple levels.

Experimental results of rutin content in Sophora japonica cv. jinhuai

The rutin content of *Sophora japonica* cv. jinhuai from different germplasm resources of *Sophora japonica* cv. jinhuai was determined by HPLC. And the quality of *Sophora japonica* from different germplasm resources was evaluated according to the content of rutin. The results are shown in *Table 6*. The content of rutin in early maturing strains such as X-3 and S-1 was 42.9% and 39.1%, respectively, and the rutin content of them was higher. But the content of rutin of the strain of L-2 was lower. The content of rutin in medium-maturing strain such as X-2, S-2 and L-1 was 34.7%, 34.8% and 36.8%, respectively. But the content of rutin of the others in medium-maturing strains was higher. The content of rutin in such as X-2 was the lowest, which was 34.7%. The rutin content of "Tehuang" (D-1) in Dongshan was the highest, which was 43.2%. The results showed that the rutin content of *Sophora japonica* was higher in early-maturing strain, medium-maturing strain and Dongshan "Tehuang", and the quality of *Sophora japonica* cv. jinhuai was better.

Table 6. *Determination results of rutin content in the germplasm resources of Sophora japonica cv. jinhuai*

Numble	X-1	X-2	X-3	X-4	J-1	S-1	S-2	L-1	L-2	D-1
Content (%)	41.4	34.7	42.9	34.9	40.6	39.1	34.8	36.8	35.2	43.2
Average content (%)	38.4									

As can be seen from the *Table 7*, the rutin content among different *Sophora japonica* cv. jinhuai germplasms varies greatly, with the maximum being between X-1 and D-1, as high as 8.5 percentage points, and the minimum being only 0.1 percentage points between S-2 and X-2. The average rutin content of early-maturing varieties is 39.45%; the average rutin content of mid-maturing varieties is 37.68%, and the rutin content of late-maturing varieties is 34.9%; indicating that the rutin content of early-maturing varieties is higher, and that of mid-maturing varieties is lower. In short, late-ripening rutin content is the lowest. At the same time, the rutin content of mature *Sophora japonica* cv. jinhuai strains also has great differences; among the early maturing strains, the rutin content also has great differences, and the rutin content difference between L-2 and X-3 is 7.7%. The difference between X-1 and D-1, which are both medium-ripe strains, is the largest, up to 8.5 percentage points. The difference between S-2 and X-2

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is the smallest, with a difference of only 0.1 percentage points. This shows that the rutin content between different strains and strains at different maturity stages is also quite different.

Table 7. *Comparison of rutin content difference among Sophora japonica cv. jinhuai*

D-value of content	L-2	L-1	X-2	S-1	X-4	S-2	D-1	X-3	X-1	J-1
L-2	0									
L-1	1.6	0								
X-2	0.5	0.1	0							
S-1	3.9	2.3	4.4	0						
X-4	0.3	1.9	0.2	4.2	0					
S-2	0.4	2	0.1	4.3	0.1	0				
D-1	6.4	6.4	8.5	4.1	8.3	8.4	0			
X-3	7.7	6.1	8.2	3.8	8	8.1	0.3	0		
X-1	6.2	4.6	6.7	2.3	6.5	6.6	1.8	1.5	0	
J-1	5.4	3.8	5.9	1.5	5.7	5.8	2.6	2.3	0.8	0

Correlation analysis between genetic distance and rutin content

Mantel analysis was performed based on the rectangular data between the genetic distance and the difference in rutin content. The results showed that there was no significant difference in the genetic distance and the difference in rutin content between the strains of *Sophora japonica* cv. jinhuai. Genetic factors are not the main factor affecting rutin content, but the correlation R between them is positive, indicating that genetic factors have a certain positive correlation and promotion effect on rutin, but it is not significant (P = 0.330). The formation of the active ingredients of plant rutin is related to a variety of factors, not only genetic factors but also changes in the environment of the place of origin, changes in ecological factors, etc.; it shows that the unique environmental climate and soil factors in northern Guangxi have an impact on the rutin content of *Sophora japonica* cv. jinhuai. It has a certain influence; this is our later research direction on the accumulation of active components of Sophora japonica cv. jinhuai rutin (*Fig. 5*).

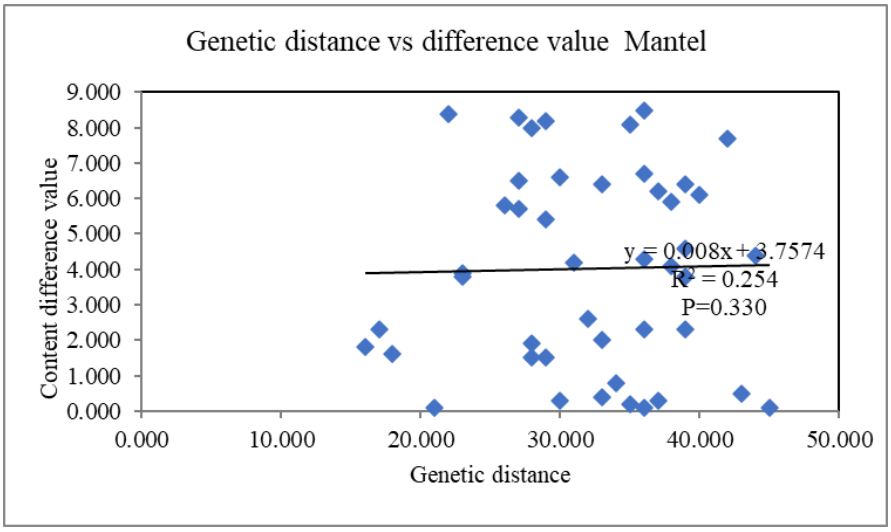

Figure 5. *Genetic distance vs difference value Mantel*

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Discussion

Nowadays, it is increasingly believed that genetic distances between different populations of the same species do not necessarily lead to genetic variation, and that factors such as genetic mutations, selection, and gene flow are the main causes of genetic differentiation between populations (Fischer and Matthies, 1009). Qin et al (2022) used ISSR molecular marker technology to analyze the genetic diversity of a very small population of *Paphiopedilum emersonii* and cluster eight wild populations of white *Paphiopedilum emersonii* based on genetic distance, and the results showed that frequent genetic exchange between populations in close proximity, strong genetic mutations and selection can lead to genetic differentiation between populations. Therefore, the unique cultivation environment has resulted in strong genetic selection in the Sophora japonica strains, resulting in more types of variation in the Sophora japonica strains. During the variety breeding process, *Sophora japonica* cv. jinhuai mainly adopts clonal methods (grafting and cuttings) (Qin et al., 2021; Xiao et al., 2023), which can timely and better preserve the traits and genetic resources of mutant lines; this is in line with Shu's study (Shu et al., 2019) of the genetic diversity of *Sophora japonica* cv. jinhuai's cultivated lines. The results were consistent with the genetic diversity being higher than that of wild Sophora japonica trees. It shows that the use of clones to breed new strains of *Sophora japonica* cv. jinhuai with high genetic diversity and excellent and stable biological traits is a fast and accurate breeding method for screening new *Sophora japonica* cv. jinhuai varieties.

Because of the limited conditions, this study only focused on some related traits of yield and quality of *Sophora japonica*. And there was no in-depth study on the growth environment, growth potential, cultivation conditions and other aspects of each variety of *Sophora japonica*. It was found in the study that the leaflets of *Sophora japonica* cv. jinhuai were not simply opposite, but opposite and alternate are spaced. The odd-numbered leaves were spaced from the even-numbered leaves. *Sophora japonica* cv. jinhuai with high yield has long branches and large leaves. The study also found that the flowering stage of the *Sophora japonica* cv. jinhuai was from June to August, which was different from what some expert studies suggested that the flowering period was from April to May (Chen and Jiang, 2013). In the survey, it was found that the planting area of the medium-maturing *Sophora japonica* is the widest, and it had a good prospect for popularization because of its advantages of tall plants and strong trees.

The amplification experiment of ISSR-PCR system of *Sophora japonica* cv. jinhuai will be influenced by reaction conditions and amplification procedures. The ISSR-PCR reaction system of *Sophora japonica* cv. jinhuai established in this study laid a foundation for its variety identification, genetic relationship analysis and genetic diversity. The results showed that the genetic information of 11 *Sophora japonica* cv. jinhuai germplasms showed different degrees of commonality and polymorphism. This result not only showed the complexity of the genetic background of the *Sophora japonica* cv. jinhuai germplasm, but also showed that there was a certain commonality among them. In the analysis of ISSR results, the results of genetic similarity coefficient and genetic distance were consistent. And the results of cluster analysis were basically consistent with the results of double principal coordinate analysis (DPCoA). Although some *Sophora japonica* cv. jinhuai come from different sources, such as L-1 and L-2, their genetic similarity coefficients are relatively high. Cluster analysis and principal coordinate analysis also showed that their genetic relationship is close, indicating that they had a close genetic relationship. Through the determination of rutin content, the

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amount of rutin content in *Sophora japonica* had no significant correlation with the variety. D-1 had the highest rutin content, so the quality of D-1 was the best among the investigated *Sophora japonica* cv. jinhuai germplasms. And it has specific traits in biological characteristics. So it is suggested that D-1 should be cultivated as a new variety and popularized.

Mantel analysis was performed based on the rectangular data between the genetic distance and the difference in rutin content. The results showed that there was no significant difference in the genetic distance and the difference in rutin content between the strains of Sophora japonica. The formation of the effective components of rutin is not only related to genetic factors but also to factors such as environmental factors in the place of origin; it shows that the unique environmental climate and soil factors in northern Guangxi have a certain impact on the rutin content of Sophora japonica; later, environmental climate, soil Factors and the influence of soil microorganisms on the accumulation of rutin content, revealing the influence mechanism and journey mechanism of Sophora rutin, is a new research direction for us to improve the accumulation of effective components of Sophora rutin (Wang et al., 2022; Zou et al., 2023).

Conclusion

In this study, the *Sophora japonica* cv. jinhuai from different germplasm resources in 6 townships of Quanzhou County, Guilin were used as materials to investigate and compare some traits of *Sophora japonica* cv. jinhuai. The ISSR analysis marker technology was used to construct a DNA molecular genetic map of *Sophora japonica* cv. jinhuai to explore Germplasm genetic relationship. High performance liquid chromatography (HPLC) was used to determine the content of rutin in various qualities of *Sophora japonica* and evaluate the quality of *Sophora japonica*. The main findings are as follows: (1) Among the 11 Sophora japonica cv. jinhuai varieties, the early-maturing X-3 has a high rutin content and has the potential to harvest two locust rice a year, and can be used as a line for new cultivation technology development; The medium-ripening strain X-1 has strong vigor and high content of golden locust trees. It has a wide adaptability area and has good promotion prospects. (2) Through ISSR analysis of *Sophora japonica* cv. jinhuai, the genetic relationship among *Sophora japonica* cv. jinhuai germplasm is relatively close. (3) The *Sophora japonica* from " Tehuang" (D-1) has the highest content of rutin than others. And this strain has specific traits that other strains do not have.

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REFERENCES

[1] Ai, X. (2007): Observation of Biological Characteristics of Some Peony Varieties such as "Golden Wheel" and Preliminary Study on Genetic Relationship. – Beijing Forestry University, Beijing.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2201_395410

[2] Chen, J. F., Jiang, R. N. (2013): Summary of planting techniques of *Sophora japonica* cv. jinhuai in Northern Guangxi. – Southern Horticulture 4(3): 48-49.
[3] Fischer, M., Matthies, D. (1998): RAPD variation in relation to population size and plant fitness in the rare Gentianella germanica (Gentianaceae). – American Journal of Botany 85(6): 811-819.
[4] Gu, S. J., Yang, N., Zhu, K. M. (2011): Study on the microwave extraction technology of rutin from *Sophora japonica* cv. jinhuai *Japonica* rice in northern Guangxi. – China Modern Applied Pharmacy 28(2): 121-124.
[5] Gu, S. J., Liu, J. N., Yao, X. L. (2012): Study on the intervention effect of *Sophora japonica* cv. jinhuai *japonica* quercetin on human nasopharyngeal carcinoma cells. – Anhui Agricultural Sciences 40(18): 9639-9640, 9668.
[6] Jiang, Y. S. (2013): Standardized planting technology of *Sophora japonica* cv. Jinhuai. – Guangxi Forestry 5: 48-49.
[7] Li, F., Li, Q. H., Li, T. L. (2008): Research progress on the determination method of the effective ingredients of *Sophora japonica*. – Traditional Chinese Medicine Information 25(4): 19-21.
[8] Li, F., Tang, H., Wei, X. (2009a): The existing problems and development countermeasures of *Sophora japonica* cv. jinhuai production in Quanzhou County, Guangxi. – Journal of Guangxi Academy of Sciences. 25(2): 952-954.
[9] Li, J. F., Xun, S. M., Li, X. F. (1999): Development of *Sophora japonica* health-care fragrant tea. – Medicine Diet Therapy Research 2: 8.
[10] Li, P. F., Zhang, H., Xiao, L., Jin, X. H. (2006): Determination of rutin in *Sophora japonica* from different places by RP-HPLC. – Chinese Herbal Medicine 37(9): 1419-1420.
[11] Li, R. H., Xia, Y. S., Liu, S. Z. (2009b): Improved CTAB method for extracting plant DNA. – Laboratory Research and Exploration 28(9): 14-16.
[12] Li, Y. S. (2014): Study on the extraction and purification of rutin and the preparation technology of its derivatives. – Chinese Pharmacy 25(27): 2587-2590.
[13] Li, Z. Z., Zhu, H., Xie, F. (2013): Determination of rutin in *Sophora Japonica* from different places. – World Traditional Chinese Medicine 8(8): 952-954.
[14] Liu, J. Y., Ding, J. C., Xu, J. H. (2006): Effect of *Sophora japonica* cv. jinhuai guanxin capsule on experimental myocardial ischemia and hyperlipidemia. – Pharmacology and Clinics of Chinese Medicine 22(5): 50-53.
[15] Ma, Q., Wei, R., Sang, Z. (2019): Neuroprotective aurones from *Sophora japonica*. – Chemistry of Natural Compounds 55(2): 103.
[16] Nanjing University of Traditional Chinese Medicine (2006): Dictionary of Traditional Chinese Medicine (Vol. 1). – Shanghai Science and Technology Press, Shanghai.
[17] Qin, F., Shi, Y. C., Qin H. Z., et al. (2021): Comparison of physiological characteristics of three 'Jinhuai' varieties. – Chinese Agricultural Science Bulletin (18),38-43.
[18] Qin, H. Z., Pan, B., Zhao, J., et al. (2022): Genetic diversity analysis by ISSR of Paphiopedilum emersonii, a plant species with extremely small population. – Guangxi Sciences 29(6): 1134-1140.
[19] Shu, W. J., Liu, J. L., Zou, R., et al. (2017): Determination of rutin content in sophora japonica by high performance liquid chromatography (HPLC). Shi Zhen Chinese Medicine 3: 709-711.
[20] Shu, W. J., Tang, J. M., Chen, Z. Y., et al. (2019): Analysis of genetic diversity and population structure in Sophora japonica Linn. in China with newly developed SSR markers. – Plant Molecular Biology Reporter 37: 87-97.
[21] Tang, J. M., Chen, Z. Y., Wei, X. (2014): Optimization of SSR-PCR reaction system and primer screening of *Camellia tunghinensis* Chang. – Genomics and Applied Biology 33(2): 398-404.
[22] Wang, J. R., Song, X. H., Li, L. Y., Gao, S. J., Shang, F. H., Zhang, X. M., Yang, Y. (2022): Metabolomic analysis reveals dynamic changes in secondary metabolites of

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2201_395410

Sophora japonica, L. during flower maturation. – Front. Plant Sci. https://doi.org/10.3389/fpls.2022.916410.
[23] Wei, R. C., Li, H., Jiang, J. G. (2012): ISSR analysis of genetic background of polyploid seedless *Siraitia grosvenorii* and its parents. – Acta Horticulturae 39(2): 387-394.
[24] Wei, R. C., Bai, L. H. (2015): ISSR analysis of genetic background of triploid female and diploid male strains of *Siraitia grosvenorii*. – Chinese Traditional and Herbal Drugs 46(06): 881-886.
[25] Wei, S. L. (2003): Geographic Variation and Provenance Selection of *Glycyrrhiza uralensis* Fich. – Northeast Forestry University, Harbin.
[26] Xiao, N. J., Deng, L. L., Wei, X., et al. (2023): Research on cutting technology of Golden Sophora, a medicinal and edible plant. – Journal of Guangxi Academy of Sciences 1: 62-70.
[27] Yang, Y. S., Chen, Z. Y., Zou, R., et al. (2023): Comparative study of endogenous hormone dynamics during the first and second growth cycles in Sophora japonica cv. jinhuai harvested twice a year. – Journal of Biotech Research 14: 59-66.
[28] Zou, J. L., Li, H. Y., Wang, Z. L., et al. (2023): Functional characterization of two efficient glycosyltransferases catalysing the formation of rutin from Sophora japonica L. – Organic & Biomolecular Chemistry 21:7913-7916. https://doi.org/10.1039/D3OB01281F.

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