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Amino Acid Content in *Rhododendron schlippenbachii* Maxim. Flowers of Different Colors

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Rhododendron **is one of the largest shrubs and is well known as a garden plant because of their evergreen leaves and various flower colors. The present study investigated the variation in amino acids in the differently colored flowers of** ***Rhododendron schlippenbachii*****. Analysis of the** ***Rhododendron*** **flowers revealed 22 types of amino acids. The amino acid content varied greatly depending on the different colors of the flowers. Violet** ***Rhododendron*** **flowers had the highest total quantity of amino acids, which was 2.24 and 1.31 times higher than the total amino acid content in the red and white flowers, respectively. Violet** ***Rhododendron*** **flowers also contained the highest quantities of aspartate, glutamate, glutamine, histidine, ^{3}-aminobutyric acid (GABA), and methionine while white** ***Rhododendron*** **flowers contained the highest quantities of serine, threonine, alanine, valine, norvaline, tryptophan, phenylalanine, isoleucine, and leucine. Compared to the violet flowers, white flowers had 1.24, 1.35, 2.34, and 2.46 times higher amounts of serine, tryptophan, phenylalanine, isoleucine, and leucine, respectively. Although the total amino acid content was the lowest in the red flowers, these flowers contained the highest quantities of Asparagine, Vitamin U, Glutamine, Glycine, Tyrosine, Cysteine, and Lysine. The content of Asparagine was much higher in red flowers than in any other color of** ***Rhododendron*** **flower. Red flowers contained 3.70 and 2.48 times higher levels of asparagine than that measured in white and violet colored** ***Rhododendron*** **flowers, respectively. Our results demonstrate that** ***Rhododendron*** **flowers with different colors contain variable quantities of amino acids, with the highest total amount of amino acids observed in the violet flowers.**

Keywords: amino acids, flower color, variation, *Rhododendron schlippenbachii***.**

Rhododendron schlippenbachii, or the royal azalea, is a species of *Rhododendron* native to the Korean Peninsula and adjacent regions of Manchuria, Japan, and the Russian Far East. It is the dominant understory shrub in many Korean hillside forests, growing at an altitude of 400–1500 m. *Rhododendron* is one of the largest shrub genera, has been distributed through most of the northern hemisphere, and their species are well-known garden plants due to their evergreen leaves and various flower colors[1]. Furthermore, the vast genus is used in the traditional medical system in China, Europe, and North America. Such application is based on a tremendous number of phytochemicals with diverse biological activities, such as anti-microbial[2], anti-inflammatory[3], anti-diabetic[4], and anti-oxidative properties[5]. The *R. schlippenbachii* Maxim. (*R. schlippenbachii* Maxim) species has traditionally been sought after as a garden plant because of its attractive and diverse flowers, but it also has potential as a source for natural medicines, because of its activities as a cholinesterase inhibitor, anti-hyperglycemic, and anti-oxidant[6-8] (Figure 1).

Amino acids are important for all life processes. They are essential for every metabolic

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process, as well as for the optimal transport and optimal storage of all nutrients (i.e., water, fat, carbohydrates, proteins, minerals, and vitamins). Many diseases such as obesity, high-cholesterol levels, diabetes, insomnia, erectile dysfunction, or arthritis can be caused by metabolic disturbances. This can also apply to hair loss and serious cases of wrinkle formation. The correct amino acid composition may be able to repair many of these metabolic deficiencies. This is confirmed by various studies that stress the importance of amino acids hair and dermal health[9-11]. Additionally, the amino acid arginine can lead to considerable expansion of the blood vessels, improving blood pressure in humans[12-14]. Another study showed that arginine is also important in the treatment of diabetes-related foot diseases[15].

More than 60% of the protein required by humans comes from plant sources. The most important function of amino acids is that they are the building blocks of proteins. Amino acids have antioxidant effects[16-19], and free amino acids are necessary in secondary plant metabolism and the biosynthesis of compounds, such as glucosinolates and phenolics, that play important roles, either directly or indirectly, in plant–environment interactions and human health[20]

Although many amino acids exist in nature, approximately 24 are reported to be essential to human nutrition[21,22]. Several previous studies have addressed the different nutritive properties of *Rhododendron*; however, to our knowledge no study has shown the amino acid content in the different species of *Rhododendron*. The objective of the present study was to determine the profile and quantity of amino acids present in the differently colored flowers of *Rhododendron.*

MATERIAL AND METHODS

Plant material

White, violet, and red flowers of three *R. schlippenbachii* Maxim cultivars were maintained at the Chungnam National University Experiment Farm, Daejeon, Korea. Flowers of these three cultivars were harvested on May 10, 2015 and immediately freeze-dried at -80°C for at least 72 h before being ground using a mortar and pestle into a fine powder for amino acid analysis.

Chemicals

We obtained trichloroacetic acid (TCA, 99.0%) from Samchun Pure Chemical Co., Ltd. (Pyeongtaek, Korea). Standards for 16 amino acids and four amino acid supplements were obtained from Agilent Technologies (Waldbronn, Germany). The vitamin U (DL-methionine methylsulfonium chloride) standards and sodium phosphate monobasic monohydrate (NaH_2PO_4) were purchased from Sigma-Aldrich (St. Louis, MO, USA). High performance liquid chromatography (HPLC)-grade acetonitrile (ACN) and methanol (MeOH) were purchased from J. T. Baker (Phillipsburg, NJ, USA). Ultrapure water with a resistivity of 18.2 M©/cm was produced using a PureLab Option system from ELGA LabWater (Model LA 621; Marlow, UK).

Extraction and HPLC of free amino acids

In a 2-mL Eppendorf tube, 100 mg of freeze-dried plant powder was suspended in 1.2 mL 5% (v/v) TCA solution. The mixture was vortexed and allowed to stand for at least 1 h at room temperature before being centrifuged at 15,000 ×*g* for 15 min at 4°C. The supernatant was filtered through a 0.45-µm hydrophilic polyvinylidene difluoride (PVDF) syringe filter (Ø13 mm, Cat. no. 6779-1304; Whatman Int. Ltd., Maidstone, UK) into an HPLC vial.

HPLC analysis of the free amino acids was performed as described previously[23]. Briefly, 20 different free amino acids were identified using an Agilent 1200 Series HPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with Zorbax Eclipse Amino Acid Analysis (AAA) columns (150 × 4.6 mm i.d., particle size 5 ¼m) and Zorbax Eclipse AAA Guard columns (12.5 × 4.6 mm i.d., particle size 5 ¼m, 4-pack). A wavelength of 338 nm, 40f, and flow rate of 2.0 mL/min were used in the HPLC conditions. The mobile phase consisted of 40 mM NaH_2PO_4 (pH 7.8, solvent A) and ACN:MeOH:H_2O (45:45:10, v/v/v) (solvent B). The HPLC gradient protocol was as follows: a linear step from 0% to 57% of solvent B from 1.9 to 21.1 min; 57% to 100% of solvent B from 21.1 to 21.6 min; isocratic conditions with 100% solvent B from 21.6 to 25.0 min; followed by a rapid drop to 0% solvent B at 25.1 min; and then isocratic conditions with 0% solvent B until completion (total 30 min). Standards were prepared as individual solutions (50 pmol/µL [0.05 mM]) of 20 amino acids. The free amino acids quantification was based on HPLC

peak areas calculated as equivalents of the standard compounds. All quantities were expressed as milligrams per 100 grams fresh weight (FW). All samples were run in triplicate.

RESULTS AND DISCUSSION

Amino acid content in white, violet, and red flowers of *R. schlippenbachii*

Analysis of the different *Rhododendron* flowers revealed 22 types of amino acids that differed in content based on flower color (Table 1). Arginine was not detected in any of the flowers and among the 21 amino acids, vitamin U and glycine were not detected in white *Rhododendron* flowers; and vitamin U, glycine, and leucine were not detected in violet *Rhododendron* flowers. In addition, no methionine, norvaline, or phenylalanine was detected in red flowers. Among the amino acids that were found in *Rhododendron*, the levels of histidine and glutamine were much higher in both violet and white flowers than in red flowers.

Table 1. Amino acid content in white, violet and red flowers of *Rhododendron schlippenbachii* Maxim

Free amio acids	Flower color (amino acid:mg/100 g dry wt.)		
	White	Violet	Red
Aspartate	41.48 ± 1.01	59.68 ± 0.22	38.96 ± 0.70
Glutamate	13.04 ± 0.23	15.21 ± 0.90	8.16 ± 0.22
Asparagine	66.22 ± 0.36	98.70 ± 0.56	244.99 ± 0.99
Serine	29.90 ± 0.06	24.09 ± 0.03	24.35 ± 1.23
Vitamin U	0.00	0.00	1.63 ± 0.00
Glutamine	114.74 ± 9.05	158.91 ± 14.29	35.42 ± 2.71
Histidine	394.29 ± 15.78	555.41 ± 27.47	2.37 ± 0.01
Glycine	0.00	0.00	3.28 ± 0.23
Threonine	21.82 ± 0.14	20.04 ± 0.41	11.65 ± 0.14
Arginine	0.00	0.00	0.00
Alanine	49.35 ± 0.33	34.27 ± 0.40	19.59 ± 0.04
GABA	18.48 ± 0.79	27.34 ± 1.77	23.38 ± 0.56
Tyrosine	10.38 ± 7.40	21.54 ± 18.59	29.42 ± 16.28
Cystine	7.79 ± 1.97	11.35 ± 1.81	11.79 ± 3.69
Valine	9.83 ± 0.03	7.85 ± 0.15	2.97 ± 0.08
Methionine	2.77 ± 0.04	3.45 ± 0.04	0.00
Norvaline	4.45 ± 0.02	3.47 ± 0.27	0.00
Tryptophan	4.48 ± 0.25	3.33 ± 0.04	3.80 ± 0.09
Phenylalanine	4.51 ± 0.04	1.93 ± 0.01	0.00
Isoleucine	5.23 ± 0.01	2.13 ± 0.34	2.60 ± 0.13
Leucine	2.17 ± 0.04	0.00	1.46 ± 0.01
Lysine	3.56 ± 0.06	2.87 ± 0.09	4.01 ± 0.02
0 Total	804.50 ± 19.19	1051.55 ± 21.44	469.84 ± 17.31

[a)]ND, not detected.

Violet *Rhododendron* flowers contained the highest total quantity of amino acids (1051.55 mg/100 g dry wt.), which was 2.24 and 1.31 times higher than that in red and white *Rhododendron*, respectively. Violet *Rhododendron* flowers had the highest quantities of aspartate, glutamate, glutamine, histidine, γ-aminobutyric acid (GABA), and methionine. The contents of these amino acids ranged 38.96–59.68, 8.16–15.21, 35.42–158.91, 2.37–555.41, 18.48–27.34, and 2.77–3.45 mg/100 g the dry weight, respectively; among the different *Rhododendron* flowers. Violet flowers contained 1.53, 1.86, 4.49, 234.35, and 1.48 times higher amounts of aspartate, glutamate, glutamine, histidine, and GABA, respectively, and red *Rhododendron* flowers had the lowest amino acid content. We observed the highest quantities of serine, threonine, alanine, valine, norvaline, tryptophan, phenylalanine, isoleucine, and leucine in white flowers. The ranges of these amino acids

were 24.09–29.90, 11.65–21.82, 19.59–49.35, 2.97–9.83, 3.47–4.45, 3.33–4.48, 1.93–4.51, 2.13–5.23, 1.46–2.17, and 2.87–4.01 mg/100 g the dry weight, respectively, among the different *Rhododendron* flowers. White flowers contained 1.87, 2.52, and 3.31 times higher amounts of threonine, alanine, and valine, respectively, than did red *Rhododendron* flowers. Further, we observed 1.24, 1.35, 2.34, and 2.46 times higher amounts of Serine, tryptophan, phenylalanine, isoleucine, and leucine, respectively, in white flowers than in violet flowers. Although the total amino acid content was the lowest in red flowers, they contained the highest quantities asparagine, vitamin U, glutamine, glycine, tyrosine, cysteine, and lysine. The asparagine content was much higher in red flowers than in any other *Rhododendron* flower. Additionally, red flowers contained 3.70 and 2.48 times higher levels of asparagine than that in white and violet flowers, respectively. Red flowers also contained 2.83 and 1.51 times higher levels of tyrosine and cysteine than did white flowers. The lysine level was 1.4 times higher in red flowers than in violet *Rhododendron* flowers.

Among the cultivars of *Momordica charantia* variation due to amino acid was noticed by Kim *et al*.[23], who showed that among all the amino acids isolated from *M. charantia*, arginine was present in remarkably high quantities, whereas cysteine and methionine were present at the lowest concentrations. Variations in amino acid content have also been observed in different organs of *Scutellaria baicalensis*[24], green and red mustard[25], and in different species of aloe[26]. Previously, Li *et al*.[27] reported that amino acid and GABA content varied in cultivars of *Liriope platyphylla*, a finding supported by the results of the present study.

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