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Comparison of tocopherols composition in mung bean [*Vigna radiata* (L.) Wilczeck] germplasms of Asia

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Abstract

The aim of the present investigation was to compare the tocopherols composition from the germplasms of mung bean collected from different regions of Asia. Tocopherols are well known for their beneficial effects on human health such as antioxidant and anti-inflammatory activities. A total of 146 mung bean (*Vigna radiata* (L.) Wilczeck) germplasms were collected from the gene bank of the Rural Development Administration (RDA, Suwon, Korea) and classified into 11 types based on their origin and was investigated for the concentrations of various tocopherols present in these groups. Our results showed that Iranian mung bean seeds (159.7 µg g^{-1}) contained the highest levels of total tocopherols. In the whole mung bean germplasm, 48 Korean mung bean varieties are divided into two groups—the landrace collection and the bred collection. The average total tocopherol concentration in the bred collection (156.4 µg g^{-1}) was higher than that in the landrace collection (145.1 µg g^{-1}). The average γ-tocopherol content in the bred and landrace varieties were 75.1 µg g^{-1} and 68.0 µg g^{-1} respectively. The average α-tocopherol concentrations in the bred and landrace collections were 44.7 µg g^{-1} and 43.1 µg g^{-1}, respectively. Additionally, δ-tocopherol concentrations in the bred and landrace collections were 36.5 µg g^{-1} and 34.0 µg g^{-1}, respectively. Thus, tocopherol content in the bred mung bean collection is higher than that in the landrace collection. These results describe the variations of tocopherol composition in the mung bean germplasm. The data obtained would be useful to both consumers and producers for manufacturing traditional mung bean are commonly used in countries.

Keywords: Mung bean; *Vigna radiata;* tocopherols; germplasm.
Abbreviations: AT-α-tocopherol; DT-δ-tocopherol; GT-γ-tocopherol; GC-Gas chromatography.

Introduction

Mung bean (*Vigna radiata* (L.) Wilczeck), a leguminous seed, has been used as a food source, and it is commonly sold in food markets, particularly in Asia. The main nutritional components of mung bean include carbohydrate and high-quality protein; and they are highly nutritious. According to Lee et al. (2000) aroma extracts of mung bean, adzuki bean, and kidney bean inhibit aldehyde oxidation. Oxidative damage is a primary factor in aging and degenerative diseases, such as heart disease, cataracts, cognitive dysfunction, and cancer (Blake and Winyard, 1995; Halliwell and Gutteridge, 1998; Pietta, 2000).

Tocopherols belong to the vitamin E group of compounds and have important roles in human health and nutrition (Munné-Bosch and Alegre, 2002). The structures of tocopherols are similar to vitamin E compounds, and they are derivatives of a 6-chromanol ring (Seppanen et al., 2010). Vitamin E was introduced by Evans and Bishop (1936) as a significant factor in animal reproduction. Additionally, vitamin E was found to be related to antioxidant activity (Moon and Shibamoto, 2009). In 1936, α-tocopherol was isolated from wheat germ oil and its chemical structure ($C_{29}H_{50}O_2$) was established (Evans et al., 1936); in the following year, β- and γ-tocopherols were isolated. In 1947 (Stern et al., 1947), δ-tocopherol was extracted from soybean oil. Antioxidants are substances that slow down damage caused to other substances by the effects of oxygen; oxidative damage causes various diseases in the human body. Tocopherols are present in fruits and seeds, roots, tubers, cotyledons, stems, leaves, and flowers of higher plants (Munné-Bosch and Alegre, 2002). α-tocopherol functions to break radical chains in membranes and lipoproteins (Kamal-Eldin and Appleqvist, 1996; Schwartz et al., 2008).

Several studies have examined vitamin E content in fruits and vegetables (Chun et al., 2006; Hamauzu and Chachin, 1995; Pirronen et al., 1986). Plant tissues show differences in their total tocopherol content and tocopherol composition (Munné-Bosch and Alegre, 2002). Variations in vitamin E are affected by various factors such as processing procedures, storage time, and conditions (Chun et al., 2006; Hamauzu and Chachin, 1995; Ruperez et al., 2001). Plants synthesize 4 different tocopherols, namely, α-,β-,γ-, and δ-tocopherols. Specifically, plant seeds contain tocopherols, which are a source of vitamin E and are used as antioxidants, (Kamal-Eldin et al., 2000) these molecules have been shown to affect various cancers and diseases.

Tocopherols, which are natural antioxidant molecules that scavenge free radicals in biological systems, are abundant in plant seeds. The 4 isomers of tocopherols- α-, β-, γ- and δ-tocopherol are identified based on the number and position of methyl substituents on the chromanol ring (Rani et al., 2007). Previous studies have demonstrated the medical benefits of tocopherols. Tocopherol intake was shown to decrease atherosclerosis, cancer, and degenerative diseases such as Alzheimer and Parkinson's disease (Buring and Hennekens, 1997; Bramley et al., 2000). One review reported that vitamin E therapy showed beneficial effects against diabetes (Levy and Blum, 2007). A previous study reported that tocopherol content shows genotypic differences (Rani et al., 2007). In this study, mung bean germplasm was collected from different origins. Based on the results obtained, mung bean seeds are an exceptionally rich source of tocopherols and are a potential source of natural neutraceutical food stuffs owing to their marked biological like antioxidant and anti-inflammatory activity.

The purpose of this study was to determine the tocopherol content in mung bean germplasm. Mung bean (*Vigna radiata* (L.) Wilczek) is an important crop in Korea; Asian people use mung bean for various purposes. However, the molecules present in these beans are not well characterized. Thus, we examined mung bean as a foundation for the mung bean breeding project as well as for other food studies.

Results and Discussion

Analysis of tocopherols content in mung bean germplasm collected from different origins of Asia

Tocopherols are vitamin E - like compounds that are classified as α-, β-, γ-, and δ-tocopherols, depending on the number and position of methyl groups on the chromanol ring. α-tocopherol is preferentially absorbed and accumulated in humans and has been reported to possess high biological activity (Rigotti, 2007). The antioxidant activity of tocopherols contributes to one of their most important roles for human health, reducing both lipid peroxidation and the risk of cancer and preventing progression of precancerous lesions (Munne-Bosch and Alegre, 2002; Pinheiro-Sant'Ana et al., 2011). Vitamin E (tocopherols) compounds are well known for their beneficial effects on human health, such as antioxidant and anti-inflammatory activities and preventing various cancers. Mung beans have been used in food production; they contain high protein and carbohydrate levels, and they are a highly nutritious source in the human diet.

A total of 146 mung bean samples were collected from the gene-bank of Rural Development Administration (RDA, Suwon, Gyeonggi-Do, Korea) and were divided into 11 groups based on their origins, which includes, China, India, Iran, Japan, Korea, Nepal, Pakistan, Philippines, Taiwan, Thailand, and Uzbekistan.

The average tocopherol concentrations from mung beans collected from different origins is presented in table 1. The samples collected from Iran yielded highest total tocopherol content of 159.7 μg g^{-1}, followed by Taiwan which yielded 159.1 μg g^{-1}. The lowest total tocopherol content was observed from the Japanese collection which yielded about 143.8 μg g^{-1}. Similar variations in the tocopherol content were observed in the soybean germplasm collected from different origins (Kim et al., 2012).

Among the 4 tocopherols groups, α-, γ-, and δ-tocopherols were detected in mung bean samples and γ-tocopherol was present at the highest level followed by α- and δ-tocopherol (Table 1). The average concentrations of γ-tocopherol showed differences among the eleven origins. The highest average γ-tocopherol content was observed in the mung bean seeds collected from Taiwan, which yielded 79.4 μg g^{-1}, followed by Iran, which yielded 78.0 μg g^{-1}. The lowest average γ-tocopherol content was observed in the mung bean seeds collected from Uzbekistan, which yielded 66.9 μg g^{-1}. Similar to our results, Kim et al., (2013) also reported variations in the tocopherol content of adzuki bean (*Vigna angularis*) collected from different origins.

A total of 48 whole mung bean germplasm samples were collected from Korea and were divided into two groups, namely, the landrace collection and bred collection. The average

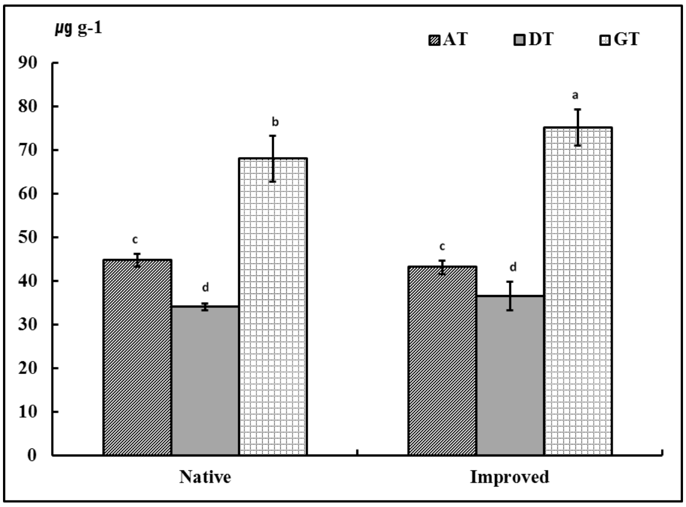

Fig 1. Comparison of individual tocopherol concentrations between landrace and bred Korean mung bean varieties. Experimental results were means ± SD of three parallel measurements. Mean values with common letters are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test (DMRT). AT; α-tocopherol, DT; δ-tocopherol, GT; γ-tocopherol.

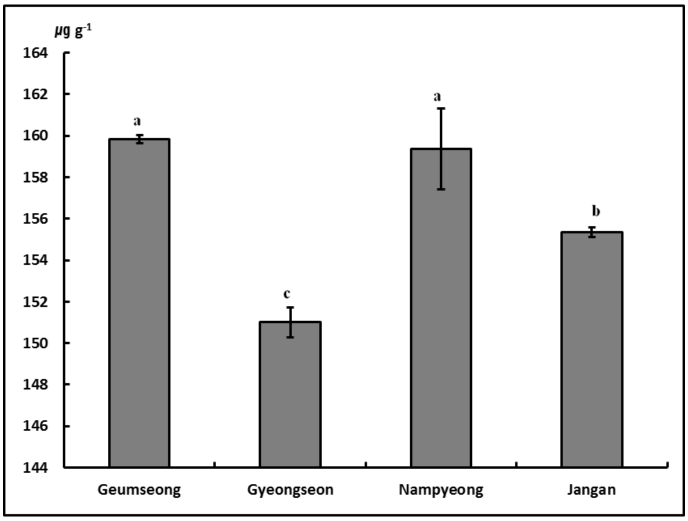

Fig 2. Comparison of total tocopherol content between bred Korean mung bean varieties. Experimental results were means ± SD of three parallel measurements. Mean values with common letters are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test (DMRT).

total tocopherol concentration of the bred collection (156.4 μg g^{-1}) was higher than that of the landrace collection (145.1 μg g^{-1}) in Korean mung bean germplasm (Fig. 1). The γ-tocopherol contents in bred and landrace varieties were 75.1 μg g^{-1} and 68.0 μg g^{-1}, respectively. The α-tocopherol contents in bred and landrace mung beans were 44.7 μg g^{-1} and 43.1 μg g^{-1}, respectively. The concentrations of δ-tocopherol in bred and landrace collections were 36.5 μg g^{-1} and 34.0 μg g^{-1} respectively.

In a previous study, γ-tocopherol showed anti-inflammatory activity (Jiang et al., 2000). Another study indicated that γ-, δ-, and α-tocopherol intake was associated with diabetes. These data suggest that γ-tocopherol may play a role in reducing the risk of diabetes (Montonen et al., 2004). Furthermore, γ-tocopherol is more efficient than α-tocopherol in trapping reactive nitrogen species (Jiang et al., 2000; Anwar et al., 2007).

Table 1. Comparison of tocopherols content in mung beans germplasm collected from different origins of Asia.

		AT	DT	GT	Total
China	Maximum (μg g^{-1})	43.7	35.9	88.9	168.4
	Minimum (μg g^{-1})	42.5	34.4	68.0	144.9
	Mean (μg g^{-1})	43.0	35.1	76.4	155.0
	CV (%)	0.3	1.5	2.8	1.3
	$LSD_{(0.05)}$	0.3	2.0	7.9	5.8
India	Maximum (μg g^{-1})	45.3	36.0	84.7	163.1
	Minimum (μg g^{-1})	43.0	34.2	66.0	144.3
	Mean (μg g^{-1})	43.8	35.0	74.4	153.2
	CV (%)	0.8	1.8	0.8	0.5
	$LSD_{(0.05)}$	0.8	1.4	1.4	1.6
Iran	Maximum (μg g^{-1})	46.7	43.3	87.3	174.3
	Minimum (μg g^{-1})	43.2	34.5	71.8	151.9
	Mean (μg g^{-1})	44.5	37.2	78.0	159.7
	CV (%)	0.9	3.7	2.7	2.2
	$LSD_{(0.05)}$	1.0	3.2	5.0	8.4
Japan	Maximum (μg g^{-1})	44.8	34.0	75.3	153.4
	Minimum (μg g^{-1})	42.6	32.8	60.2	136.7
	Mean (μg g^{-1})	43.5	33.2	67.2	143.8
	CV (%)	1.1	0.4	0.9	0.5
	$LSD_{(0.05)}$	1.2	0.3	1.6	1.7
Korea	Maximum (μg g^{-1})	49.2	41.7	80.7	159.8
	Minimum (μg g^{-1})	41.7	33.1	59.8	135.0
	Mean (μg g^{-1})	43.2	34.2	68.6	146.0
	CV (%)	1.2	1.0	2.6	1.5
	$LSD_{(0.05)}$	1.1	0.7	3.5	4.5
Nepal	Maximum (μg g^{-1})	44.9	36.7	79.0	158.4
	Minimum (μg g^{-1})	43.1	33.9	67.1	144.4
	Mean (μg g^{-1})	44.1	34.9	72.8	151.8
	CV (%)	0.8	1.2	1.2	0.6
	$LSD_{(0.05)}$	0.8	1.0	2.0	2.3

Table 1. Continued.

		AT	DT	GT	Total
Pakistan	Maximum (μg g^{-1})	45.3	38.3	84.4	164.9
	Minimum (μg g^{-1})	42.3	34.1	59.6	139.4
	Mean (μg g^{-1})	44.3	35.5	73.4	153.2
	CV (%)	1.5	1.1	1.3	0.9
	$LSD_{(0.05)}$	1.7	1.0	2.2	3.4
Philippines	Maximum (μg g^{-1})	45.2	40.8	81.5	161.2
	Minimum (μg g^{-1})	41.7	33.6	61.6	138.7
	Mean (μg g^{-1})	43.2	34.9	72.7	150.8
	CV (%)	0.9	0.7	0.8	0.5
	$LSD_{(0.05)}$	0.8	0.5	1.2	1.4
Taiwan	Maximum (μg g^{-1})	45.1	35.9	81.5	162.4
	Minimum (μg g^{-1})	43.4	34.6	76.1	156.1
	Mean (μg g^{-1})	44.4	35.3	79.4	159.1
	CV (%)	0.9	0.8	0.6	0.5
	$LSD_{(0.05)}$	1.1	0.8	1.4	2.2
Thailand	Maximum (μg g^{-1})	46.6	36.7	91.4	174.7
	Minimum (μg g^{-1})	42.9	33.4	63.6	141.3
	Mean (μg g^{-1})	44.3	34.6	73.3	152.1
	CV (%)	1.0	0.9	0.5	0.4
	$LSD_{(0.05)}$	0.9	0.7	0.8	1.4
Uzbekistan	Maximum (μg g^{-1})	43.7	39.4	73.3	155.5
	Minimum (μg g^{-1})	39.5	32.5	47.0	119.0
	Mean (μg g^{-1})	42.4	35.0	66.9	144.3
	CV (%)	1.4	1.0	0.5	0.4
	$LSD_{(0.05)}$	1.3	0.8	0.8	1.4

The α- and δ-tocopherols exhibit the highest antioxidant activities (Anwar et al., 2007). Among the mung bean samples, 4 varieties of the Korean mung bean collections showed differences in their tocopherol concentrations. Geumseong Nokdu showed the highest tocopherol levels (159.8 μg g^{-1}). Gyeongseon Nokdu (151.0 μg g^{-1}), Nampyeong Nokdu (159.4 μg g^{-1}), and Jangan Nokdu (155.4 μg g^{-1}) showed levels lower than that of Geumseong Nokdu (Fig. 2).

Thus, functional molecules in bred varieties have been increased through hybridization, which improved mung bean genetic characteristics. Additionally, genetic characteristic such as seed weight and other environmental factors, including temperature, cultivation site, and cultivation time, have influenced tocopherol concentration in mung bean seeds.

Materials and Methods

Plant materials

A total of 146 mung bean samples were collected from the gene-bank of Rural Development Administration (RDA, Suwon, Gyeonggi-Do, Korea). They were grouped into 11 groups according to their origins, which includes China (4 genotypes), India (11 genotypes), Iran (7 genotypes), Japan (5 genotypes), Korea (48 genotypes), Nepal (7 genotypes), Pakistan (6 genotypes), Philippines (28 genotypes), Taiwan (4 genotypes), Thailand (17 genotypes), and Uzbekistan (9 genotypes). All the samples were cultivated at the Konkuk University farm. Preparation of mung bean analysis sample, experimental conditions, climatic conditions, and plot management are according to the method of Kim et al. (2012). The characteristics of the mung bean germplasms are presented in the supporting information (Supplementary table 1).

Table 2. Calibration curves equation of tocopherol standards.

Vitamin E	Equation	r^2
α-tocopherol	y = 3057.9x - 28978	0.999
β-tocopherol	y = 2788.3x - 20325	0.999
γ-tocopherol	y = 2807.3x - 21501	0.999
δ-tocopherol	y = 2929.6x - 22957	0.999

Sample extraction

The extraction of tocopherols was carried out according to the method of Kim et al. (2012) with some modifications. In brief, 0.5 g of ground mung bean sample was added to a 50-mL falcon tube containing 10 mL ethanol (HPLC grade, J. T. Baker, Phillipsburg, NJ, USA) and 0.1 g of ascorbic acid (L(+)-ascorbic acid). The samples were placed in a shaking hot water bath at 80°C for 10 min. Later, 300 μL of 44% potassium hydroxide (KOH, Samchun, Seoul, Korea) was added for saponification and the sample was shaken for an additional 10 min at the same temperature. After saponification, the solution was quickly cooled on ice for 30 min, after which 10 mL of

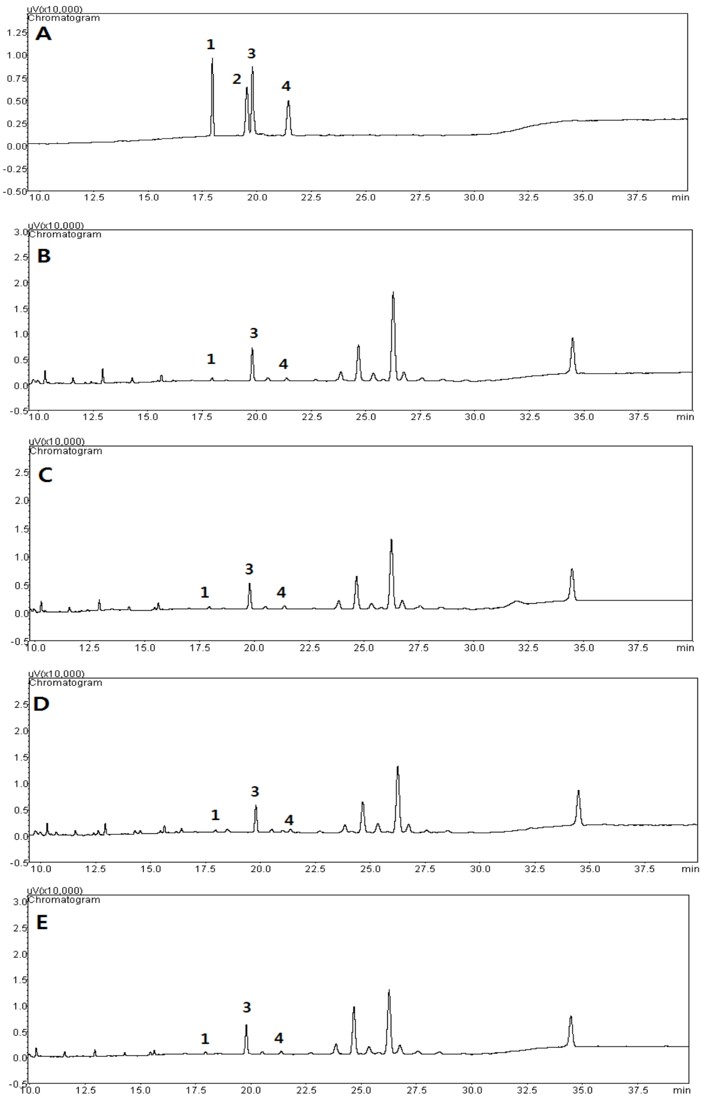

Fig 3. Chromatograms of tocopherols among four different varieties.. (A) standard chromatogram, (B) Geumseong Nokdu, (C) Kyeongseon Nokdu, (D) Nampyeong Nokdu, (E) Jangan Nokdu, 1: δ-tocopherol, 2: β-tocopherol, 3: γ-tocopherol, 4: α-tocopherol).

hexane (HPLC grade, J. T. Baker) and distilled water (HPLC grade, J. T. Baker) was added. The samples were thoroughly mixed and centrifuged for 4 min at 4000 rpm. The hexane layer was collected and 10 mL of hexane was added; the sample was centrifuged in the same condition. This hexane-extraction process was repeated two additional times and the collected hexane layers were pooled and washed twice with distilled water. After removing the water layer, the collected hexane layer was filtered through a funnel containing anhydrous sodium sulfate (Na_2SO_4) (Yakuri Chemical, Kyoto, Japan); the sample was concentrated using a vacuum evaporator. The residues were redissolved with 2 mL of iso-octane (HPLC grade, J. T. Baker).

Analysis of tocopherols in mung bean germplasm

For tocopherol analysis, tocopherol standards (α-, β-, γ-, δ-tocopherol) were purchased from Toronto Research Chemicals, Inc. (North York, Ontario, Canada). Tocopherol standards were determined based on their retention times and a standard calibration curve was plotted with different concentrations (1, 50, and 100 ppm). High linearity of $r^2 > 0.996$ was obtained from each curve. Gas chromatographic analysis was conducted on a Shimadzu GC 2010 with a 2010 autosampler using flame ionization detector (FID). The injector and detector temperatures were 290°C and 320°C respectively, and a Varian GC column (50 m × 0.32 mm I.D) was used for separation. The GC oven temperature was initially held at 220°C for 2 min and then increased from 220 to 290°C for 14 min at 5°C min^{-1} followed by an increase from 290 to 310°C for 18 min at 10°C min^{-1} to obtain the desired separation. A 2.0-μL sample was injected into the GC column using a split injection with a 1: 20 split. Standard stock solutions were prepared using 2,2,4-trimethylpentane (iso-octane). The 4 vitamin E (tocopherols) standards were used to establish calibration curves (Table 2).

Statistical analysis

Statistical analyses were conducted using the general linear model procedure (GLM) of SAS. The experimental design was a completely randomized design and triplicated. The least significant difference (LSD) test was based on the 0.05 probability level.

Conclusions

In our present study, we investigated the distribution of tocopherol composition on mung bean germplasm collected from different origins. The samples collected from Iran yielded highest total tocopherol content of 159.7 μg g^{-1}, followed by Taiwan which yielded 159.1 μg g^{-1}. The lowest total tocopherol content was observed from the Japanese collection which yielded about 143.8 μg g^{-1}. Among the 4 tocopherols, γ-tocopherol was highest in all the mung bean germplasm followed by α- and δ-tocopherols. The highest average γ-tocopherol content was observed in the mung bean seeds collected from Taiwan, which yielded 79.4 μg g^{-1}, followed by Iran, which yielded 78.0 μg g^{-1}. The lowest average γ-tocopherol content was observed in the mung bean seeds collected from Uzbekistan, which yielded 66.9 μg g^{-1}. These results will be useful to breeders for germplasm selection and for other food stuffs industries producing high-quality mung bean collections. Further research on the antioxidant activity in mung bean germplasm of different origin is in progress.

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