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Variation in Amino Acid Contents of White and Red Sword Bean [*Canavalia gladiata* (Jacq.) D.C.]

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Sword bean (*Canavalia gladiata*) belongs to the genus *Canavalia*, a neglected group of legumes with many valuable characteristics, in addition to containing a variety of amino acids. The present study investigated amino acid variation in different cultivars of white and red sword bean. Twenty-one different amino acids were identified from different cultivars of white and red sword bean and levels of these amino acids varied significantly depending on the cultivar. The white sword bean cultivar had a total amino acid concentration that was 1.54 times higher than red sword bean. White sword bean contained higher levels of nine different amino acids, whereas red sword bean contained higher levels of 12 different amino acids. Among amino acids, glycine concentrations were the highest irrespective of cultivar. The range of glycine was 2616.73 to 4363.12 mg/100 g the dry weight of each cultivar. The sword bean cultivar contained 1.67 times higher levels of glycine than that of red sword bean. The amino acids found in the highest quantities in white sword bean varied marginally compared to red sword bean. However, in red sword bean, the amino acids found at higher levels varied widely compared to those of white sword bean. The results of this study demonstrate that sword bean cultivars contain varying amounts of amino acids, suggesting that these vegetable cultivars have the potential of serving as excellent sources of amino acids.

Keywords: *Canavalia gladiata*, Amino acids, Cultivars, Grain legume.

INTRODUCTION

Sword bean (*Canavalia gladiata*) is a legume that is mainly cultivated as an annual vegetable [1]. The seeds are oblong-ellipsoidal in shape and highly variable in colour, ranging from red to red-brown and even white or black. The hilum is as long as the seed and is dark brown in colour [2]. Although a neglected crop at the international level, sword bean is of the utmost importance in some areas [1]. Its forage and seeds have many nutritional and ethnomedicinal properties.

In India, sword bean seed are consumed by certain ethnic groups and poor village people [3]. In Asia, the young pods and seeds of sword bean are used as green vegetable. The roasted seed are used to prepare a coffee- like drink in Latin America [4]. In Nigeria, it's usually used as an ornamental plant, grown near houses and allow to trail on the walls and trees [5,6].

The high rate of at which world population is growing, the world food supply should grow at the same rate if not faster. The most affected countries were the developing countries. Therefore, it is essential that cheaper sources of protein and other nutrients be found. The role of legumes seed in the diets of animal and man in developing countries cannot be over-emphasized. They are rich in nutrients such as digestible protein with good array of amino acids and minerals [7]. The percentage crude proteins of most legumes vary from 20 to 50 [8] and been judged a good source of minerals. Leguminous seed have been reported to be excellent source of energy [9,10] in animal and human diets. Most of the protein required by humans is derived from plant sources [11]. The most important function of amino acids is their role as the building blocks of proteins. Previous research has identified the antioxidant effects of several amino acids [12-15]. Free amino acids are required for plant secondary metabolism and biosynthesis of different compounds that play important roles in plant-environment interactions and human health [16].

Many amino acids exist in nature, but not all are essential for human health. Twenty-four different amino acids are reported to be essential to human nutrition [17,18]. Several studies have addressed management strategies, different uses and nutritional values of sword bean. To date, no study has clearly reported the amino acid content in different cultivars of sword bean despite it being a protein-rich, leguminous crop. The objective of the present study was to identify and quantify the amino acids present in a white and red cultivar of sword bean cultivars.

EXPERIMENTAL

White and red sword bean (*Canavalia gladiata*) were used as a planting materials in this study. Both cultivars were grown in the greenhouse of Chungnam National University Experiment Farm located at Daejeon, Korea. For each cultivars twenty plants were considered to grow in a greenhouse maintaining the temperature of 25 °C and 70 % RH for a 14:10 light:dark cycle. Seeds of both sword bean cultivars were harvested on 15 October, 2015 and freeze-dried at -80 °C immediately after collection for at least 72 h and then ground using a mortar and pestle into a fine powder for the analysis of amino acid.

Trichloroacetic acid (TCA, 99.0 %) was purchased from Samchun Pure Chemical Co., Ltd. (Pyeongtaek, Korea). Necessary standards (Sixteen amino acid) and four amino acid supplements were bought from Agilent Technologies (Waldbronn, Germany). Standard for vitamin U (DL-methionine methylsulfonium chloride) and sodium phosphate monobasic monohydrate (NaH_2PO_4) were obtained from Sigma-Aldrich (St. Louis, MO, USA). HPLC-graded acetonitrile (ACN) and methanol (MeOH) were collected J.T. Baker (Phillipsburg, NJ, USA). Ultrapure water with a resistivity of 18.2 MΩ/cm was produced by using a PureLab Option system from ELGA LabWater (Model LA 621; Marlow, UK).

Extraction procedure: An amount of 100 mg of freeze-dried plant powder of both sword bean cultivars was placed in a 2 mL Eppendorf tube and then 1.2 mL 5 % (v/v) trichloroacetic acid solution was added. After being vortexed, the mixture was allowed to stand for at least 1 h at room temperature and then 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 (Ø 13 mm, Cat. no. 6779-1304; Whatman Int. Ltd., Maidstone, UK) into an HPLC vial.

HPLC analysis for free amino acids: The amino acids analysis through HPLC was done following the methods described by Kim *et al*. [19]. Twenty different 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 µm) and Zorbax Eclipse AAA Guard columns (12.5 × 4.6 mm i.d., particle size 5 µm, 4-pack). The HPLC systems were set at a wavelength of 338 nm, 40f and a flow rate of 2.0 mL/min. The mobile phase was 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 gradient protocol of HPLC was as follows: a linear step from 0 to 57 % of solvent B from 1.9 to 21.1 min; from 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 the end (a total of 30 min). A solution (50 pmol/µL [0.05 mM]) for all the amino acids (20 amino acids) was prepared as a standard. The level of amino acids was detected based on HPLC peak areas calculated as equivalents of the standard compounds and all quantities were expressed as milligrams per 100 g fresh weight (FW). All the samples were run for three times.

RESULTS AND DISCUSSION

Twenty-one different amino acids were found at varying concentrations in both white and red sword bean cultivars (Table-1). The amino acid methionine was not detected in white sword bean, whereas threonine was absent in red sword bean. White sword bean contained 15.07 mg/100 g dry weight threonine, whereas red sword bean contained 18.15 mg/100 g dry weight methionine. White sword bean contained 1.54 times higher concentration of total amino acids (4706.07 mg/100 g dry wt) than red sword bean (3059.52 mg/100 g dry wt). The white sword bean cultivar contained higher quantities of nine different amino acids (*i.e.*, aspartate, glutamate, asparagine, glutamine, glycine, threonine, tyrosine, tryptophan and lysine). By contrast, the red sword bean cultivar contained higher quantities of 12 different amino acids (*i.e.*, serine, histidine, arginine, alanine, γ-amino butyric acid, cystine, valine, methionine, norvaline, phenylalanine, isoleucine and leucine). Among all amino acids, the level of glycine was much higher irrespective of cultivar. The cultivar sword bean contained 1.67 times higher levels of glycine than that of red sword bean. The range of glycine in all cultivars was 2616.73 to 4363.12 mg/100 g the dry weight.

TABLE-1
AMINO ACID CONTENT IN WHITE AND RED SWORD BEAN [*Canavalia gladiata* (Jacq.) D.C.]

Amino acid	Cultivars (amino acid: mg/100 g dry wt.)	
	White sword bean	Red sword bean
Aspartate	57.75 ± 0.47	35.23 ± 0.28
Glutamate	25.92 ± 0.91	20.45 ± 0.06
Asparagine	67.27 ± 0.24	63.93 ± 0.54
Serine	1.61 ± 0.02	10.73 ± 0.31
Glutamine	6.35 ± 0.46	3.31 ± 0.26
Histidine	6.87 ± 0.08	11.15 ± 0.04
Glycine	4363.12 ± 40.56	2616.73 ± 15.04
Threonine	15.07 ± 0.15	0.00
Arginine	44.03 ± 0.41	73.52 ± 0.38
Alanine	19.52 ± 0.13	48.63 ± 0.18
γ-Amino butyric acid	46.78 ± 0.28	54.23 ± 0.18
Tyrosine	1.68 ± 0.11	1.60 ± 0.03
Cystine	6.37 ± 0.01	8.98 ± 0.07
Valine	9.75 ± 0.11	45.41 ± 0.12
Methionine	0.00	18.15 ± 0.00
Norvaline	9.71 ± 0.15	11.13 ± 0.09
Tryptophan	1.99 ± 0.00	1.67 ± 0.25
Phenylalanine	4.92 ± 0.03	8.41 ± 0.04
Isoleucine	2.37 ± 0.03	9.98 ± 0.06
Leucine	1.64 ± 0.09	6.10 ± 0.08
Lysine	10.19 ± 0.38	10.18 ± 0.09
Total	4706.07 ± 40.01	3059.52 ± 16.46

The values represent the mean ± SD

The amino acids present in higher levels in white sword bean varied marginally compared to red sword bean. Levels of aspartate, glutamate, asparagine, glutamine, tyrosine and tryptophan were 1.64, 1.27, 1.1, 1.92, 1.05 and 1.20 times higher, respectively, in white sword bean than in red sword bean. However, the amino acids present at higher levels in red sword bean varied widely compared to those in white sword bean. The red sword bean cultivar contained 6.67, 1.62, 1.67, 2.49, 1.16, 1.41, 4.66, 1.15, 1.71, 4.21 and 3.72 times higher levels of serine, histidine, arginine, alanine, γ-amino butyric acid, cystine, valine, norvaline, phenylalanine, isoleucine and leucine, respectively, than white sword bean.

Variation in amino acid profile among cultivars was previously reported by Kim *et al*. [19], who found that of all the amino acids isolated from *Momordica charantia*, arginine was present in remarkably high quantities, whereas cystine and methionine were present in low concentrations. Similar variation has also been observed in the different organs of *Scutellaria baicalensis* [20] and green and red mustard [21] and in different species of aloe [22]. Similar results regarding variation in the amino acid content of different of sword bean cultivars were observed in this study. Li *et al*. [23] reported that the amino acid and γ-amino butyric acid content varied by cultivar, which is supported by the findings of the current study.

Conclusion

The levels of amino acids in white and red sword bean cultivars varied greatly. The white sword bean cultivar contained the highest total amount of amino acids and the highest levels of nine different amino acids, including threonine. In contrast, no threonine was detected in the red sword bean cultivar. Although the total amino acid content was lower in red sword bean, this cultivar contained higher levels of 12 different amino acids, including methionine. The results of our study demonstrate that the seeds of different sword bean cultivars exhibit variable amino acid concentrations. The cultivar-specific amino acid profiles generated by this study suggest that sword bean is potentially a significant source of amino acids.

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