Fatty Acid Composition, Isoflavone and L-3,4-dihydroxyphenylalanine (L-dopa) Contents in Different Parts of Faba Bean (*Vicia faba*) Genotypes

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ABSTRACT The faba bean, one of the few crops that produces L-3,4-dihydroxy-phenylalanine (L-dopa) naturally, is also a good source of fatty acids and isoflavones. This study analyzed fatty acids, L-dopa and isoflavones in the leaves, immature pods and seeds of selected high-yielding faba bean genotypes spring-sown in Korea. The most abundant fatty acids in the leaves, immature pods and seeds were linolenic acid (58.6-63.7%), linoleic acid (46.9-58.9%), and linoleic acid (45.7-63.6%), respectively. The L-dopa content was highest in the leaf for all the faba bean genotypes except PI430715 and PI614810 where it was highest in the immature pods. The α -linolenic acid and L-dopa contents of the seed and immature pod of the PI430715 and PI614810 genotypes were the highest of all the genotypes. Four isoflavones (daidzin, genistin, daidzein and genistein) were detected with levels of daidzin and genistin generally higher than those of daidzein and genistein in each part of the faba bean. The L-dopa content was correlated ($P \leq 0.05$) with those of five fatty acids (oleic, gondoic, phytanic, α -linolenic, and behenic acid). Overall, the PI430715 and PI614810 faba bean genotypes with their higher L-dopa and α -linolenic acid contents were the most functional. This study may help identify optimal genotypes for breeding new cultivars which provide functional compounds to treat Parkinson's disease or use in foods.

Keywords Correlation analysis, GC-MS, Faba bean, UPLC

INTRODUCTION

The faba bean (*Vicia faba* L.) is one of the most common legume crops in many countries, traditionally used for animal feeding and as human food (Goyoaga *et al.* 2008). The faba bean is an erect, coarse, leafy annual herb whose pods may be greenish black, brown or black in color. Its seeds may be oblong, oval, flattened and rounded whose color can vary from white, reddish brown, greenish brown, and purple to black. The seeds vary greatly in size depending on the variety. Adsule and Akpapunam (1996) reported that the faba bean can be divided into three

botanical varieties on the basis of their seed size: smaller seeds (*V. faba* L. var. minor), medium seeds (*V. faba* L. var. equina), and larger seeds (*V. faba* L. var. major). The faba bean is known by a variety of common names such as faba bean, field bean, broad bean, horse bean, tick bean, and Windsor bean. Faba beans form an important part of the diet for people in China, Egypt, Europe and Latin America (Adsule and Akpapunam 1996). The seeds are rich in protein and starch, and valued for use in human food and animal feed (Crepon *et al.* 2010). The immature pods of faba beans are soft in texture and can be added to stews and soups or used as a vegetable. The young leaves of the faba

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bean plant can also be used as a vegetable and as animal feed (Goyoaga *et al.* 2008; Crepon *et al.* 2010).

The faba bean is one of the best natural plant sources of L-3,4-dihydroxy-phenylalanine (L-dopa). L-dopa is the precursor of the neurotransmitter dopamine and its synthesis involves the use of tyrosinase to convert tyrosine to L-dopa, a useful drug for the treatment of Parkinson's disease (Fox and Brotchie 2014). In humans, L-dopa can cross the blood-brain barrier and be converted to dopamine in the brain. Chemically-synthesized L-dopa is the main ingredient of several prescription drugs used in therapy for dopamine replacement in patients with Parkinson's disease (Rabey *et al.* 1993; Hu *et al.* 2015). It is well known that the L-dopa content of the faba bean is generally affected by genetic and environmental factors such as cultivar type, harvest time, location and temperature (Goyoaga *et al.* 2008; Crepon *et al.* 2010; Hu *et al.* 2015). Although different parts of the faba bean are used, there have been no studies on the L-dopa content of faba beans grown in the environment conditions of Korea.

The widespread use of legume crops makes this food group an important and diverse source of fatty acids and isoflavones in human nutrition (Kirakosyan *et al.* 2004; Yoshida *et al.* 2009). Therefore, evaluating the fatty acid and isoflavone contents of legume crops is important and beneficial for the food and pharmaceutical industries (Hamberg and Fahistadius 1992; Kirakosyan *et al.* 2004;

Yoshida *et al.* 2009). Recently, breeders have developed new varieties of legume which are more productive and nutritious with improved levels of fatty acids and isoflavones. Although the fatty acid and isoflavone contents of the faba bean seeds, leaves and stems have been studied (Robert and Griffiths 1984; Hamberg and Fahistadius 1992; Kirakosyan *et al.* 2004; Yoshida *et al.* 2009), there have been no studies on the immature pod or the fatty acid and isoflavone contents of faba beans grown in Korea. The aim of the present study is therefore to investigate the L-dopa, isoflavone and fatty acid contents of the leaves, immature pods and seeds of selected faba bean varieties with a high yield, which are spring-sown in Korea.

MATERIALS AND METHODS

Plant materials and harvesting

Ten faba bean genotypes were obtained from the United States Department of Agriculture (USDA) in 2014 (Table 1). These genotypes were then cultivated from 2014 to 2015 at the breeding farm of the Advanced Radiation Technology Institute at the Korean Atomic Energy Research Institute (Jeong-eup, Republic of Korea). The faba beans were selected on the basis of high seed and biomass production after spring sowing in early March. To

Table 1. Origin, morphological characteristics, and yield traits of the faba bean genotypes used in this study.

No.	Accession number	Origin	Leaf size	Leaf shape	Pod size	Seed size	Seed color	Flowering time	No. of Seed (ea/plant)	One hundred seed weight (100 ea/g)
1	PI252004	Turkey	Small	Spatulate	Small	Medium	Purple	29. Apr	141.0c ²⁾	98.9b
2	PI284345	Italy	Meddle	Elliptic	Small	Medium	Brown	6. May	75.4f	97.8b
3	PI366039	Argentina	Meddle	Elliptic	Small	Medium	Black	13. May	123.3d	93.3b
4	PI369511	Turkey	Meddle	Elliptic	Small	Medium	Brown	29. Apr	88.5e	85.4b
5	PI430715	Hungary	Large	Spatulate	Small	Medium	Brown	6. May	155.0b	86.4b
6	PI469147	Iran	Large	Ovate	Meddle	Medium	Brown	6. May	122.0d	87.9c
7	PI533728	Spain	Meddle	Ovate	Large	Medium	Brown	13. May	145.7bc	87.8d
8	PI567883	Turkey	Meddle	Spatulate	Large	Large	Yellow	13. May	87.0e	122.2a
9	PI614810	USA	Meddle	Ovate	Small	Small	Brown	13. May	160.5a	38.6e
10	PI655319	Morocco	Meddle	Spatulate	Large	Large	Yellow	6. May	65.8g	135.7a

²⁾The letters above each point indicate a significant difference at the 0.05 probability level (Duncan's multiple range tests, n = 3).

select the superior genotypes, we evaluated their morphological and growth characteristics and their leaf size, pod size, seed size, seed color, flowering time, seed yield, and the 100-seed weight. We classified the genotypes into three botanical varieties on the basis of their seed size and 100-seed weight (Agung and McDonald 1998); the latter as under 80 g (*V. faba* L. var. minor), 80-120 g (*V. faba* L. var. equina), and over 120 g (*V. faba* L. var. major). Faba beans collected from three biological replicates were used for each sample. The leaves, immature pods, and seeds of the faba bean plants were harvested (Fig. 1) and then freeze dried for later analysis. The representative weather conditions at the breeding farm during the faba

bean cultivation are shown in Table 2.

Fatty acids analysis

For GC-MS analysis, the fatty acids were extracted as described by Ryu *et al.* (2017), with the following modifications. The powdered freeze-dried material (100 g) of the leaf, immature pod, or seed was extracted in 100 mL *n*-hexane for 4 hours, then 1 mL 2 N potassium hydroxide in methanol was added. After the sample centrifugation for 5 minutes at $3000 \times g$, the collected supernatant was filtered using a $0.45\text{-}\mu\text{m}$ syringe filter. The fatty acid composition of the different parts of the faba bean plant was analyzed using a GC-MS (Plus-2010, Shimadzu, Japan) instrument

Fig. 1. Morphological profiles of different parts of the faba bean genotypes. (A) seed, (B) immature pod, (C) leaf, 1-10: accession numbers listed in Table 1.

Table 2. The weather conditions of cultivation area.

	Temperature (°C)			Humidity (%)			Wind speed (m/s)		Solar radiation (w/m ²)	Rainfall (mm)
	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Max.	Mean	Total
March	8.26	-3.6	25.9	72.71	13	113	0.87	10	177.45	38.0
Apr.	15.64	5.2	29.2	87.57	15	113	0.77	9	240.59	122.5
May	20.80	7.4	35.1	72.77	16	113	0.81	10	281.15	43.0
Jun.	24.62	14.3	34.8	89.07	22	113	0.57	13	261.03	68.5

equipped with an HP-88 capillary column (J&W Scientific, 60 m × 0.25 mm × 0.25 m) under the following conditions: ionization voltage, 70 eV; mass scan range, 50-450 mass units; injector temperature, 230°C; detector temperature, 230°C; injection volume, 1 µL; split ratio, 1 : 30; carrier gas, helium; and flow rate, 1.7 mL/minute. The column temperature program specified an isothermal temperature of 40°C for 5 minutes increasing to 180°C at a rate of 5°C/minute then a subsequent increase to 28°C at a rate of 1°C/minute. We identified the substances present in the extracts according to their retention time (RT) and using the mass spectra database (NIST 62 Library).

L-dopa and isoflavones analyses

The L-dopa and isoflavone compounds were analyzed using a UPLC system (CBM-20A, Shimadzu Co., Kyoto, Japan) with two gradient pump systems (LC-30AD, Shimadzu), an UV detector (SPD-M30A, Shimadzu), an auto sample injector (SIL-30AC, Shimadzu), and a column oven (CTO-30A, Shimadzu).

L-dopa was extracted from each sample as described by Takashi *et al.* (2011), with some modifications. Briefly, 0.5 g of the freeze-dried sample were treated with 5 mL acetonitrile : water : formic acid (80 : 20 : 1, v/v) for 24 hours, with periodic vortexing. The material was filtered through a 0.45-µm membrane filter. Three replicates of the extract were analyzed from each sample. Separation was achieved on an XR-ODS column (3.0 × 100 mm, 1.8 µm, Shimadzu) using a linear gradient elution program with a mobile phase containing solvent A (water with 0.1% trifluoroacetic acid) and solvent B (acetonitrile with 0.1% trifluoroacetic acid). The L-dopa content was analyzed using the following gradient: 0-5 minutes, 0-10% B; 5-10 minutes, 10-20% B; 10-15 minutes, 20-30% B; 10-17

minutes, 30-50% B; and 17-21 minutes, 50-0% B. The flow rate of the mobile phase was adjusted to 0.3 mL/minute and the column temperature was set at 40°C. L-dopa was detected at 280 nm and identified using commercial standards (Sigma, St. Louis, MO, USA).

The isoflavone extracts from different parts of the faba bean were determined as described by Tepavcevic *et al.* (2010), with some modifications. Five mL of 80% MeOH and 0.5 g of each sample were placed in a tube for 4 hours then hydrolyzed with 200 µL of 2 N NaOH. The solutions were neutralized with 50 µL of glacial acetic acid. After the sample was centrifuged for 5 minutes at 3000 rpm, the collected supernatant was filtered using a 0.45-µm syringe filter. The mobile phase contained solvent A (water with 0.05 % formic acid) and solvent B (acetonitrile with 0.05% formic acid). The isoflavone contents were analyzed using the following gradients: 0-10 minutes, 15-35% B; 10-11 minutes, 35-15% B; and 11-12.5 minutes, 15% B. The flow rate of the mobile phase was adjusted to 0.4 mL/min, and the column temperature was set at 35°C. The isoflavones, daidzin, genistin, daidzein, and genistein were detected at 254 nm and identified using commercial standards (LKT Laboratories Inc., St. Paul, MN, USA).

Statistical analysis

The chemical analysis data were subjected to the analysis of variance and multiple comparison procedures of the statistical software package SPSS, Version 12 (SPSS Inc., Chicago, IL, USA). The differences were determined as significant at $P \leq 0.05$. When the treatment effect was significant, the means were separated using Duncan's multiple range tests.

RESULTS

Morphological characteristics and seed yield traits

The evaluations of the morphological characteristics are presented in Table 1 and Fig. 1. Ten faba bean genotypes were divided into three botanical varieties on the basis of seed size; PI614810 with small seeds (*V. faba* L. var. minor), seven genotypes (PI252004, PI284345, PI366039, PI369511, PI430715, PI469147 and PI533728) with medium seeds (*V. faba* L. var. equina) and two genotypes (PI567883 and PI655319) with large seeds (*V. faba* L. var. major). The faba bean genotypes were divided according to their leaf shape into three types: four genotypes with spatulate, three genotypes with elliptic, and three genotypes with ovate leaves. Significant differences in the leaf and pod size were observed between all the genotypes as well as in most of the seed yields including the number of seeds and the 100-seed weights. The number of seeds from all the genotypes ranged from 65.8 to 160.5. The PI614810 genotype had the highest number of seeds whereas PI655319 had the lowest. The 100-seed weight was recorded for the PI655319 genotype (135.7 g) as the highest value and for the PI614810 genotype (38.6 g) as the lowest value.

Fatty acids analysis

The fatty acids identified in the different parts of the faba bean genotypes, along with their retention time (RT) and percentage content, are shown in Table 3. The leaf extract contained eight fatty acids: myristic acid (C14:0, tetradecanoic acid), palmitic acid (C16:0, hexadecanoic acid), palmitoleic acid (C16:1, 9-hexadecenoic acid), stearic acid (C18:0, octadecanoic acid), oleic acid (C18:1, 9-octadecenoic acid), linolelaidic acid (C18:2, trans-9,12-octadecadienoic acid), phytanic acid (C16:0-(CH₃)₄, 3,7,11,15-tetramethyl hexadecanoic acid), and α -linolenic acid (C18:3, $n = 3$, 9,12,15-octadecatrienoic acid). Palmitic, linolelaidic and α -linolenic acids were found to be the major fatty acids present in the faba bean leaf (Supplementary Fig. S1). All of the genotypes had high contents of α -linolenic acid, the highest (63.7%) being found in the leaf of the PI567883 genotype. The leaf of the PI369511 genotype had the highest linolelaidic acid

content (13.5%). The leaf of the PI284345 genotype showed the highest palmitic acid content (15.4%). The leaf contained eight fatty acids, of which α -linolenic acid was the most abundant (58.6-63.7%).

The extract from the immature pod contained eleven fatty acids: myristic acid, palmitic acid, palmitoleic, stearic acid, oleic acid, linoleic acid, γ -linolenic acid (C18:3, $n = 6$, cis-6,9,12-octadecadienoic acid), arachidic acid (C20:0, eicosanoic acid), phytanic acid, α -linolenic acid, gondoic acid (C20:1, $n = 9$, cis-11-eicosenoic acid). Palmitic, linoleic and α -linolenic acids were the principal fatty acids presented in the immature pod (Supplementary Fig. S2). All the immature pods of the genotypes had a high content of linoleic acid, with the PI366039 genotype being the highest (58.9%) and the PI430715 genotype being the lowest (46.9%). The highest content of palmitic acid in the immature pod was shown to PI614810 genotype (25.8%), while the lowest content was observed to PI252004 genotype (21.7%). The PI430715 genotype had the highest content of α -linolenic acid in the immature pod (20.4%) where other genotypes exhibited PI614810 (18.2%), PI284345 and PI469147 (12.4%), PI655319 (11.0%), PI533728 (10.8%), PI567883 (10.3%), PI252004 (9.3%), PI369511 (8.9%), and PI366039 (8.4%).

Ten different fatty acids were identified in the extract of the faba bean seed: palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, γ -linolenic acid, arachidic acid, α -linolenic acid, gondoic acid and behenic acid (C22:0, Docosanoic acid). Palmitic, oleic and linoleic acids were presented at high levels in the seed (Supplementary Fig. S3). The highest content of palmitic acid was found in the seed of the PI614810 genotype (20.8%), whereas the lowest content was in the PI430715 genotype (16.9%). The linoleic acid contents of seeds of the faba bean genotypes were ranged from 45.7% in PI430715 to 63.6% in PI252004. The α -linolenic acid contents of the seeds were ranged from 4.9% to 11.1% with the PI430715 genotype having the highest value, approximately 18% to 66% higher than that of the other faba bean genotypes.

L-dopa content

The L-dopa and isoflavone contents of different parts of the faba bean (Table 4) were significantly different ($P \leq$

Table 3. Fatty acid compositions in different parts of faba bean genotypes.

No.	RT ⁽²⁾	Name of the compound	Total (%)									
			PI252004	PI284345	PI366039	PI369511	PI430715	PI469147	PI533728	PI567883	PI614810	PI655319
Leaf												
1	13.7	Myristic acid	0.1b ^{y)}	0.2a	0.0c	0.0c	0.1b	0.1b	0.0c	0.0c	0.0c	0.2a
2	15.2	Palmitic acid	13.1b	15.4a	12.0b	12.1b	13.0b	13.1b	12.2b	12.1b	11.9b	15.2a
3	15.7	Palmitoleic acid	3.9ab	3.0bc	3.5ab	4.0a	3.6ab	3.1bc	3.3bc	3.9ab	3.5ab	2.8c
4	17.2	Stearic acid	2.1c	2.7a	1.4e	1.6de	2.3b	1.7d	1.8d	1.9cd	1.3e	2.4b
5	17.8	Oleic acid	3.6cd	4.3a	3.1f	3.7cd	3.2ef	4.1ab	3.8bc	3.5de	4.1ab	4.1ab
6	18.8	Linolelaidic acid	13.3ab	13.1cd	13.2bc	13.5a	13.2bc	13.0d	12.8e	11.0h	12.7e	11.5g
7	19.8	Phytanic acid	3.5ab	3.0ab	3.4ab	3.5ab	3.3ab	3.4ab	2.9b	3.9a	3.4ab	2.7b
8	20.4	α -linolenic acid	60.5c	58.6d	63.6a	61.6b	61.4b	61.6b	63.2a	63.7a	63.1a	61.2b
Immature pod												
1	13.7	Myristic acid	0.5a	0.4b	0.5a	0.3b	0.0d	0.0d	0.0d	0.5a	0.0d	0.2c
2	15.1	Palmitic acid	21.7c	23.7ab	23.0bc	23.2ab	22.8ab	23.0ab	25.3ab	22.0c	25.8a	25.2ab
3	15.3	Palmitoleic acid	0.5c	0.6e	0.0g	0.4d	1.4b	1.0a	0.3e	0.5c	0.5g	0.2f
4	17.0	Stearic acid	1.9d	2.2b	2.2b	2.2b	1.9d	2.1c	2.1c	1.9d	2.6a	1.9d
5	17.7	Oleic acid	6.1b	7.9a	6.3b	7.6a	3.0e	5.4c	5.9bc	7.4a	3.0e	4.4d
6	19.0	Linoleic acid	58.7a	51.7d	58.9a	56.0b	46.9f	52.6d	53.9c	55.5b	49.4e	56.0b
7	19.2	γ -linolenic acid	1.3d	0.7f	0.3g	0.9e	2.6a	2.4b	1.0e	1.4c	0.0h	1.0e
8	19.6	Arachidic acid	0.6b	0.7a	0.8a	0.6b	1.2c	1.1c	0.8c	0.9c	0.5c	0.3d
9	19.8	Phytanic acid	0.0c	0.3b	0.0c	0.0c	0.5a	0.0c	0.0c	0.0c	0.5a	0.0c
10	20.4	α -linolenic acid	9.3f	12.4c	8.4g	8.9fg	20.4a	12.4c	10.8de	10.3e	18.2b	11.0d
11	20.7	Gondoic acid	0.0d	0.0d	0.0d	0.0d	0.7a	0.6a	0.3c	0.4b	0.0d	0.0d
Grain												
1	15.0	Palmitic acid	17.6cd	19.6ab	18.7ab	18.2bc	16.9d	19.2ab	18.6ab	19.9ab	20.8a	20.4ab
2	15.3	Palmitoleic acid	0.3d	0.0e	0.0e	0.0e	3.1a	0.7c	0.0e	0.0e	1.2b	0.0e
3	17.0	Stearic acid	2.2b	2.4a	2.5a	2.4a	2.1bc	2.1bc	2.0c	2.5a	1.6d	2.5a
4	17.7	Oleic acid	10.2cd	12.6a	10.8bc	12.7a	6.1f	8.8e	9.8d	11.4b	5.5f	11.3b
5	19.0	Linoleic acid	63.6a	58.2e	61.2c	59.2d	45.7g	59.1d	62.4b	57.3f	56.7f	57.4f
6	19.2	γ -linolenic acid	0.0e	0.4d	0.0e	0.6d	12.3a	2.4c	0.0e	0.0e	4.2b	0.0e
7	19.5	Arachidic acid	1.0b	1.2a	1.4a	1.3a	0.0d	0.9b	0.8b	1.2a	0.5c	1.3a
8	20.4	α -linolenic acid	5.2e	5.0e	4.9e	5.0e	11.1a	6.8c	6.0d	7.0c	9.1b	7.2c
9	20.7	Gondoic acid	0.0c	0.0c	0.0c	0.0c	2.7a	0.0c	0.0c	0.0c	0.4b	0.0c
10	23.0	Behenic acid	0.0d	0.5bc	0.6ab	0.6ab	0.0d	0.0d	0.4c	0.7a	0.0d	0.0d

^{a)}Retention time.^{y)}The letters above each point indicate a significant difference at the 0.05 probability level (Duncan's multiple range tests, n = 3).

Table 4. L-dopa and isoflavone content in different parts of the faba bean genotypes.

Accession No.	L-dopa (mg/g)	Daidzin (mg/100 g)	Genistin (mg/100 g)	Daidzein (mg/100 g)	Genistein (mg/100 g)
Leaf					
PI252004	28.19±6.09 ^{c2)}	10.05±2.07a	2.00±0.03c	0.97±0.27a	0.34±0.02b
PI284345	39.82±0.28a	0.97±0.01c	0.32±0.05f	0.17±0.02c	0.69±0.21a
PI366039	34.61±3.77ab	1.40±0.53c	0.38±0.04f	0.09±0.01c	0.66±0.23a
PI369511	34.63±6.30ab	4.33±1.21b	4.90±0.52b	0.18±0.02c	0.72±0.06a
PI430715	29.02±5.26bc	3.71±1.47b	2.40±0.02c	0.27±0.03c	0.85±0.44a
PI469147	38.63±6.97ab	0.15±0.00c	0.68±0.12ef	0.26±0.05c	–b
PI533728	32.42±4.11ab	1.83±0.65c	1.33±0.24d	0.46±0.08b	–b
PI567883	35.26±4.52ab	1.31±0.53c	0.56±0.10f	0.10±0.01c	0.65±0.18a
PI614810	33.76±7.28ab	1.84±0.37c	11.46±0.66a	0.39±0.09bc	–b
PI655319	32.79±4.94ab	1.53±0.44c	1.13±0.22de	0.23±0.02c	0.07±0.01b
Immature pod					
PI252004	19.99±0.16f	2.42±0.75bc	0.86±0.05c	–c	0.08±0.00cd
PI284345	23.71±0.11e	2.36±0.74bc	1.12±0.26c	0.24±0.02a	0.31±0.04a
PI366039	34.29±0.07d	3.11±1.11b	1.11±0.27c	–c	0.15±0.01b
PI369511	20.01±0.18f	2.37±0.98bc	1.27±0.22c	0.10±0.01b	0.10±0.00c
PI430715	79.58±0.40a	3.16±0.68b	3.46±0.64a	0.23±0.05a	0.10±0.01c
PI469147	43.89±0.50c	0.31±0.05c	2.37±0.38b	0.26±0.04a	0.05±0.01d
PI533728	20.46±0.04f	1.45±0.42bc	0.81±0.15c	–c	0.07±0.00d
PI567883	17.65±0.06g	1.81±0.55bc	1.31±0.16c	–c	0.07±0.00d
PI614810	71.37±0.50b	6.58±3.15a	2.72±0.64b	–c	–e
PI655319	16.31±0.02h	1.57±0.49bc	1.27±0.14c	–c	0.09±0.00cd
Grain					
PI252004	0.72±0.03g	0.60±0.07d	0.91±0.10d	–c	–c
PI284345	3.97±0.01c	3.13±0.92b	1.91±0.41c	0.86±0.68b	0.05±0.01c
PI366039	1.76±0.68f	–e	2.85±0.33bc	1.60±0.50a	0.31±0.04b
PI369511	3.98±0.01c	3.75±0.35b	3.01±0.22b	–c	–c
PI430715	15.69±0.10a	1.99±0.07c	5.32±0.91a	0.38±0.03b	0.94±0.27a
PI469147	5.07±0.05c	–e	4.70±0.20a	0.43±0.24b	0.84±0.28a
PI533728	3.36±0.02d	6.50±0.19a	2.97±0.52b	0.03±0.00c	–c
PI567883	2.36±0.00e	3.27±0.23b	3.16±0.40b	0.05±0.01c	0.03±0.01b
PI614810	10.43±0.03b	6.79±0.40a	2.76±0.97bc	0.06±0.03c	0.16±0.02b
PI655319	3.52±0.05d	3.30±0.02b	2.38±0.50bc	0.23±0.05c	0.16±0.02b

²⁾The letters above each point indicate a significant difference at the 0.05 probability level (Duncan's multiple range tests, n = 3).

0.05) among the faba bean genotypes. Of the different parts, the leaf exhibited the highest L-dopa content of all the different faba bean genotypes except for PI430715 and PI614810 where the highest content was in the immature pod (Supplementary Fig. S4). The L-dopa content in the leaf ranged from 28.19 mg/g for PI252004 up to 39.82 mg/g for PI284345, with a mean value of 33.91 ± 5.65 mg/g. The PI430715 genotype exhibited the highest content of L-dopa in the immature pod (79.58 ± 0.40 mg/g)

and other genotypes contained relatively low, presenting 71.37 ± 0.50 mg/g in PI614810, 43.89 ± 0.50 mg/g in PI469147, 34.29 ± 0.07 mg/g in PI366039, 23.71 ± 0.11 mg/g in PI284345, 20.46 ± 0.04 mg/g in PI533728, 20.01 ± 0.18 mg/g in PI369511, 19.99 ± 0.16 mg/g in PI252004, 17.65 ± 0.06 mg/g in PI567883, and 16.31 ± 0.02 mg/g in PI655319, respectively. The L-dopa content in the seed ranged from 0.72 ± 0.03 mg/g for PI252004 up to 15.69 ± 0.10 mg/g for PI430715, with a mean value of 5.08 ± 4.39

Table 5. Correlation coefficients between L-dopa and fatty acid content for faba bean genotypes.

Compounds	Oleic acid	Arachidic acid	Phytanic acid	α -linolenic acid	Behenic acid
L-dopa	-0.779**	-0.560**	0.408*	0.459*	-0.498**

* $P < 0.05$, ** $P < 0.01$.

mg/g. The L-dopa content of the seed of the PI430715 genotype was approximately 1.4 to 15 times higher than that of the other faba bean genotypes.

The daidzin content in the leaf of the genotypes ranged from 0.15 ± 0.00 to 10.05 ± 2.07 mg/100 g. The PI252004 genotype exhibited the highest content of daidzin, approximately 2.3 to 10 times higher than that of the other faba bean genotypes. The genistin content in the leaf of the genotypes ranged from 0.32 ± 0.10 mg/100 g for the PI284345 genotype to 11.46 ± 0.66 mg/100 g for the PI614810 genotype. The genistin content in the leaf of the PI614810 genotype was approximately 2.3 to 10 times higher than in that of the other faba bean genotypes. The daidzein contents in the leaf of the genotypes ranged from 0.09 to 0.97 mg/100 g with the highest content in the PI252004 genotype. The genistein contents in the leaf of seven genotypes ranged from 0.07 to 0.72 mg/100 g whereas no genistein detected in the PI469147, PI533728 and PI614810 genotypes. The highest level of daidzin content in the immature pod was found in the for PI614810 genotype (6.58 ± 3.15 mg/100 g), and the lowest value was shown in that of the PI469147 genotype (0.31 ± 0.05 mg/100 g). The genistin content in the immature pods of all genotypes was ranged from 0.81 mg/100 g for PI533728 up to 3.46 ± 0.64 mg/100 g for PI430715. The daidzein contents in the immature pod of all genotypes were less than 0.26 mg/100 g. The highest genistein content in the immature pod was observed in the PI284345 genotype. The daidzin contents in the seed of eight genotypes were ranged from 0.60 up to 6.79 mg/100 g. The higher contents of daidzin were shown in the PI533728 and PI614810 genotypes while no daidzin was detected in the PI366039 and PI469147 genotypes. The genistin contents in the seed of the genotypes were ranged from 0.91 to 5.32 mg/100 g with the highest content in PI430715 genotype. The daidzein in the seeds of the genotypes was not detected in PI252004 and PI369511, but it was contained up to 1.60

mg/100 g in the PI366039 genotype. The genistein contents in the seeds of all the genotypes were less than 0.94 mg/100 g (Supplementary Fig. S5).

Correlation analysis

The values of Pearson's correlation coefficient are shown in Table 5. The L-dopa content was positively correlated ($P \leq 0.05$) with the contents of phytanic acid and α -linolenic acid. The L-dopa content was negatively correlated ($P \leq 0.01$) with the contents of oleic acid, arachidic acid, and behenic acid. The morphological characteristics were not significantly correlated with the contents of fatty acids, L-dopa, and isoflavones. The L-dopa content was significantly correlated ($P \leq 0.05$) with the fatty acid contents.

DISCUSSION

Faba bean (*Vicia faba* L.) is one of the most important legumes for its functional compounds. This is the first report on the L-dopa, isoflavone, and fatty acid value of the different parts of the selected faba bean in Korea environment. Faba bean is widely used in cereal-dominated rotations as break crop, partly because of its high input of nitrogen in the soil. Korean imports for large amount of legumes every year. Faba bean is most advantageous as a legume crop that can be cultivated in the winter or spring season. The results of this study revealed the presence of functional compounds in the different part of selected faba bean with high yield by spring sowing. It is routinely used to access faba bean cultivates and breeding programs. Unfortunately, Kim (2006) reported that faba bean in fall season seeding showed higher yield with large grain size expressing earlier maturing than those of spring seeding in southern Korea. Thus, study on the winter cultivation is necessarily. The most important characteristics for im-

proving the seed yield of faba bean were reported to be 100-seed weight, number of seeds per plant and seed size (Agung and McDonald 1998). Normally, the large and medium seeds genotypes offered more seed yield than small seeds (Agung and McDonald 1998; Kim 2006).

This study revealed the presence of fatty acid in the different parts of the faba bean by GC-MS. The leaf contained 8 fatty acid, of which α -linolenic acid was the most fatty acid (58.6-63.7%). Similarly, Arbaoui and Link (2008), reported that the main fatty acid (51-57%) in the leaf and stem of faba bean was α -linolenic acid. The present study has detected 11 fatty acids in the immature pod, of which linoleic acid was the most abundant. There have been no studies on the fatty acid composition of the immature pod of faba bean. The levels of linoleic acid in the immature pod of the faba bean genotypes were higher than those reported for Japanese soybean cultivars, except for the PI430715 and PI614810 genotypes which had similar linoleic acid contents (Kumar *et al.* 2007). The linoleic acid content was lower in the immature pods of the PI430715 and PI614810 genotypes than in those of the other genotypes. In contrast, the α -linolenic acid content of the immature pod of the PI430715 and PI614810 genotypes was approximately twice that in pods of the other genotypes. The seeds contained ten fatty acids, of which linoleic acid was the most abundant (45.7-63.6%), findings similar to those of Yoshida *et al.* (2009) (43.6-69.8% for triacylglycerols obtained from four faba bean cultivars). The α -linolenic acid contents of the seeds of the PI430715 and PI614810 genotypes were higher than those of the other genotypes. α -linolenic acid, a polyunsaturated fatty acid abundant in plant vegetable oils, has medicinal properties so is used to treat heart disease, high cholesterol level, and high blood pressure (Furuhashi and Hotamisligil 2008). Our results suggest that PI430715 and PI614810 are potentially the most appropriate faba bean genotypes for improving health benefits through their high contents of functional compounds.

Nowadays, it has been trying that use of genetically modified plants to produce L-dopa for the treatment of Parkinson's disease has been proposed as an alternative to produce it synthetically (Longo *et al.* 1974; Rabey *et al.* 1993; Burbano *et al.* 1995; Ando *et al.* 1999; Randhir and

Shetty 2004; Goyoaga *et al.* 2008; Fox and Brothie 2014). We observed significant differences in L-dopa content between the different parts of the faba bean genotypes. The leaf and immature pod showed higher levels of L-dopa than the seed so that the tissues could be a good source for producing the compound promising for treating Parkinson's disease. Previous studies have revealed that L-dopa presents in several tissues of the faba bean plant: the sprout 2-3%, leaf 6%, immature pod 6%, flower 7%, immature seed 4%, and mature seed less than 1% (Burbano *et al.* 1995; Goyoaga *et al.* 2008; Hu *et al.* 2015). Burbano *et al.* (1995) reported that the L-dopa content decreased significantly as the seed was developed. The L-dopa levels of the leaf observed in the present study were similar to those reported by Hu *et al.* (2015) for six faba bean cultivars (18.2-48.7 mg/g dry weight). The L-dopa concentrations in the seed and immature pod for the PI430715 and PI614810 genotypes were higher than in those of the other genotypes and also higher than those reported by Kirakosyan *et al.* (2004) in the seed. The L-dopa concentrations observed in the immature pod were similar to those reported by Goyoaga *et al.* (2008) for the embryo axes of two faba bean cultivars harvested up to 9 days after imbibition. This also suggests that the PI430715 and PI614810 genotypes are potentially the most appropriate materials for helping treat Parkinson's disease (Goyoaga *et al.* 2008; Hu *et al.* 2015).

In this study, we analyzed the isoflavone content in the different parts of faba bean genotypes. We observed significant differences ($P < 0.05$) for most of the isoflavone content including daidzin, genistin, daidzein and genistein. The levels of daidzin and genistin were generally higher than those of daidzein and genistein in each part. The data also indicate that is wide variation among genotypes and parts in the levels of isoflavones analyses. These findings are similar to those described by Kirakosyan *et al.* (2004). Faba bean that have traditionally been utilized in animal feed are known to be relatively rich in isoflavones (Kirakosyan *et al.* 2004; Crepon 2010). We therefore analyses the distribution of isoflavones in different parts of faba bean. These results are important particularly for industries that produce products since it can grind and processing to avoid excessive losses of isoflavones.

The L-dopa content was significant correlated ($P < 0.05$) with fatty acids. Previous studies have revealed that unsaturated fatty acids, such as oleic, linoleic, and α -linolenic acids, can decrease the tyrosinase levels, while saturated fatty acids, such as palmitic and stearic acids, can increase them (Ando *et al.* 1999). L-dopa can also be synthesized by the hydroxylation of tyrosinase (L-tyrosine) through a series of enzymatic and chemical steps (Shetty *et al.* 2002). Julien *et al.* (2006) have reported that administering L-dopa to Parkinsonian monkey's brain decreased their level of unsaturated fatty acids.

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