Variation in Antioxidant Activity and Flavonoid Aglycones in Eggplant (*Solanum melongena* L.) Germplasm

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ABSTRACT Eggplant (*Solanum melongena* L.) is an excellent source of vitamins A and C and of flavonoid compounds, which are important antioxidant components believed to reduce the risk of various diseases. We investigated the antioxidant activity and flavonoid content in eggplant leaves and fruits to identify genetic resources with high antioxidant capacity for use in food or as feed additives, and also determined the influence of days to flowering, leaf blade colors, and latitudes of origin on the antioxidant activity and flavonoid content in eggplant leaves. The accessions originating from 45°N showed the highest flavonoid contents (AVG. = 15.4 $\mu\text{g mg}^{-1}$) followed by accessions from 30°~45°N (AVG. = 13.0 $\mu\text{g mg}^{-1}$), 15°~30°N (AVG. = 11.0 $\mu\text{g mg}^{-1}$), and 0°~15°N (AVG. = 9.5 $\mu\text{g mg}^{-1}$). The same pattern was also found in 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 1,1-diphenyl-2-picryl-hydrazil (DPPH) antioxidant activities. High ABTS and DPPH activity and flavonoid content were found in the early-flowering accessions. All flavonoids of the greenish violet leaves were significantly higher than those of green leaves. The flavonoid concentration in eggplant leaves was 10 to 20 fold greater, at an average of 15.6 $\mu\text{g mg}^{-1}$, than that of the fruit (AVG. = 0.9 $\mu\text{g mg}^{-1}$). Taken together, eggplant leaves represent a potential source of natural antioxidants due to their high flavonoid content.

Keywords Antioxidant activity, Eggplant, Flavonoid aglycone, Germplasm

INTRODUCTION

Eggplant (*Solanum melongena* L.) is a vegetable crop from the nightshade family (Solanaceae) that originated in warm regions of India and China (Lawande and Chavan 1998). Eggplant is an excellent source of vitamin A and C and of flavonoid compounds, which are important antioxidant components that may reduce the risk of disease (George 1985; Konys 1993). Flavonoid compounds and antioxidant activity occur in most parts of the eggplant, including the calyx, leaf, fruit, and stem (Saddiqe *et al.* 2011). Methods using the stable 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) or 1,1-diphenyl-2-picryl-hydrazil (DPPH) radicals are widely used to evaluate the free radical-scavenging ability of antioxidants (Nabavi *et al.*

2009). Both methods have excellent reproducibility under certain assay conditions, but they show significant differences in their responses to antioxidants (Arnao 2000). The range of flavonoids occurring in plant materials is usually large, and every plant has an original and unique flavonoid profile, which makes quantification difficult. For this reason, flavonoid aglycones are frequently used to determine total flavonoid content, which can reduce the variability (Webb and Harborne 1991). These aglycones can be quantified using ultraviolet (UV) spectrophotometric methods, as described previously (Miean and Mohamed 2001), and several high-performance liquid chromatography procedures have been used to quantify flavonoid aglycones. Quercetin, myricetin, kaempferol, and isorhamnetin are the four most widespread flavonoid aglycones (Olszewska 2007).

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Although previous studies have revealed that ethanol (EtOH) extracts of eggplant leaves have higher antioxidant activity and total flavonol content than do their fruits (Jung *et al.* 2011), most eggplant leaves are discarded as waste. To the best of our knowledge, no published studies have determined the influences of agronomic traits and latitudes of origin of specific accessions on the antioxidant activity and flavonoids in eggplant leaves. In this study, we investigated the antioxidant activity and flavonoid content in eggplant leaves and fruits to identify genetic resources with a high antioxidant capacity for use in food or as feed additives, and also determined how days to flowering, leaf blade colors, and latitudes of origin influenced the antioxidant activity and flavonoid content in eggplant leaves.

MATERIALS AND METHODS

Materials

In total, 102 eggplant accessions collected from 15 countries (Table 1, Fig. 1) were classified into four clusters by their latitudes of origin, 45°N ($n = 9$), 30°~45°N ($n = 31$), 15°~30°N ($n = 22$), and 0°~15°N ($n = 40$), and then divided into three groups based on their days to flowering (80 days, $n = 26$; 80~90 days, $n = 44$, and 90 days, $n = 32$). All accessions were sown on April 4 in the plug plate and planted on June 5, 2013 in an open field at National Agrobiodiversity Center in Suwon, Korea. The experiment was laid out in completely randomized design (CRD) with three replications. A plot was consisted of six plants of each accession in one row. Plant spacing was 100 cm between rows and 30 cm between plants.

Table 1. NAC²⁾ registration numbers and origins of 102 eggplant accessions investigated in this study.

NAC registration number	Country of origin	NAC registration number	Country of origin	NAC registration number	Country of origin	NAC registration number	Country of origin	NAC registration number	Country of origin
1. IT219140	CAN	22. IT189726	JPN	43. IT136561	NPL	64. IT201082	PHL	85. IT218791	RUS
2. IT170475	CHN	23. IT189727	JPN	44. IT136562	NPL	65. IT201084	PHL	86. IT218790	RUS
3. IT208418	CHN	24. IT189728	JPN	45. IT136564	NPL	66. IT201087	PHL	87. IT224506	RUS
4. IT213107	CHN	25. IT189763	JPN	46. IT136566	NPL	67. IT201088	PHL	88. IT224477	RUS
5. IT218742	CHN	26. IT189764	JPN	47. IT189771	NPL	68. IT201089	PHL	89. IT218616	THA
6. IT218770	CHN	27. IT189769	JPN	48. IT200247	NPL	69. IT201090	PHL	90. IT218694	THA
7. IT218771	CHN	28. IT189770	JPN	49. IT200249	PHL	70. IT201091	PHL	91. IT218697	THA
8. IT180718	EGY	29. IT102771	KOR	50. IT201053	PHL	71. IT201092	PHL	92. IT218699	THA
9. IT180719	EGY	30. IT103969	KOR	51. IT201057	PHL	72. IT201096	PHL	93. IT218700	THA
10. IT189729	IDN	31. IT104244	KOR	52. IT201059	PHL	73. IT201100	PHL	94. IT218772	THA
11. IT136568	IND	32. IT136571	KOR	53. IT201060	PHL	74. IT201101	PHL	95. IT218774	THA
12. IT136569	IND	33. IT220040	KOR	54. IT201063	PHL	75. IT201102	PHL	96. IT218775	THA
13. IT136570	IND	34. IT203193	MDA	55. IT201071	PHL	76. IT201103	PHL	97. IT218776	THA
14. IT208416	IND	35. IT183769	MYS	56. IT201072	PHL	77. IT201112	PHL	98. IT203186	UKR
15. IT208417	IND	36. IT110943	NPL	57. IT201073	PHL	78. IT201113	PHL	99. IT203178	UZB
16. IT218701	IND	37. IT136552	NPL	58. IT201074	PHL	79. IT201116	PHL	100. IT203179	UZB
17. IT189720	JPN	38. IT136553	NPL	59. IT201077	PHL	80. IT201117	PHL	101. IT218709	UZB
18. IT189721	JPN	39. IT136554	NPL	60. IT201078	PHL	81. IT201118	PHL	102. IT218722	UZB
19. IT189722	JPN	40. IT136556	NPL	61. IT201079	PHL	82. IT201124	PHL		
20. IT189724	JPN	41. IT136557	NPL	62. IT201080	PHL	83. IT203188	RUS		
21. IT189725	JPN	42. IT136558	NPL	63. IT201081	PHL	84. IT203189	RUS		

²⁾ National Agrobiodiversity Center

Chemicals

DPPH, l-ascorbic acid, ABTS, 6-hydroxy-2,5,7,8-tetra-methylchroman-2-carboxylic acid (Trolox), quercetin, apigenin, kaempferol, and isorhamnetin were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other reagents were of analytical grade.

Sample preparation

Crude extracts were produced using 7 g of oven-dried eggplant leaves and fruits from each accession using an ASE-200 extractor (Dionex, Sunnyvale, CA, USA). Extractions were performed in 40 ml of 75% EtOH under nitrogen gas at 1500 psi and 70°C. Extracted samples were dried using an HT-4X vacuum concentrator (Genevac, Stone Ridge, NY, USA).

DPPH assay

The free radical-scavenging activity of the extracts was assessed by the DPPH method proposed by Lee and Lee (2004), with slight modifications. A DPPH solution (150 µl; 150 µM in anhydrous EtOH) was added to 100 µl of sample solution. The mixture was shaken vigorously and left to stand at 25°C in the dark for 30 min. Absorbance at 517 nm was then measured with a ELISA (Bio-Teck, Epoch, US). DPPH free radical scavenging activity was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = [1 - (A_0 - A_1)/(A_2 - A_3)] \times 100$$

where A0, A1, A2, and A3 are the absorbance of the sample, the sample blank, the control, and the control blank, respectively. The radical scavenging effect is expressed as micrograms l-ascorbic acid equivalent antioxidant capacity (ASC) per 1 mg dried extract (µg ASC mg⁻¹ dry weight).

ABTS assay

ABTS radical scavenging activity was estimated using the method in Re *et al.* (1999) with some modifications. The ABTS radical cation was generated by adding 7 mM ABTS to 2.45 mM potassium persulfate, followed by overnight incubation of the mixture in the dark at room temperature. The ABTS radical cation solution was diluted with methanol (MeOH) to obtain an absorbance of 0.7 ± 0.02 at 735 nm. A diluted ABTS radical cation solution (190 µl) was added to 10 µl of sample solution. After 6 min, absorbance at 735 nm was determined using ELISA. The capability to scavenge the ABTS radical was calculated using the following equation:

$$\text{ABTS scavenging effect (\%)} = [1 - (A_0 - A_1)/(A_2 - A_3)] \times 100$$

where A0, A1, A2, and A3 are defined as above. The free radical scavenging effect of each sample was reported as the Trolox equivalent antioxidant activity obtained by comparing the changes in absorbance at 735 nm in reaction mixtures containing a sample eggplant extract or a Trolox equivalent.

Fig. 1. Distribution of 102 eggplant accessions by their countries of collection.

Flavonoid aglycones assay

Flavonoid aglycone contents of the eggplant fruits and leaves were investigated using high-performance liquid chromatography (Thermo Scientific, Waltham, MA, USA) with a hypersil ODS column (125 × 4 mm, 5- μ m particle, HP). Flavonoid aglycone contents were estimated using the method in Olszewska (2007) with some modifications. Fifty milligrams of eggplant leaf and fruit extracts was heated at 90°C for 2 h with 10 ml of 1 N hydrochloric acid, followed by shaking for 2 h with 10 ml of MeOH. The hydrolysate was diluted with MeOH to 25 ml using a volumetric flask and filtered through a PTFE syringe filter (13 mm, 0.45- μ m pore; Whatman, Maidstone, Kent, UK). Detection was performed at 370 nm.

Statistical analyses

Duncan's multiple range tests (DMRTs) were carried out to test for significant differences among eggplant germplasms using SAS (version 9.1; SAS Institute, Inc., Cary, NC, USA). Principal components analysis (PCA) was applied to identify the antioxidant activity and flavonoids, which were the main source of the variability, and to explain the genetic diversity in eggplant germplasm.

RESULTS

Four typical flavonoid aglycones were observed in all eggplant leaf samples, which were identified as quercetin, apigenin, kaempferol, and isorhamnetin by comparing them with the retention times of standard compounds (Fig. 2). Flavonoid aglycone concentrations in eggplant green leaves, greenish violet leaves, and fruits are compared in Fig. 3. All flavonoids of the greenish violet leaves were significantly higher than those of green leaves. The flavonoid concentration in eggplant leaves was 10 to 20 fold higher (with an average of 15.6 μ g mg⁻¹) than that of the fruit (AVG. = 0.9 μ g mg⁻¹), and apigenin and isorhamnetin were not detected in eggplant fruit, which suggests that eggplant leaves represent a potential source of natural antioxidants due to their very high flavonoid concentrations. ABTS (73.9 μ g Trolox mg⁻¹) and DPPH (53.1 μ g ASC mg⁻¹) activity, and quercetin (3.0 μ g mg⁻¹), apigenin (1.6 μ g mg⁻¹), kaempferol (8.8 μ g mg⁻¹), and total flavonoid aglycone concentrations (12.9 μ g mg⁻¹) in the early-flowering accessions (days to flowering <80 days) were found to be significantly higher than those in other accessions (Table 2). The accessions originating from 45°N showed the highest flavonoid concentrations (AVG. = 15.4 μ g mg⁻¹), followed by accessions that originated from 30°~45°N

Fig. 2. Standard and sample chromatograms of flavonoid aglycones in eggplant.

(AVG. = $13.0 \mu\text{g mg}^{-1}$), $15^{\circ} \sim 30^{\circ}\text{N}$ (AVG. = $11.0 \mu\text{g mg}^{-1}$), and $0^{\circ} \sim 15^{\circ}\text{N}$ (AVG. = $9.5 \mu\text{g mg}^{-1}$). The same patterns were also found in ABTS and DPPH antioxidant activities (Table 3). The PCA using a correlation matrix and Pearson correlation coefficients indicated that the first three principal

components (PCs) explained 79% of the total variance among accessions; PC1, PC2, and PC3 explained 40%, 25%, and 14% of the variance, respectively. Thus, based on PC1, ABTS and DPPH antioxidant activity and all flavonoid aglycones displayed relatively high contributions to the

*The same letter in each column indicates no significant difference by Duncan's multiple range test, $p < 0.05$

Fig. 3. Flavonoid contents classified by leaves and fruits in 102 eggplant germplasm.

Table 2. Flavonoid contents and antioxidant activity classified by days to flowering in germplasm of leaves of 102 eggplant accessions.

Days to flowering	Flavonoid aglycones ($\mu\text{g mg}^{-1}$)					Antioxidant activity	
	Quercetin	Apigenin	Kaempferol	Isorhamnetin	Total flavonoid	DPPH ^{y)}	ABTS ^{x)}
<~80 ($n = 26$)	$3.0 \pm 1.1\text{a}^z)$	$1.6 \pm 0.5\text{a}$	$8.8 \pm 2.5\text{a}$	0.3 ± 0.1	$12.9 \pm 3.7\text{a}$	$53.1 \pm 12.1\text{a}$	$73.9 \pm 11.2\text{a}$
81~90 ($n = 44$)	$2.0 \pm 0.6\text{b}$	$1.3 \pm 0.5\text{b}$	$6.5 \pm 1.7\text{b}$	0.4 ± 0.1	$9.5 \pm 2.8\text{b}$	$50.9 \pm 12.2\text{ab}$	$62.9 \pm 10.1\text{b}$
> 91 ($n = 32$)	$1.7 \pm 0.5\text{b}$	$1.2 \pm 0.4\text{b}$	$5.5 \pm 1.1\text{b}$	0.3 ± 0.1	$8.5 \pm 2.0\text{b}$	$46.3 \pm 13.3\text{b}$	$56.2 \pm 10.8\text{b}$

^{z)} The same letter in each column indicates no significant difference by Duncan's multiple range test, $p < 0.05$

^{y)} $\mu\text{g ASC mg}^{-1}$

^{x)} $\mu\text{g Trolox mg}^{-1}$

Table 3. Flavonoid contents and antioxidant activity classified by origin latitude in germplasm of leaves of 102 eggplant accessions.

Latitude	Flavonoid aglycones ($\mu\text{g mg}^{-1}$)					Antioxidant Activity	
	Quercetin	Apigenin	Kaempferol	Isorhamnetin	Total flavonoid	DPPH ^{y)}	ABTS ^{x)}
45°N ($n = 9$)	2.5 ± 0.5	$1.9 \pm 0.4\text{a}^z)$	$10.9 \pm 1.6\text{a}$	0.3 ± 0.2	$15.4 \pm 2.7\text{a}$	$56.5 \pm 16.3\text{a}$	$75.6 \pm 10.5\text{a}$
$30 \sim 45^{\circ}\text{N}$ ($n = 31$)	2.5 ± 0.8	$1.4 \pm 0.5\text{ab}$	$8.5 \pm 2.0\text{b}$	0.4 ± 0.1	$13.0 \pm 3.7\text{ab}$	$52.2 \pm 15.1\text{b}$	$67.1 \pm 12.3\text{ab}$
$15 \sim 30^{\circ}\text{N}$ ($n = 22$)	1.9 ± 0.6	$1.4 \pm 0.6\text{ab}$	$6.8 \pm 1.5\text{c}$	0.3 ± 0.1	$11.0 \pm 3.3\text{bc}$	$51.8 \pm 16.1\text{b}$	$66.5 \pm 17.3\text{ab}$
$0 \sim 15^{\circ}\text{N}$ ($n = 40$)	1.8 ± 0.6	$1.1 \pm 0.5\text{b}$	$6.3 \pm 1.9\text{c}$	0.3 ± 0.1	$9.5 \pm 2.8\text{c}$	$51.4 \pm 15.0\text{b}$	$60.6 \pm 12.4\text{b}$

^{z)} The same letter in each column indicates no significant difference by Duncan's multiple range test, $p < 0.05$

^{y)} $\mu\text{g ASC mg}^{-1}$

^{x)} $\mu\text{g Trolox mg}^{-1}$

total variance, whereas PC2 was characterized mainly by ABTS and DPPH antioxidant activity (Table 4). The biplot of the first two PCs, including loading of the ABTS and DPPH antioxidant activity and flavonoid aglycone content, is given in Fig. 4. This figure indicates that the 45°N group including Canada, Russia, Ukraine, and Moldova resources had high ABTS and DPPH antioxidant activity, whereas high flavonoid level resources were clustered in the 30° ~ 45°N group. The first two components accounted for 65% of the total variance in 102 eggplant accessions. IT213107 from China was recommended as a potential source of natural antioxidants due to its high ABTS (83.6 $\mu\text{g Trolox}$

mg^{-1}) and DPPH (73.6 $\mu\text{g ASC mg}^{-1}$) antioxidant activity and high total flavonoid content (21.0 $\mu\text{g mg}^{-1}$) among accessions.

DISCUSSION

The range of flavonoids occurring in plant materials is generally reported to be large, and every plant has an original and unique flavonoid profile, which makes quantification difficult. For this reason, flavonoid aglycones are frequently used to determine the total flavonoid content, which can

Fig. 4. Plot of the first and second principal components classified by the latitude of origin for 102 eggplant leaves.

Table 4. Principal components analysis of germplasm of 102 eggplant accessions, with the eigenvalues and eigenvectors of antioxidant activity and flavonoids.

Variables	Comp 1	Comp 2	Comp 3
Quercetin	-0.46	-0.37	0.27
Apigenin	-0.46	-0.27	0.18
Kaempferol	-0.32	-0.08	-0.94
Isorhamnetin	-0.46	-0.26	0.07
DPPH	-0.36	0.61	0.06
ABTS	-0.38	0.59	0.10
Contribution	40%	25%	14%
Cumulative contribution	40%	65%	79%
Eigenvalue	2.06	1.27	0.72

lower the variability (Webb and Harborne 1991). So in this study, eggplant flavonoids were investigated by calculating the flavonoid aglycone concentrations of quercetin, apigenin, kaempferol, and isorhamnetin. Quercetin has been reported to be a major flavonoid in most plant extracts (Miean and Mohamed 2001). However, in this study, eggplant leaves were found to mainly accumulate kaempferol, which accounted for more than 70% of the total flavonoids. This finding will provide valuable information for developing resources having high kaempferol concentrations. A previous study indicated that the temperature of the place of collection showed a negative correlation with antioxidant activities and flavonoids (Åkerstöm *et al.* 2010). Similar results were found in our study; accessions originating from 45° ~ 60°N displayed the highest flavonoid concentrations, followed by accessions originating, in order, from 30° ~ 45°N, 15° ~ 30°N, and 0° ~ 15°N. This result concurred with the PCA reported earlier in this paper, which showed that the 0 ~ 15°N group and 45° ~ 60°N group were different (Fig. 4). These results can be explained by the other finding, namely that either low latitude or high temperature at the geographical origin may lead to low antioxidant activity (Ghasemi *et al.* 2011; Piao *et al.* 2013). Previous studies reported that EtOH extracts of eggplant leaves had higher DPPH radical scavenging activity and total flavonol content than did their fruits (Jung *et al.* 2011; Madukwe *et al.* 2013). Similarly, in this study, eggplant leaves had significantly higher flavonoid concentrations than their fruit had (Fig. 3). From these results, it can be concluded that eggplant leaves represent a potential source of natural antioxidants due to their very high flavonoid concentrations. The biplot (Fig. 4) indicates that the 45°N group had high ABTS and DPPH antioxidant activity, whereas high flavonoid-level resources were clustered in the 30° ~ 45°N group. Thus, this plot can be used to categorize genetic entities in making eggplant breeding decisions.

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REFERENCES

- Akerstöm A, Jaakola L, Bang U, Jäderlund A. 2010. Effects of latitude-related factors and geographical origin on anthocyanidin concentrations in fruits of *Vaccinium myrtillus* L. *Agric. Food Chem.* 58: 11939-11945.
- Arnao MB. 2000. Some methodological problems in the determination of antioxidant activity using chromogen radicals: a practical case. *Trends Food Sci. Technol.* 11: 419-421.
- George R. 1985. Vegetable seed production. London and New York, pp. 223-229.
- Ghasemi K, Ghasemi Y, Ehteshamnia A, Nabavi SM, Nabavi SF, Ebrahimzadeh MA, Pourmorad F. 2011. Influence of environmental factors on antioxidant activity, phenol and flavonoids contents of walnut (*Juglans regia* L.) green husks. *J. Med. Plant Res.* 5: 1128-1133.
- Jung EJ, Bae MS, Jo EK, Jo YH, Lee SC. 2011. Antioxidant activity of different parts of eggplant. *J. Med. Plant Res.* 5: 4610-4615.
- Konys E. 1993. Warzywa psiankowate. Gapiński M., (red.), Warzywa mało znane i zapomniane. PWRiL, Poznań, pp. 78-82.
- Lawande KE, Chavan JK. 1998. Eggplant (Brinjal), handbook of vegetable science and technology. Production, composition, storage and processing. New York, pp. 225-244.
- Lee DJ, Lee JY. 2004. Antioxidant activity by DPPH assay. *Korean J. Crop Sci.* 49: 187-194.
- Madukwe EU, Ani PN, Ezeonu C. 2013. Comparative Analysis of Antioxidant Vitamins in Extracts of Three Vegetables among the Commonly Consumed in South-Eastern Nigeria. *Global Res. Anal.* 2: 94-95.
- Miean KH, Mohamed S. 2001. Flavonoid (myricetin, quercetin, kaempferol, luteolin, and apigenin) content of edible tropical plants. *J. Agric. Food Chem.* 49: 3106-3112.
- Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Fazelian M, Eslami B. 2009. In vitro antioxidant and free radical scavenging activity of *Diospyros lotus* and *Pyrus boissieriana* growing in Iran. *Pharmacog. Mag.* 5: 122-126.
- Olszewska M. 2007. Quantitative HPLC analysis of flavonoids and chlorogenic acid in the leaves and inflorescences of *Prunus aerotina* Ehrh. *Acta Chromatogr.* 19: 253-269.
- Piao XM, Jang EK, Chung JW, Lee GA, Lee HS, Sung JS, Jeon YA, Lee JR, Kim YG, Lee SY. 2013. Variation in antioxidant activity and polyphenol content in tomato

- stems and leaves. Plant Breed. Biotechnol. 1: 366-373.
- Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorisation assay. Free Rad. Biol. Med. 26: 1231-1237.
- Saddiqe Z, Naeem I, Mughal T, Taskeen A, Mubeen H. 2011. Characterization of flavonoid aglycones in aerial parts of *Hypericum oblongifolium* L. Asian J. Chem. 23: 939-940.
- Webb ME, Harborne JB. 1991. Leaf flavonoid aglycone patterns and sectional classification in the genus *Vicia* (Leguminosae). Biochem. Syst. Ecol. 19: 81-86.