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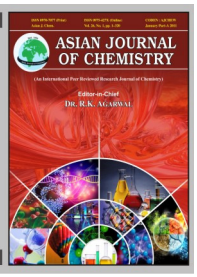

Accumulation of Free Amino Acids in Different Organs of Green and Red Mustard Cultivars

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Mustard (*Brassica juncea*) is an important oil crop in the Brassicaceae family, containing a variety of amino acids. The present study investigated the variation in amino acids among the different tissues of green and red mustard cultivars. The levels of these amino acids varied significantly among tissues and between cultivars. A markedly higher quantity of all of the amino acids was observed in young leaf tissue, compared to other parts of the plant, in both cultivars. Among the free amino acids, concentrations of leucine, lysine, arginine, alanine, valine and phenylalanine were much higher than those of any other amino acids. In green mustard cultivars, young leaf tissue contained 3.6, 1.3, 17.5 and 63.2 times greater total amino acid content than stem, flower, seed and root, respectively. Young leaf tissue of red mustard cultivars contained 11.56, 1.1, 9.6 and 35.1 times greater total amino acid content than stem, flower, seed and root, respectively. Considering the total amino acid contents from all organs, the green cultivar contained 1.3 times more amino acids than the red cultivar. Present results demonstrate that mustard cultivars contain varying amounts of free amino acids among plant tissues and suggest that these cultivars have excellent potential to serve as a source of amino acids in vegetable plants.

Keywords: Free amino acids, Cultivars, Young leaf, Flower, Mustard.

INTRODUCTION

The family Brassicaceae includes plant species that represent major vegetable crops and that can play an important role in maintaining a well-balanced diet[1]. Seeds are pressed to make mustard oil and edible leaves can be eaten as salads or mixed with other salad greens[2]. An inverse correlation between consumption of Brassicaceae and risk of cancer has been reported[3]. Globally, mustard (*Brassica* spp.) crops are considered to be the third most important sources of vegetable oil after soybean and groundnut[4]. Among the different species, *Brassica juncea* (L.) Czern. is an important oil producing crop and has been cultivated in Asia and Europe for thousands of years.

Most (65 %) of the human requirement for protein comes from plant sources, with 45-50 and 10-15 % from cereals and legumes or vegetables, respectively[5]. In the average diet, the importance of plant proteins varies from highly developed regions (where animal production is particularly abundant) to the least developed regions (where animal proteins are scarce). However, the tendency toward consumption of proteins from legume and vegetable sources is increasing throughout the world.

Awareness about food composition has increased among consumers and comprehensive information beyond that available in food composition tables is often demanded. Information on amino acid composition is limited in such tables and when it is included it is primarily provided in terms of free and bound amino acids. The most important functions of amino acids include their role as building blocks of proteins. Numerous researchers have reported on antioxidant effects of several amino acids[6-9]. Free amino acids are needed in secondary plant metabolism and in the biosynthesis of compounds such as glucosinolates and phenolics that play important roles, either directly or indirectly, in plant-environment interactions and human health[10].

Many amino acids exist in nature, although not all are necessary for human health. Approximately twenty four different amino acids are vital to human nutrition[11,12]. Mustard is an important source of amino acids, but insufficient information exists regarding the composition of amino acids in mustard cultivars. In this study, we investigated the content of free amino acids in different organs of two cultivars (green and red) of mustard.

EXPERIMENTAL

B. juncea seeds were purchased from Asia Seed Co., Ltd (Seoul, Korea). Seeds of both cultivars were grown under light (16 h) and dark (8 h) conditions in the greenhouse (25 ºC and 50 % humidity) at Chungnam National University (Daejeon, Korea) beginning March 28, 2012. Young leaves were harvested 3 weeks after sowing and other organs (stem, flower and root) were collected after flowering. Collected samples were freeze-dried at -80 ºC for at least 72 h. Dried samples were ground into a fine powder using a mortar and pestle for analysis by high-performance liquid chromatography (HPLC).

Trichloroacetic acid (TCA, 99.0 %) was obtained from Samchun Pure Chemical Co., Ltd. (Pyeongtaek, Korea). Sixteen amino acid standards and 4 amino acid supplements were obtained from Agilent Technologies (Waldbronn, Germany). Vitamin U (DL-methionine methylsulfonium chloride) standards and sodium phosphate monobasic monohydrate (NaH_2PO_4) were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade acetonitrile and methanol were supplied by J.T. Baker (Phillipsburg, NJ, USA). Ultrapure water having a resistivity of 18.2 MΩ/cm was produced by PureLab Option from ELGA LabWater (Model LA 621, Marlow, UK).

Extraction and HPLC analysis of free amino acids: One hundred milligrams of freeze-dried plant powder was weighed into a 2 mL Eppendorf tube, to which 1.2 mL of 5 % (v/v) trichloroacetic acid (TCA) solution was then added. After vortexing, the mixture was allowed to stand for at least 1 h at room temperature and was then centrifuged at 15,000 rpm, at 4 ºC for 15 min. The supernatant was filtered through a 0.45 µm pore-size hydrophilic PVDF Syringe Filter (Ø 13 mm, Cat. no. 6779-1304, Whatman Int. Ltd.) into an HPLC-vial. HPLC analysis of free amino acids was conducted according to the 'Rapid, accurate, sensitive and reproducible HPLC analysis of amino acids analysis' using Zorbax Eclipse-AAA Columns and the Agilent 1100 HPLC (http://www.chem.agilent.com/Library/chromatograms/ 59801193.pdf). Briefly, 20 different free amino acids were determined using Agilent Technologies 1200 series HPLC system equipped with Zorbax Eclipse AAA analytical column (150 mm × 4.6 mm i.d., particle size 5 µm) and Zorbax Eclipse AAA guard column (12.5 mm × 4.6 mm i.d., 5 µm, 4-Pack). The HPLC conditions were set at 338 nm wavelength, 40 ºC oven temperature and 2 mL/min flow rate. The mobile phase consisted of 40 mM NaH_2PO_4 (pH 7.8, solvent A) and CH_3CN:MeOH:H_2O (45:45:10, v/v/v) (solvent B). The gradient programs were as follows: a linear step from 0-57 % of solvent B from 1.9-21.1 min, from 57-100 % of solvent B from 21.1-21.6 min, isocratic conditions with 100 % solvent B from 21.6-25.0 min, followed by a rapid drop to 0 % solvent B at 25.1 min and then isocratic conditions with 0 % solvent B to 30 min (total 30 min). A 50 pmol/µL (0.05 mM) solution of the 20 amino acids was prepared as the standard. The quantification of free amino acids was based on HPLC peak areas and calculated as equivalents of standard compounds and all amounts were expressed as milligram per 100 g fresh weight (FW). All samples were run in triplicates.

RESULTS AND DISCUSSION

Free amino acids in young leaf tissue: From the analysis of both green and red mustard cultivars, 20 different free amino acids were recorded (Table-1). A markedly higher amount of all of the amino acids was observed in the young leaf tissue of both mustard cultivars compared to other parts of the plant (Table-1). Among the free amino acids, the levels of leucine, lysine, arginine, alanine, valine and phenylalanine were much higher than those of any other compounds (Table-1). Although the variation in amino acid content of leaf tissue between the green and red cultivars was relatively minor, the green cultivar contained larger quantities of all but 3 amino acids and its total amino acid content was 1.3 times greater than that of the red cultivar. Among the amino acids, the accumulation of leucine was the highest (152.83 and 126.06 mg/100 g FW, green and red cultivars, respectively) and vitamin U was the lowest (6.68-6.80 mg/100 g FW, respectively, to green and red cultivar).

Free amino acids in stem tissue: The same 20 amino acids identified in young leaf tissue were also identified during the analysis of stem tissue in both mustard cultivars (Table-1). On the basis of total free amino acid content in different tissues of mustard cultivars, stem ranked third after young leaf and flower. The trend of amino acid content in stem tissue was different from that of young leaf tissue. Variability between cultivars was higher in stem compared to young leaf. Variation in the content of lysine, leucine, isoleucine, phenylalanine and tyrosine in the green cultivar was much higher than in the red cultivar. The green mustard cultivar displayed 4.3 times higher total amino acid content than the red cultivar. Among the amino acids, lysine was the most abundant (40.53 mg/100 g FW in green cultivar). Of the 20 amino acids identified, glutamate was the only one with a higher concentration in the red cultivar (7.07 mg/100 g FW in red and 2.49 mg/100 g FW in green).

Free amino acids in flower tissue: The same 20 amino acids identified in young leaf and stem tissues were found in the flower tissue of each mustard cultivar (Table-1). Flowers contained the second highest total amino acid content of the tissues examined. The trend in amino acid content in flowers was the same as that in young leaves and differed little between the two mustard cultivars. Glutamine content was much higher in flower tissue than in the other plant tissues, in both cultivars (419 and 353.18 mg/100 g FW in green and red cultivars, respectively). Both cultivars showed almost the same total amino acid content, with just slightly higher concentrations in the green compared to the red cultivar. The green cultivar contained higher concentrations of 10 amino acids (glutamate, asparagine, serine, glutamine, threonine, arginine, tyrosine, tryptophan, phenylalanine and lysine) and the red cultivar contained higher concentrations of aspartate, vitamin U, histidine, glycine, alanine, cystine, valine, methionine, isoleucine and leucine.

Free amino acids in the seed: As for the other plant tissues, the same 20 amino acids were identified in seeds of both mustard cultivars (Table-1), with the exception of vitamin U, which was not found in seeds of the green cultivar. Seed contained lower concentrations of amino acids than all of the other plant tissues except for roots. However, tyrosine concentrations in

TABLE-1
VARIATION OF FREE AMINO ACID CONTENTS (mg/100 g FRESH WEIGHT) IN DIFFERENT TISSUES (YOUNG LEAF, STEM, FLOWER, SEED, AND ROOT) OF GREEN AND RED MUSTARD CULTIVARS

Amino acids	Young leaves		Stem		Flower		Seed		Root	
	Green mustard	Red mustard	Green mustard	Red mustard	Green mustard	Red mustard	Green mustard	Red mustard	Green mustard	Red mustard
Aspartic acid	57.62±0.52	36.47±0.23	16.47±1.26	6.48±0.67	37.04±3.81	41.67±2.02	2.93±0.05	8.87±0.07	2.85±0.38	3.48±0.34
Glutamic acid	17.73±1.59	16.34±0.87	2.49±0.40	7.07±0.1	29.32±4.49	28.18±4.21	3.45±0.05	0.64±0.18	0.62±0.34	2.15±0.82
Asparagine	42.19±1.21	30.02±1.47	11.43±0.08	1.49±0.12	112.23±6.22	98.94±5.63	6.27±0.07	8.87±0.04	0.40±0.13	0.60±0.06
Serine	86.59±2.30	62.75±1.53	27.42±1.74	7.23±0.10	66.37±2.78	65.17±2.46	0.89±0.02	0.45±0.02	1.07±0.56	1.79±0.06
Vitamin U	6.68±0.80	6.80±0.23	3.46±0.32	1.77±0.06	21.74±0.72	23.08±0.44	ND[a)]	0.28±0.08	0.37±0.11	0.43±0.04
Glutamine	69.50±3.52	52.29±2.32	29.24±1.50	13.07±1.28	419.00±9.85	353.18±13.14	0.80±0.01	0.54±0.05	4.27±0.35	3.58±0.16
Histidine	36.11±2.40	28.22±0.74	8.51±1.13	1.30±0.03	29.23±2.87	34.50± 3.48	0.57±0.02	0.72±0.04	0.95±0.30	0.90±0.32
Glycine	70.01±2.41	45.98±1.40	11.44±0.55	24.85±0.92	95.05±3.95	112.74±5.72	0.73±0.01	1.07±0.02	3.84±0.23	8.35±0.35
Threonine	83.06±1.20	62.13±1.26	21.39±1.27	4.99±0.19	41.10±1.09	34.30±0.64	0.78±0.01	0.57±0.10	1.52±0.11	2.27±0.10
Arginine	124.13±3.24	97.92±3.03	35.21±2.31	3.56±1.41	41.18±2.05	32.75±2.96	2.71±0.02	3.11±0.10	1.76±0.03	1.54±0.31
Alanine	108.92±2.17	73.99±1.09	30.76±1.14	7.07±0.38	58.29±1.37	67.05±1.44	1.89±0.00	0.96±0.01	0.95±0.01	1.33±0.04
Tyrosine	75.14±1.38	74.53±2.73	24.04±3.08	0.97±0.05	11.97±2.55	9.40±1.71	52.49±0.27	69.48±0.23	0.28±0.04	0.26±0.01
Cystine	32.28±2.29	33.01±0.84	19.78±1.80	5.70±0.71	20.23±0.77	24.69±0.52	3.02±0.53	6.42±0.20	3.23±0.36	3.00±0.20
Valine	107.35±1.95	65.47±2.19	28.96±1.03	3.04±0.06	30.53±0.61	35.32±0.79	0.58±0.03	0.99±0.13	0.52±0.02	0.70±0.01
Methionine	31.74±2.53	34.33±2.15	8.26±0.28	1.36±0.40	6.04±1.31	6.37±1.20	0.13±0.00	0.73±0.04	ND	0.21±0.00
Tryptophan	28.24±4.66	27.70±3.77	6.78±0.64	1.44±0.00	14.26±1.50	11.88±1.57	2.20±0.01	4.24±0.05	ND	ND
Phenylalanine	101.51±2.07	84.68±1.78	27.00±1.10	1.94±0.48	15.40±0.94	10.54±0.33	1.39±0.10	1.90±0.17	ND	0.53±0.42
Isoleucine	84.79±1.18	55.69±1.17	23.55±1.23	1.99±0.24	21.02±0.52	24.89±0.43	0.43±0.02	0.60±0.21	ND	0.61±0.27
Leucine	152.83±3.12	126.06±1.98	36.93±2.03	1.32±0.01	11.22±0.58	11.93±0.42	0.74±0.13	1.05±0.04	0.27±0.02	0.26±0.01
Lysine	151.66±4.74	116.76±3.45	40.53±3.90	1.17±0.02	19.70±0.55	16.17±0.41	0.62±0.02	0.60±0.01	0.34±0.04	0.26±0.01
Total	1468.05	1131.15	413.68	97.83	1100.90	1042.75	83.82	117.63	23.23	32.24

the seeds of both cultivars were much higher than those of the other tissues, except for young leaf.

Free amino acids in root tissue: Trace amounts of various amino acids were detected in the roots of both mustard cultivars. The concentrations of amino acids were much lower than those of the other plant tissues. No methionine, phenylalanine, or isoleucine were detected in the roots of the green cultivar and tryptophan was not detected in either cultivar.

The results of this study show that the young leaf tissue of mustard contained far greater amino acid contents than in other plant parts. In green mustard cultivars, young leaf tissue contained 3.6, 1.3, 17.5 and 63.2 times higher total amino acid content than stem, flower, seed and root, respectively. The young leaf tissue of red mustard cultivars contained 11.56, 1.1, 9.6 and 35.1 times higher total amino acid content than the stem, flower, seed and root, respectively. Concentrations of proline in flower buds and flowers reported by Xue *et al.*[13] were significantly higher than those in vegetative organs of *Brassica napus*, consistent with our results. We also found that amino acids content varied between the cultivars. It is notable that similar cultivars do not follow precisely the same trends in amino acids content in different tissue types. For example, both cultivars contained much higher concentrations of glutamine in the flowers compared to the other tissues. Considering total amino acid contents from all organs, the green cultivar contained 1.3 times more amino acid than the red cultivar. Variation in amino acids among cultivars was reported previously by Kim *et al.*[14] , who found that, among all the amino acids isolated from *Momordica charantia*, arginine was present in remarkably high quantities while cysteine and methionine showed the lowest concentrations. Similar results regarding variation of amino acids content in mustard cultivars were studied in this study.

Conclusion

The levels of amino acids in different parts of mustard varied widely. Young leaf contained the highest levels of all the amino acids. Such contents also varied between the cultivars. The results of this study demonstrate that green and red mustards exhibit varying amount of amino acids accumulation among different organs of the cultivar showing higher amount of accumulation in the green cultivar. Cultivar-specific amino acid profiles have a high potential as a good source in vegetable plants.

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REFERENCES

1. F. Ferreres, C. Sousa, P. Valentao, R.M. Seabra, J.A. Pereira and P.B. Andrade, *Food Chem.,* **101**, 549 (2007).
2. D. Zohary and M. Hopf, Domestication of Plants in the Old World, University Press, Oxford, edn 3 (2000).
3. B. Kusznierewicz, A. Bartoszek, L. Wolska, J. Drzewiecki, S. Gorinstein and J. Namiesnik, *LWT-Food Sci. Technol*., **41**, 1 (2008).
4. S.I. Warwick, A. Francis and I.A. Al-Shehbaz, *PI Syst. Evol.,* **259**, 249 (2006).
5. S. Mahe, N. Gausseres and D. Tome, *Grain Legumes (AEP)*, **7**, 15 (1994).
6. R. Marcuse, *J. Am. Oil Chem. Soc.*, **39**, 97 (1962).
7. H.Y. Fu, D.E. Shieh and C.T. Ho, *J. Food Lipids*, **9**, 35 (2002).
8. S.H. Sha and J. Schacht, *Hearing Res.*, **142**, 34 (2000).
9. M.E. Carlotti, M. Gallarate, M.R. Gasco, S. Morel, A. Serafino and E. Ugazio, *Int. J. Pharm.*, **155**, 251 (1997).
10. M.H. Gomes and E. Rosa, *J. Sci. Food Agric.*, **81**, 295 (2000).
11. G.S. Gilani, C. Xiao and N. Lee, *J. AOAC Int.*, **91**, 894 (2008).
12. D.J. Millward, D.K. Layman, D. Tomé and G. Schaafsma, *Am. J. Clin. Nutr.,* **87**, 1576 (2008).
13. X. Xue, A. Liu and X. Hua, *BMB reports*, **42**, 28 (2009).
14. Y.K. Kim, H. Xu, N.I. Park, Ho, Boo, S.Y. Lee and S.U. Park , *J. Med. Plants Res*., **3**, 897 (2009).