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# AL-7, A Novel *Rosa roxburghii* Genotype with Superior Nutritional and Antioxidant Value

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**Keywords.** amino acid, antioxidant activity, AsA, germplasm, phenolic compounds, *Rosa roxburghii*, triterpenoid, tyrosinase inhibitory activity

*Rosa roxburghii* Tratt, a perennial rosebush native to China, is widely distributed in the southwestern provinces of China (Lu et al. 2021). According to local folk traditions, various parts of the *R. roxburghii* plant are used as herbal products or functional foods. For example, its roots are considered effective in the treatment of diarrhea, whereas its leaves are used in the treatment of dyspepsia and to make Chinese tea. It is also a good natural source of antioxidant phytochemicals and essential oils (Liu et al. 2016). In particular, the fruit of this species is well-known for its high L-ascorbic acid (AsA) content, and a wide range of nutritional and medicinal components, including amino acids, phenolic compounds, and triterpenoids, and is believed to have antioxidant and tyrosinase inhibitory activities (Jiang et al. 2022; Li et al. 2022; Lu et al. 2017; Zeng et al. 2017). A range of functional foods and health care products for clinical applications have been developed from this fruit and its organic extracts.

Due to its favorable agronomic traits, the cultivar Guinong 5 has been developed and commercially grown on ~130,000 ha in China. Compared with other fruits, such as kiwifruit, strawberry, and orange, *R. roxburghii* fruit (e.g., ‘Guinong 5’) has an unusually high AsA content of ~861 mg per 100 g of fresh fruit (Li et al. 2022). As an important functional phytonutrient, AsA, also known as vitamin C,

is of vital importance to human beings, as it protects the body against oxidative stress, acts in collagen synthesis reactions, promotes a healthy immune system and wound healing, and aids in iron absorption (Padayatty et al. 2003). It also plays an important role in delaying the aging of human chondrocytes (Chang et al. 2015) and has anticancer properties (Reang et al. 2021). Because human beings do not have the ability to synthesize or store AsA in their bodies, this vitamin must be acquired regularly from dietary uptake. Thus, the natural level of AsA in foods is an important quality indicator in evaluating the nutritional value of fruits and vegetables. Amino acids are another important type of biologically active compound. Amino acids are the building blocks of proteins and polypeptides, and the quantification of free amino acids (FAAs) in biological fluids or tissues provides nutritional information used in the diagnosis of certain diseases (Nagao and Kimura 2020). The metabolism of FAAs has been related to periodontal disease (Balci et al. 2021), fatty liver disease (Yamakado et al. 2017), and Alzheimer’s disease (Fonteh et al. 2007). In addition to nutritional and clinical properties, some FAAs taste sweet or are delicious to humans (Lu et al. 2015), and therefore contribute substantially to food flavor. Phenolic compounds were considered to contribute to the antioxidant and tyrosinase inhibitory activities (Oskoueian et al. 2011).

As part of our continuing work investigating the concentration of AsA and amino acids in ‘Guinong 5’ fruit (Lu et al. 2017), we have carried out an extensive investigation on wild germplasms of the *R. roxburghii* species to identify elite genotypes of this rosebush. Here, we report a novel germplasm of *R. roxburghii*, coded as AL-7, whose fruit showed significantly higher concentrations of AsA, triterpenoids, amino acids, phenolic compounds, and antioxidant and tyrosinase inhibitory activity

compared with ‘Guinong 5’. Based on this, we have characterized the AsA and triterpenoid concentration, amino acid and phenolic compounds profile, and antioxidant and tyrosinase inhibitory activity of two *R. roxburghii* germplasms. The novel genotype offers potential as a functional food ingredient with improved nutritional characteristics when compared with the current commonly grown cultivars.

## Origin

AL-7 was discovered in Anlong county, Guizhou Province (lat. 24°55' N–25°33' N, long. 104°59' E–105°41' E) in 2015, and was retrieved for asexual reproduction in the fruit germplasm repository of Guizhou University (lat. 26°42.408'N, long. 106°67.353' E). The decision was made to name this variant after 6 to 7 years of observation and evaluation.

## Material and Methods

### Plant material

*R. roxburghii* fruits of ‘Guinong 5’ and a novel germplasm (AL-7, Fig. 1A) were used in this experiment, grown in the fruit germplasm repository of Guizhou University, Guiyang, China (lat. 26°42.408'N, long. 106°67.353'E). Fruits (Fig. 1B and C) were collected in September of 2021 and each sample consisted of 20 uniformly sized fresh fruits that were randomly selected. After being washed with deionized water and dried, fruits were weighed and measured for size as shown in Table 1. Fresh fruits were frozen in liquid nitrogen immediately and then stored at –72 °C for AsA quantification. Water content was determined by weighing the sample before and after drying, which occurred at 55 °C until a constant weight was obtained. The dried samples were ground briefly in a mortar and then passed through a 65-mesh screen for further analysis. All chemicals, reagents, and standards used in this research were analytical or high-performance liquid chromatography (HPLC) grade from Sigma Chemical Co. (St. Louis, MO, USA), unless indicated otherwise.

### Data collection

**HPLC method for AsA quantification.** AsA was measured using HPLC (Rassam and Laing, 2005). Specifically, 0.5 g of frozen fruits were added to 5 mL of 6% metaphosphoric acid. A C18 column was used and eluted at 1 mL/min at 35 °C. A 0.2% solution of metaphosphoric acid was used in the mobile phase. A set of standards containing 2 to 250 mg/L of AsA were prepared and analyzed in the same way as the samples. AsA concentrations were calculated from the standard curve and expressed in mg/100 g fresh weight (FW).

**Amino acid analysis.** Amino acid analysis was performed by acid hydrolysis. Sample preparation was carried out according to the method described by Gehrke et al. (1985) and Lu et al. (2017) except for cysteine, which was 50 nmol/mL. Another standard sample (Trp: L-tryptophan) was solely prepared to 100 µmol/mL then diluted to 100 nmol/mL. Finally, the essential amino acid content (EAA),

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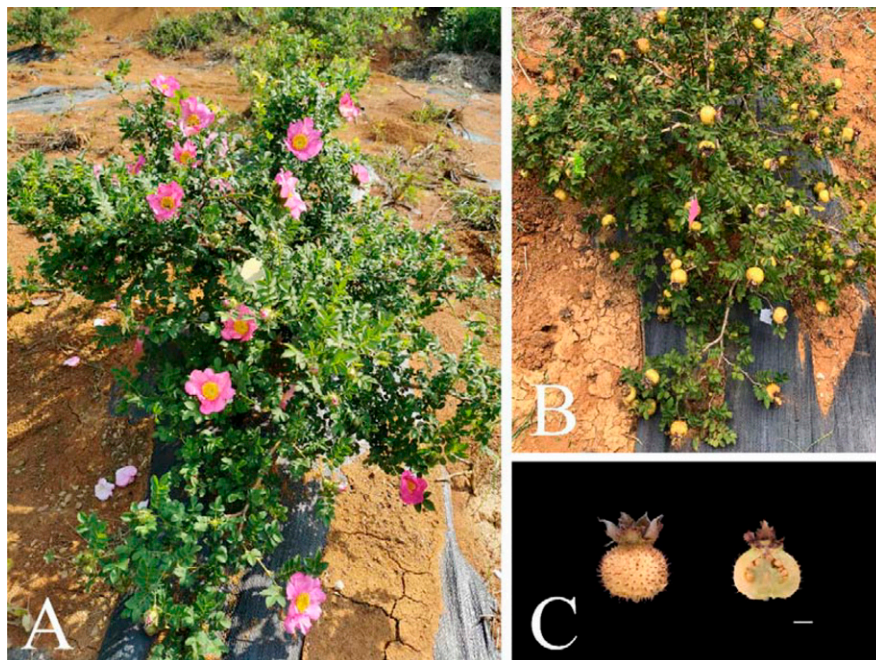


Fig. 1. A novel *Rosa roxburghii* genotype AL-7. (A) The plant in bloom. (B) The fruiting plant. (C) The whole mature fruit and its longitudinal section. Scale bar = 1 cm.

amino acid ratio (RAA) of two different *R. roxburghii* proteins was calculated separately.

**FAAs and metabolites.** Sample preparation was carried out according to the method described by Lu et al. (2017). Each amino acid and its metabolite was 100 nmol/mL, with the exception of cysteine which was 50 nmol/mL.

**Amino acid composition and content analysis.** Filtrate (20  $\mu$ L) was measured in an A300 automatic amino acid analyzer (MembraPure, Berlin, Germany), with a column packed with custom ion-exchange resin (4.6 mm  $\times$  100 mm, particle size 7  $\mu$ m). A column temperature of

30 to 70  $^{\circ}$ C was used and the reactor was held at 115  $^{\circ}$ C, with a sample injection rate of 250  $\mu$ L/min, and monitoring occurred at 570 nm and 440 nm. Amino acid concentrations were expressed in g or mg/100 g dry weight (DW).

**Determination of triterpenoids.** Triterpenoid content was analyzed by extracting samples using the method outlined by Zeng et al. (2017) with slight modifications. One gram of each sample was placed into a volumetric flask, with 15 mL ethanol (75%) and placed in an ultrasonic cleaning bath at 40 kHz for 50 min at 50  $^{\circ}$ C. After filtration through filter

paper, anhydrous ethanol was added to take the final volume of the extract to 150 mL. Aliquots of 300  $\mu$ L *R. roxburghii* fruit extract were transferred to a tube and heated to dryness at 100  $^{\circ}$ C in a water bath. After that, 500  $\mu$ L vanillin–acetic acid solution (5:95, w/v) and 800  $\mu$ L perchloric acid were added to the dried sample and incubated at 60  $^{\circ}$ C in a water bath for 15 min. Then, they were transferred into an ice-water bath, followed by an addition of 5 mL acetic acid, and placed at room temperature for 15 min. With a blank solution as a reference, the absorbance was determined using a spectrophotometer at 548 nm. Ursolic acid was used as a standard, and results were expressed as mg ursolic acid per 100 g DW.

**2,2-Diphenyl-1-picrylhydrazyl free radical scavenging activity.** 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity was performed according to the method of Zeng et al. (2017), with minor modifications. The extracts were diluted to a proper concentration, and then 2 mL of the extract was added to 4 mL DPPH solution (0.10 mM). After 30 min in the dark at room temperature, the absorbance was measured relative to a reagent blank also incubated at the same condition at 519 nm. The DPPH radical scavenging rate (%) was calculated as follows:

$$\text{Radical scavenging rate (\%)} =$$

$$(A_0 - A_x + A_{x0})/A_0 \times 100,$$

where  $A_x$  is the absorbance of test compounds,  $A_{x0}$  is the absorbance of test compounds with 4 mL anhydrous ethanol as the blank reaction, and  $A_0$  is the absorbance of ethanol solution (60%, v/v) as the blank reaction. DPPH radical scavenging rates of different concentrations from each sample were measured, and results were expressed in micromoles of Trolox equivalent antioxidant capacity per liter DW ( $\mu$ mol TE/L).

**Ferric-reducing antioxidant power assay.** The ferric-reducing antioxidant power (FRAP) assay was performed according to the method of Zeng et al. (2017), with slight modifications. The FRAP reagent was prepared by mixing 0.2 M sodium acetate with acetic acid (pH 3.6), TPTZ solution (10 mM in 40 mM HCl), and 20 mM  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  solution in a ratio of 10:1:1 (by volume) before evaluation. An aliquot of 100  $\mu$ L of the extract was added to 4.9 mL of FRAP reagent, transferred into vials, and incubated at 37  $^{\circ}$ C for 10 min. Absorbance was measured at 593 nm and results were expressed in micromoles of Trolox equivalent antioxidant capacity per liter DW ( $\mu$ mol TE/L).

**Tyrosinase inhibitory activity determination.** Tyrosinase inhibitory rate of the isolated compounds was measured spectrophotometrically by the method proposed by Zeng et al. (2017). Sample extract (400  $\mu$ L) was added to a reaction mixture containing 800  $\mu$ L of 12 mM DOPA solution, 2 mL of 0.05M sodium phosphate buffer (pH 6.5), and 800  $\mu$ L of mushroom tyrosinase solution (110 units/mL).

Table 1. Weight, dimension, water content, L-ascorbic acid (AsA) concentration, triterpenoid concentration, phenolic compounds, antioxidant capacity, and tyrosinase inhibitory activity of *Rosa roxburghii* fruit.

	'Guinong 5'	AL-7
Weight (g)/fruit	14.22 $\pm$ 1.28 a	12.94 $\pm$ 1.32 a
Length (cm)	2.53 $\pm$ 0.62 a	2.13 $\pm$ 0.23 a
Width (cm)	3.62 $\pm$ 0.54 a	3.40 $\pm$ 0.41 a
Water (%)	84.83 $\pm$ 0.69 b	86.48 $\pm$ 0.57 a
AsA (mg/100 g FW)	1277.92 $\pm$ 25.69 b	2274.02 $\pm$ 48.36 a
Triterpenoid (mg ursolic acid/100 g DW)	3531.82 $\pm$ 100.14 b	4333.06 $\pm$ 252.09 a
Total phenolics (mg gallic acid/100 g DW)	7042.60 $\pm$ 244.33 a	6808.86 $\pm$ 202.13 a
phenolic compounds (mg/100 g DW)		
Gallic acid	5.55 $\pm$ 0.04 b	12.11 $\pm$ 0.72 a
(+)-Catechin hydrate	141.06 $\pm$ 2.57 b	333.95 $\pm$ 4.58 a
Chlorogenic acid	46.43 $\pm$ 0.31 b	65.54 $\pm$ 0.15 a
Epicatechin	247.54 $\pm$ 37.91 b	299.74 $\pm$ 9.62 a
Caffeic acid	3.66 $\pm$ 0.31 b	5.7 $\pm$ 0.35 a
Polydatin	44.13 $\pm$ 0.34 a	30.84 $\pm$ 0.89 b
Ferulic acid	8.44 $\pm$ 0.58 a	6.97 $\pm$ 0.18 b
Rutin	44.04 $\pm$ 2.65 b	72.96 $\pm$ 2.53 a
Ellagic acid	22.1 $\pm$ 0.36 b	31.47 $\pm$ 0.02 a
Antioxidant and tyrosinase inhibitory activity		
DPPH ( $\mu$ mol TE/L)	3173.65 $\pm$ 184.42 a	3110.71 $\pm$ 127.62 a
FRAP ( $\mu$ mol TE/L)	7045.27 $\pm$ 111.27 b	7642.86 $\pm$ 254.48 a
TIR (mg kojic acid/100 g DW)	232.51 $\pm$ 10.41 b	263.86 $\pm$ 13.64 a

FW = fresh weight; DW = dry weight; TE = Trolox Equivalent antioxidant capacity. Means followed by the same letter within each row are not significantly different (Tukey's honestly significant difference). Values after  $\pm$  are standard errors (SE).

The reaction mixture was incubated at 37 °C for 2 min, after which the amount of dopachrome produced was measured at 475 nm, using a spectrophotometer. Kojic acid was used as a positive control and ethanol was used as the blank. The percentage of tyrosinase inhibition was calculated as follows:

$$\text{Inhibition rate (\%)} = (A - B)/A \times 100,$$

where A is the absorbance of blank and B is the absorbance of test samples. Tyrosinase inhibitory rate (TIR) of different concentrations of each sample was measured, and results were expressed in milligram kojic acid per 100 g DW (mg kojic acid/100 g DW).

#### Determination of total phenolic compounds.

The total phenolics of the samples was determined according to the Folin-Ciocalteu method described by Zeng et al. (2017) with minor modifications. Briefly, 1 mL of each extract was mixed with 5 mL of 10% (w/v) Folin-Ciocalteu reagent and 4 mL of 7.5% (w/v) sodium carbonate. The volumetric flasks were shaken and allowed to stand for 1 h at room temperature, and the absorbance was measured with a spectrophotometer at 765 nm. Gallic acid was used as a standard, and results were expressed as milligram gallic acid per 100 g DW (mg gallic acid/100 g DW).

#### Analysis of phenolic composition by HPLC.

Determination of the phenolic composition was conducted as follows: after filtration through a Millipore membrane (0.45 µm), 20 µL of the solution was injected into the HPLC system. Chromatographic separation was performed using a reverse-phase column (Phenomenex-C18, 250 mm × 4.6 mm i.d., 5 µm particle size) and an ultraviolet-vis detector. The mobile phase was composed of A (H<sub>2</sub>O/acetic acid solution, 80:20, v/v) and B (methyl alcohol). The gradient elution was performed as follows: 0~10 min, 5%~20% B, 10~25 min, 20%~30% B, 25~40 min, 30%~50% B, 40~50 min, 50%~70% B, 50~52 min, 70%~5% B. The column temperature was maintained at 30 °C, and the flow rate was kept at 1.0 mL/min. The detection wavelengths were set at 280 nm to detect flavonoids and phenolic acids. Quantitative determinations were performed using an external standard method as previously described by Rehman et al. (2017). The identification of compounds was achieved by comparing their retention time values and ultraviolet spectrograms with those of the appropriate standards and the results were expressed in milligrams per 100 g DW (mg/100 g DW). All samples were analyzed using three replicates.

**Statistical analysis.** Statistical analysis was performed using SPSS 19 software (SPSS Inc., Chicago, IL, USA). Analysis of variance was conducted, and Tukey's honestly significant difference determined the significant difference between the cultivars (α = 0.05).

## Results

**AsA.** There were no differences in the appearance index, such as weight, length, and width, between AL-7 and 'Guinong 5'; however, water

content in AL-7 was higher than that of 'Guinong 5' (Table 1). In the fruit, AsA accumulated to 2274.02 ± 48.36 mg/100 g FW (equivalent to 129.20 µmol/g) until maturity of the fruit in AL-7 (Table 1), which was significantly more than in 'Guinong 5', which was 1277.92 ± 25.69 mg/100 g FW. This represents an increase of 77.95%, and a much higher concentration of AsA than in other high fruits and vegetables that are considered high in AsA, such as acerola (~70 µmol/g, Badejo et al. 2007), kiwifruits (800 mg/100 g FW, Bulley et al. 2009), and peppers (239 mg/100 g FW, Alós et al. 2013). This cultivar may have the potential to produce even more abundant AsA.

**Triterpenoids.** Triterpenoids are an important compound class in *R. roxburghii*, as they play a major role in the antioxidant capacity of *R. roxburghii* fruit (Jiang et al. 2022). The triterpenoid content of AL-7 accumulated to 4333.06 ± 252.09 mg/100 g DW (Table 1), which was significantly more than in 'Guinong 5' (3531.82 ± 100.14 mg/100 g DW).

**Phenolic compounds.** Among the nine monomeric phenols detected, the content of seven monomeric phenols in AL-7 was significantly higher than that of 'Guinong 5'. Namely, gallic acid, catechin hydrate, chlorogenic acid, epicatechin, caffeic acid, rutin, and ellagic acid in AL-7 were higher than those in 'Guinong 5', and they increased by 118.20%, 136.74%, 41.16%, 21.09%, 55.74%, 65.67%, and 42.40%, respectively. Overall, the concentration of catechin hydrate and epicatechin are the highest, and the contents of caffeic acid and ferulic acid are the lowest (Table 1).

#### Antioxidant and tyrosinase inhibitory activity.

The free radical scavenging ability and tyrosinase inhibitory activity analysis of AL-7 and 'Guinong 5' are shown in Table 1. There was no significant difference in the ability of free radical scavenging between the two types of *R. roxburghii*. The FRAP radical scavenging

capacity and tyrosinase inhibitory activity of AL-7 were 8.48% and 13.48% higher than those of 'Guinong 5', respectively.

AL-7 has strong tyrosinase inhibitory activity, so its extract is expected to be processed into various skin care products and drugs for the treatment of hyperpigmentation disorders, which will bring new prospects for people's health.

**Amino acids.** The amino acid content of *R. roxburghii* fruits is shown in Table 2. Seventeen amino acids were detected in both *R. roxburghii* fruits, in which Cys was absent, Trp, Met, and His were relatively low in abundance, and Glu, Asp, and Leu were highly abundant, which was largely similar to previous findings reported in other *R. roxburghii* fruits (He et al. 1988). In terms of absolute abundance, in AL-7 the amino acid concentration was 4.21 ± 0.22 g/100 g, which was 38.03% higher than in 'Guinong 5', at 3.05 ± 0.20 g/100 g. Of the detected 17 amino acids, 13 in AL-7 were significantly more abundant than those in 'Guinong 5', with the exception of four EAAs: Thr, Met, Phe, and Lys. Because of this, the essential amino acid concentration in AL-7 was 1.51 ± 0.07 g/100 g, which was indeed higher than that of 'Guinong 5' at 1.21 ± 0.09 g/100 g. Because of the higher concentrations of nonessential amino acids, they only accounted for 35.80% of the total amino acids, as opposed to 39.03% in 'Guinong 5'.

The distribution and concentration of FAAs and metabolites in two *R. roxburghii* fruits are listed in Table 3 in the order of elution positions, which contained 19 generally coded amino acids and eight amino acid metabolites (P-Ser, α-AAA, α-ABA, β-Ala, GABA, Orn, EtNH<sub>2</sub>, and Hypro). The other metabolites, Tau, PEA, Urea, Sar, Cit, Cys-tha, Nle, H-Cysteine, β-AiBA, 3Mehis, 1Mehis, Car, Ans, and Hyllys, were not detected in these two fruits. Total N-containing compounds in AL-7 were 706.41 ± 52.53 mg/100 g,

Table 2. Concentration of amino acids (following acid hydrolysis of proteins) in *Rosa roxburghii* fruits.

Amino acid	'Guinong 5'		AL-7	
	g/100 g dry mass	% of total	g/100 g dry mass	% of total
Asp	0.30 ± 0.03 b	9.81	0.43 ± 0.06 a	10.17
Thr	0.17 ± 0.03 a	5.43	0.21 ± 0.01 a	5.08
Ser	0.19 ± 0.02 b	5.87	0.24 ± 0.01 a	5.58
Glu	0.36 ± 0.01 b	11.76	0.56 ± 0.02 a	13.30
Gly	0.18 ± 0.01 b	6.14	0.23 ± 0.01 a	5.46
Ala	0.19 ± 0.01 b	6.35	0.32 ± 0.01 a	7.48
Val	0.19 ± 0.02 b	7.67	0.24 ± 0.00 a	5.75
Met	0.04 ± 0.02 a	1.23	0.06 ± 0.00 a	1.35
Ile	0.13 ± 0.00 b	5.50	0.21 ± 0.01 a	4.92
Leu	0.24 ± 0.01 b	8.03	0.38 ± 0.01 a	8.98
Tyr	0.11 ± 0.00 b	3.71	0.23 ± 0.01 a	5.54
Phe	0.19 ± 0.00 a	6.28	0.15 ± 0.02 a	3.44
His	0.09 ± 0.00 b	3.10	0.14 ± 0.00 a	3.42
Lys	0.23 ± 0.01 a	4.17	0.23 ± 0.02 a	5.44
Arg	0.25 ± 0.00 b	7.92	0.31 ± 0.02 a	7.32
Pro	0.17 ± 0.03 b	6.31	0.25 ± 0.01 a	5.92
Trp	0.02 ± 0.00 b	0.72	0.04 ± 0.00 a	0.83
EAA	1.21 ± 0.09 b	39.03	1.51 ± 0.07 a	35.80
TAA	3.05 ± 0.20 b	100.00	4.21 ± 0.22 a	100.00

EAA = essential amino acids (sum of Thr, Val, Met, Ile, Leu, Phe, Lys and Trp); TAA = total amino acids (sum 17 amino acids). Means followed by the same letter within each row are not significantly different (Tukey's honestly significant difference). Values after ± are standard errors (SE).

Table 3. Concentration of free amino acids (FAAs) and amino acid metabolites in *Rosa roxburghii* fruits.

Amio acid	'Guinong 5'		AL-7	
	g/100 g dry mass	% of total	g/100 g dry mass	% of total
<b>P-Ser</b>	65.73 ± 3.25 b	13.45	270.35 ± 12.54 a	38.27
Asp	3.44 ± 0.12 b	0.70	13.14 ± 1.52 a	1.86
Thr	6.92 ± 0.57 a	1.42	4.62 ± 0.32 b	0.65
Ser	6.76 ± 0.41 a	1.38	3.62 ± 0.18 b	0.51
Asn	20.34 ± 2.58 a	4.16	18.07 ± 0.96 a	2.56
Glu	0.00 ± 0.00 b	0.00	11.27 ± 1.43 a	1.60
<b>α-AAA</b>	2.21 ± 0.12 b	0.45	10.96 ± 0.65 a	1.55
Gly	1.29 ± 0.08 b	0.26	1.67 ± 0.12 a	0.24
Ala	24.47 ± 4.26 a	5.01	18.38 ± 2.07 a	2.60
<b>α-ABA</b>	2.57 ± 0.36 a	0.53	1.05 ± 0.21 b	0.15
Val	14.09 ± 2.13 a	2.88	5.64 ± 0.56 b	0.80
Cys	0.00 ± 0.00 b	0.00	1.70 ± 0.06 a	0.24
Met	0.66 ± 0.03 b	0.13	0.96 ± 0.03 a	0.14
Ile	6.70 ± 0.32 a	1.37	7.66 ± 0.65 a	1.08
Leu	4.63 ± 0.15 b	0.95	16.44 ± 0.98 a	2.33
Tyr	9.91 ± 0.89 b	2.03	15.68 ± 1.02 a	2.22
Phe	3.54 ± 0.54 b	0.72	11.20 ± 0.88 a	1.59
<b>β-Ala</b>	20.00 ± 1.36 b	4.09	38.98 ± 2.63 a	5.52
<b>GABA</b>	131.76 ± 9.68 a	26.97	111.03 ± 15.32 a	15.72
His	4.17 ± 0.65 b	0.85	8.13 ± 0.74 a	1.15
Trp	3.90 ± 0.69 a	0.80	2.85 ± 0.35 a	0.40
<b>Orn</b>	34.49 ± 2.32 a	7.06	5.30 ± 0.48 b	0.75
Lys	4.72 ± 0.31 b	0.97	7.97 ± 0.95 a	1.13
<b>EtNH2</b>	4.12 ± 0.54 a	0.84	3.70 ± 0.23 a	0.52
Arg	83.60 ± 7.35 a	17.11	93.64 ± 6.25 a	13.26
<b>Hypro</b>	10.10 ± 0.79 a	2.07	7.80 ± 0.42 b	1.10
Pro	18.40 ± 1.68 a	3.77	14.64 ± 0.98 b	2.07
EAA	45.15 ± 4.74 b	9.24	57.33 ± 4.72 a	8.12
TAA	217.52 ± 22.76 a	44.53	257.25 ± 20.05 a	36.42
Metabolites	270.97 ± 18.42 b	55.47	449.16 ± 32.48 a	63.58
Total N-containing compounds	488.49 ± 41.18 b	100.00	706.41 ± 52.53 a	100.00

EAA = essential amino acids (sum of Thr, Val, Met, Ile, Leu, Phe, Lys and Trp); TAA = total amino acids (sum of 19 amino acids); Metabolites = sum of P-Ser, α-AAA, α-ABA, β-Ala, GABA, Orn, EtNH2 and Hypro. Means followed by the same letter within each row are not significantly different (Tukey's honestly significant difference). Values after ± are standard errors (SE).

which remarkably was 44.61% higher than in 'Guinong 5' (488.49 ± 41.18 mg/100 g). Likewise, the levels of total EAAs (Thr, Val, Met, Ile, Leu, Phe, Trp, and Lys) were 26.98% higher, and total metabolites were 65.76% greater. Arg was the most abundant FAA in the two *R. roxburghii* fruits, followed by Ala and Asn. Cys and Glu were only found in the fruit of AL-7, and only in a small amount. For amino acid metabolites, GABA was abundant in both *R. roxburghii* fruits, but in AL-7, the P-ser content was 4.11-fold that of 'Guinong 5'.

**Propagation.** AL-7's root system is shallow, the primary root is underdeveloped, but there are numerous lateral roots and fibrous roots, with the root system being mostly distributed in the top centimeter of the soil layer. The root readily produces adventitious buds, so it is easy to propagate from root cuttings. Taking cuttings is best performed in Autumn, when the air temperature is cool and the soil temperature and moisture levels are relatively stable. The successful rooting rate is high and it is generally suitable to cultivate *R. roxburghii* in mid-September to late October in Guizhou province. It is easiest to select root segments with a thickness of 0.3~1.5 cm and a length of 15 cm, and it is best to cultivate in a deep soil layer with medium fertility. The main disease of *R. roxburghii* is powdery mildew, which is most prevalent in Guizhou province from June to July; fungicides including Triadimefon can be sprayed for control.

### Availability

Enquiries about AL-7 germplasm resources should be made to the Agricultural College of Guizhou University.

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