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Variation in Amino Acid Contents in the Different Cultivars of *Rehmannia glutinosa* Liboschitz

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The present study investigated the composition of amino acids in different cultivars of *Rehmannia glutinosa*; 21 amino acids were detected in the nine cultivars investigated in this study. The level of amino acids varied markedly among the nine cultivars. The Wongang cultivar had the highest amount of amino acids (aspartic acid, glutamic acid, asparagine, serine, glutamine, histidine, phenylalanine, isoleucine and leucine), which was 4.64, 2.34, 1.93, 1.76, 1.73, 1.62, 1.29 and 1.22 times higher than that in Togang, Yeongang, Keumsanjaerae, Daegyung, Dagang, Jihwang1, Goryeo-jihwang and Gogang, respectively. The variation in the levels of glutamine, asparagine and leucine was considerably higher among the cultivars; the levels of glutamine and asparagine were 12.68 and 7.29 times higher, respectively, in Wongang cultivar than in Togang cultivar, which had the lowest content of amino acids. The levels of threonine, tyrosine, valine and methionine were the highest in the Goryeo-jihwang cultivar. The content of γ-aminobutyric acid was considerably higher in Keumsanjaerae and was 7.43, 2.75, 3.52, 3.48, 4.06, 1.69, 5.26 and 4.35 times higher than that in Yeongang, Gogang, Dagang, Daegyung, Goryeo-jihwang, Wongang, Jihwang1 and Togang, respectively. The levels of argentine, tryptophan and lysine were the highest in Gogang cultivar. The level of glycine was 1.83 times higher in Yeongang cultivar than that in Daegyung cultivar. Present results suggested that *R. glutinosa* cultivars contain varying amounts of amino acids and the Wongang cultivar might have an excellent potential to be used as a source of amino acids.

Keywords: Amino acids, Cultivars, *Rehmannia glutinosa*, Tuberous roots.

INTRODUCTION

Rehmannia glutinosa Libosch., a well-known species of the family Scrophulariaceae, is one of the most important medicinal herbs distributed in China, Japan and Korea [1]. The fresh and dried tuberous roots of this species are used as a traditional Chinese medicinal ingredient, especially in the cases of sedation, diabetes, hematological conditions and insomnia [2-4]. The extract of the tuberous roots contains iridoids, monoterpenes, glycosides, fatty acids, amino acids and microelements that have been shown to have various beneficial effects and pharmacological actions on the blood system, immune system, endocrine system, cardiovascular system and nervous system [5-10]. Therefore, the economic importance of *R. glutinosa*can be attributed to the pharmaceutical effects of the tuberous root extract [11].

Around 65 % of human protein requirement is fulfilled through plant sources [12]. The importance of plant proteins varies from highly developed to poorly developed regions, depending on the average local diets. The most important function of amino acids is their role as protein building blocks. Numerous researchers have investigated the antioxidant effects of several amino acids [13-16]. Free amino acids are needed during secondary plant metabolism and for the biosynthesis of compounds such as glucosinolates and phenolics that play important roles, either directly or indirectly, in plant-environment interactions and human health [17].

Numerous amino acids exist in nature, although not all are essential for human health. In all, approximately 24 different amino acids are known to be essential to human nutrition [18,19]. Previously, several studies have addressed the medicinal values of *R. glutinosa* from fresh or dried roots. However, the amino acid content of the different cultivars of *R. glutinosa* has not yet been reported. This study aimed to determine the amino acid composition of the different cultivars of *R. glutinosa*.

EXPERIMENTAL

The roots of nine cultivars of *R. glutinosa* (Yeongang, Gogang, Dagang, Keumsanjaerae, Daegyung, Goryeo-jihwang, Wongang, Jihwang1 and Togang) were transplanted at the

Ginseng & Medicinal Plant Research Institute, Keumsan, Korea. The crops were harvested after six months. Immediately after harvesting, the samples (root) were freeze-dried at -80 °C for at least 72 h and then ground into a fine powder by using a mortar and pestle for amino acid analysis.

Extraction and analysis of free amino acids: The amino acid extraction and analysis methods were as described by Kim *et al*. [20]. Amino acids were extracted from the freeze-dried plant tissues (1 g) with 30 mL of 70 % ethanol at 80 C for 20 min; this step was repeated three times. After the ethanol was evaporated, the residual water phase (30 mL) was mixed with ethyl ether (30 mL) by using a separation funnel. After separation, the water phase was freeze-dried. The extract was re-suspended in 3 mL of 0.02 N HCl and filtered using a 0.45 μm syringe filter.

The amino acids in the extract were identified using an amino acid analyzer (HITACHI L-8900; Japan) equipped with a HITACHI HPLC column packed with ion-exchange resin No. 2622 PF (4.6 mm × 60mm) and a UV detector (VIS1, 570 nm; VIS2, 440 nm). Wako L-8500 buffer solutions PF-1, PF-2, PF-3, PF-4 and RG were used. Next, 20 μL of each sample was injected and identified using the ninhydrin reagent set (Wako Chemical Inc., Japan). Sample preparation and analysis were repeated three times.

RESULTS AND DISCUSSION

In all, 22 amino acids were compared to investigate the composition of amino acids of the nine cultivars of *R. glutinosa;* 21 amino acids were found in the cultivars (Table-1). Vitamin U was not detected in any of the cultivars, whereas norvaline

TABLE–1
AMINO ACID CONTENT IN *Rehmannia glutinosa* Liboschitz (mg/100 g dry wt.)

Amino acid	Yeongang	Gogang	Dagang	Keumsan Jaerae	Daegyung	Goryeo-jihwang	Wongang	Jihwang 1	Togang
Aspartic acid	86.99 ± 0.94	85.88 ± 0.23	105.77 ± 0.09	105.06 ± 0.23	104.18 ± 1.14	85.19 ± 0.09	110.74 ± 0.15	71.68 ± 0.69	66.77 ± 0.11
Glutamic acid	26.79 ± 0.39	17.92 ± 0.42	17.82 ± 0.68	22.21 ± 0.24	22.54 ± 0.77	20.07 ± 2.47	28.86 ± 3.10	15.87 ± 1.66	10.38 ± 0.52
Asparagine	15.84 ± 0.12	32.02 ± 0.08	25.16 ± 0.30	17.81 ± 0.33	21.38 ± 0.06	31.08 ± 0.22	39.35 ± 0.31	23.17 ± 0.48	5.40 ± 0.14
Serine	3.89 ± 0.05	3.26 ± 0.14	3.33 ± 0.31	8.48 ± 0.13	3.84 ± 0.16	6.27 ± 0.76	9.99 ± 0.17	4.84 ± 0.36	3.16 ± 0.68
Glutamine	166.44 ± 6.28	358.94 ± 18.80	300.34 ± 21.43	285.09 ± 22.18	213.59 ± 17.59	448.59 ± 46.01	538.08 ± 75.13	237.23 ± 32.58	42.45 ± 20.70
Histidine	6.75 ± 0.26	12.21 ± 2.82	7.66 ± 0.17	7.63 ± 0.22	7.76 ± 0.76	10.25 ± 0.58	13.12 ± 0.07	8.47 ± 0.05	–
Glycine	2.08 ± 0.00	–	1.91 ± 0.67	3.12 ± 0.01	1.14 ± 1.61	1.80 ± 0.19	–	1.46 ± 0.08	1.36 ± 1.92
Threonine	2.12 ± 0.01	2.43 ± 0.09	4.92 ± 0.01	2.92 ± 0.02	2.16 ± 0.08	6.66 ± 0.06	5.91 ± 0.12	2.46 ± 0.14	3.12 ± 2.03
Arginine	208.77 ± 0.37	484.98 ± 3.21	233.01 ± 0.74	101.18 ± 0.55	309.20 ± 2.10	324.12 ± 1.33	449.79 ± 1.57	386.63 ± 1.86	102.87 ± 35.42
Alanine	7.06 ± 0.14	7.31 ± 0.14	3.83 ± 0.06	11.45 ± 0.03	7.79 ± 0.01	7.93 ± 0.02	5.03 ± 0.12	7.59 ± 0.05	9.39 ± 3.23
γ-Aminobutyric acid	11.50 ± 0.09	31.11 ± 0.45	24.30 ± 0.04	85.41 ± 0.78	24.58 ± 0.00	21.07 ± 0.08	50.79 ± 0.19	16.24 ± 0.26	19.64 ± 6.51
Tyrosine	–	4.12 ± 0.41	3.30 ± 1.81	2.28 ± 0.82	2.52 ± 0.22	10.74 ± 0.82	9.34 ± 0.11	4.89 ± 1.37	4.13 ± 5.84
Cysteine	7.32 ± 0.52	6.06 ± 0.35	5.68 ± 0.21	9.35 ± 1.01	7.35 ± 0.05	8.59 ± 0.25	6.96 ± 0.34	5.26 ± 0.17	8.18 ± 2.68
Valine	–	1.83 ± 0.09	2.73 ± 0.13	3.05 ± 0.43	–	5.78 ± 0.15	5.33 ± 0.08	2.24 ± 0.18	–
Methionine	2.16 ± 0.06	1.86 ± 0.44	2.16 ± 0.38	–	2.04 ± 0.05	4.28 ± 0.00	3.40 ± 0.20	–	2.77 ± 0.59
Norvaline	–	–	–	–	–	–	1.01 ± 1.42	–	–
Tryptophan	8.38 ± 0.05	10.93 ± 0.26	6.06 ± 0.24	2.96 ± 0.24	5.53 ± 0.06	8.23 ± 0.05	8.73 ± 0.11	9.14 ± 0.04	–
Phenylalanine	–	2.80 ± 0.02	–	–	–	1.91 ± 0.15	4.14 ± 0.09	2.61 ± 0.07	–
Isoleucine	–	4.57 ± 0.01	2.40 ± 0.64	3.90 ± 0.04	3.02 ± 0.15	5.08 ± 0.59	8.09 ± 0.30	4.08 ± 0.00	–
Leucine	2.31 ± 1.13	3.36 ± 0.57	3.21 ± 0.34	4.26 ± 1.07	2.60 ± 0.30	4.78 ± 0.55	6.01 ± 0.58	2.16 ± 0.08	–
Lysine	5.21 ± 0.09	12.35 ± 0.29	7.35 ± 0.14	8.25 ± 0.27	7.52 ± 0.11	8.62 ± 0.21	10.15 ± 0.32	9.67 ± 0.07	4.04 ± 1.43
Total	563.59 ± 7.51	1083.92 ± 26.37	760.95 ± 18.79	684.40 ± 26.47	748.72 ± 11.33	1021.06 ± 44.95	1314.83 ± 73.79	815.72 ± 26.15	283.63 ± 76.09

The values represent the mean ± standard deviation (n = 3).

was detected only in Wongang cultivars. Further, phenylalanine was found only in Gogang, Goryeo-jihwang, Wongang and Jihwang1 cultivars. Glycine was not detected in Gogang and Wongang cultivar, whereas methionine was absent in Keumsanjaerae and Jihwang1 cultivars. Tryptophan, phenylalanine, isoleucine and leucine were not found in Jihwang1 cultivars. The amino acid content differed widely among the nine cultivars of *R. glutinosa*. The Wongang cultivar contained the highest number of total amino acids; further, the levels of aspartate, glutamate, asparagine, serine, glutamine, histidine, phenylalanine, isoleucine and leucine were the highest in this cultivar. The variation of amino acid level, especially those for glutamine, asparagine and leucine, was considerably higher among the cultivars; the levels of glutamine and asparagine were 12.68 and 7.29 times higher, respectively, in Wongang cultivars than in Togang cultivars, which had the lowest number of amino acids. Even the cultivar Wongang contained 3.17, 2.78 and 1.67 times higher levels of serine, glutamate and aspirate, respectively, than those in Togang. The cultivar Wongang contained 3.37 times and 1.95-fold higher leucine and phenylalanine than those in Jihwang1and 2.17 higher isoleucine than that in Dagang. The level of threonine and tyrosine were the highest in Goryeo-jihwang cultivar; their levels were 3.15 and 4.71 times higher than those in Yeongang and Keumsanjaerae cultivars, respectively, which had the lowest amino acid contents. Further, the levels of valine and methionine were 3.16 and 2.31 times higher, respectively, in Goryeo-jihwang cultivar than those in Gogang. The cultivar Keumsanjaerae contained the highest levels of alanine, γ-aminobutyric acid and cysteine; the content of γ-aminobutyric acid was 7.43, 2.75, 3.52, 3.48, 4.06, 1.69, 5.26 and 4.35 times higher than those in Yeongang, Gogang, Dagang, Daegyung, Goryeo-jihwang, Wongang, Jihwang1 and Togang, respectively. The level of alanine was 2.99 times higher in Keumsanjaerae than that in Dagang cultivar, which had the lowest alanine content. Further, Keumsanjaerae cultivar contained 1.78 times higher cysteine than that in Jihwang1 cultivar. The levels of arginine, tryptophan and lysine were the highest in Gogang cultivar. Arginine content varied considerably among the *R. glutinosa* cultivars. The cultivar Gogang contained 2.33, 2.09, 4.80 and 4.72 times higher arginine than those in Yeongang, Dagang, Keumsanjaerae and Togang, respectively and 3.70 and 3.06 times higher tryptophan and lysine than those in Keumsanjaerae and Togang cultivars, respectively. Glycine content was the highest in Yeongang cultivar and was 1.83 times higher than that in Daegyung cultivar. Among the cultivars of *R. glutinosa*, cultivar Wongang contained the highest amount of total amino acids, which was 4.64, 2.34, 1.93, 1.76, 1.73, 1.62, 1.29 and 1.22 times higher than that of Togang, Yeongang, Keumsanjaerae, Daegyung, Dagang, Jihwang1, Goryeo-jihwang and Gogang, respectively. Variation in amino acids among cultivars has been reported by Kim *et al.* [20], who found that, among all the amino acids isolated from *Momordica charantia*, arginine was present in remarkably high quantities, whereas cysteine and methionine were present in the lowest concentrations. Amino acid content was reported to vary in the different organs of *Scutellaria baicalensis* [21] and green and red mustard [22] and in the different species of aloe [23]. The content of γ-aminobutyric acid was considerably higher in Keumsanjaerae and was 7.43, 2.75, 3.52, 3.48, 4.06, 1.69, 5.26 and 4.35 times higher than that in Yeongang, Gogang, Dagang, Daegyung, Goryeo-jihwang, Wongang, Jihwang1 and Togang, respectively. This is consistent with the findings of Li *et al*. [24], who reported that amino acid as well as γ-aminobutyric acid contents varied across different cultivars.

Conclusion

The levels of amino acids in the different cultivars of *R. glutinosa* varied remarkably. The cultivar Wongang contained the highest amount of total amino acids as well as the highest level of nine amino acids. Thus, cultivars of *R. glutinosa* accumulate varying amounts of amino acids. The cultivar-specific amino acid profiles identified in this study might have future implications of using *R. glutinosa* cultivars as a potential source of amino acids.

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