

Research Article	Pak-Euro Journal of Medical and Life Sciences	
DOI: 10.31580/pjmls.v8i2.3368	Copyright © All rights are reserved by Corresponding Author	
Vol. 8 No. 2, 2025: pp. 407-412		
www.readersinsight.net/pjmls	Revised: June 08, 2025	Accepted: June 22, 2025
Submission: March 28, 2025	Published Online: June 30, 2025	

VARIATION IN RUTIN AND AMINO ACID CONTENT AMONG NATIVE COMMON BUCKWHEAT (*Fagopyrum esculentum* MOENCH) GENOTYPES OF BALTISTAN

Ishtiaq Hussain^{1,2*}, Asghari Bano², Faizanullah³, Ihsan-ul-Haq⁴, Naeem Khan⁵, Asia Nosheen

¹Department of Botany, University of Baltistan, Skardu, Pakistan

²Department of Plant Sciences, Quaid-i-Azam University, Islamabad, Pakistan

³Department of Botany, UST, Bannu, KPK, Pakistan

⁴Department of Pharmacy, Quaid-i-Azam University, Islamabad, Pakistan

⁵Department of Agronomy, University of Florida, Gainesville FL 32611, USA

⁶Department of Bioscience, COMSATS (CUI), Islamabad, Pakistan

*Correspondence author: Ishtiaq Hussain. E. mail: ishtiaq.mondoo@uobs.edu.pk



Abstract

Common buckwheat is grown in high-altitude mountains of Pakistan (Gilgit-Baltistan), is used as a functional food around the world and is considered an important part of the human diet. Matured seeds of twelve (12) local genotypes grown at Khaplu, Ganche, were collected and ground into fine powder after removing hulls. Rutin and amino acid content among these genotypes were determined by employing the High Performance Liquid Chromatography (HPLC) method for rutin and an automatic amino acid analyzer for amino acid. The highest rutin content (32.4 mg/100g) in flour was observed by local genotype BWS-14, followed by genotype BWK-3 (25.3mg/100g), and the lowest rutin content (5.8 mg/100g) was reported by genotype BWSN-05. A significant correlation was observed between genotype BWZ-08 and BWD-1 ($r= 0.98$) at $P < 0.05$ for Amino Acid content. Whereas the amino acid content in selected common buckwheat genotypes exhibited a wide variation. The local genotype BWSU-10 and BWA-7 were observed as a good source of lysine and arginine content. Diversity in rutin as well as amino acid contents among native genotypes revealed a potential source of nutraceutical and an important alternative food crop for mountain communities of Baltistan. All these genotypes showed diversity in metabolites, and such varietal differences revealed the worth of conservation of native germplasms in local and national gene banks. Furthermore, this crop can be cultivated at a commercial scale to meet the industrial demand for nutraceutical food in Pakistan.

Keywords: Amino acid, Buckwheat, Gilgit-Baltistan, Nutraceutical, Rutin

INTRODUCTION

Buckwheat, a pseudo cereal crop is used mainly for human consumption. Among many species of buckwheat, common buckwheat (*Fagopyrum esculentum* Moench) has been a commonly grown as a food crop based on their diet and cultural uses in cold regions of the Indo-Himalayas (1), Central Karakorum (2, 3) and Kashmir regions (4). Buckwheat has received increasing interest in past few decades due to the medicinal, functional properties, short growth period and high adaptability to grow under adverse environments. (3, 5, 6) and exhibits morphological changes, polyphenolic contents and yield under various environments (7, 8). Antioxidant and biochemical diversity among such varieties and genotypes of buckwheat have been reported previously (9, 10).

Common buckwheat being an underutilized crop possesses nutritional properties and health benefits for human (6). Its seed, flour and whole plant is abundant in nutrients, vitamins, protein, amino acids, minerals, phenolic compounds rutin and antioxidant properties (11-13) and holds great nutritive value due to thiamin binding proteins (14). Unlike common cereals which are deficient in lysine, buckwheat is a richer source (15). Arginine (an immune stimulant and a wound-healing agent) has also been reported richer in buckwheat (16). Being only source of rutin among cereals and pseudocereal crops and polyphenols with health-promoting effects (13, 17), the rutin content varies from 0.01% to 8.23% in various parts



depending on growth stages and different genotype (5, 7, 18). Such variations in seeds were attributed to products, plant parts and growth stages (19-21) and capable to grow in multiple buckwheat producing countries (21).

In mountain areas of Pakistan (Gilgit-Baltistan) buckwheat is widely cultivated after barley and used as food as well as medicinal purpose. Local genotypes with respect to nutraceutical properties and rutin content need to be investigated for future breeding program. The present study aims to investigate genotypic variation with respect to rutin and amino acid composition and identification of better yielding genotype for selection varietal development program.

METHODOLOGY

For collection and preparation of samples, the matured seeds of twelve (n=12) local genotypes grown in field at Khaplu, Ganche (2200 masl, N35° 09.163E76° 20.132), were collected and ground to fine powder after removing hulls. These local genotypes (n=12) were designated as BWD-1 BWS-2 BWK-3 BWB-4 BWSN-5 BWSI-6 BWA-7 BWZ-8 BWSU-10 BWY-11 BWST-14 BWM-16. The rutin extracts were analyzed through HPLC system (Agilent 1200, Germany) using C-18 column (4.6 × 250 mm, 5 µm Supelco USA). The identification of rutin was measured at wavelength of 360nm by adopted method with slight modifications (22). Rutin was used as standard (Sigma Chemicals Co. Ltd. USA) and expressed as mg/100g rutin.

The amino acid content of buckwheat seed samples was quantified according to the method of (23) by using automatic amino acid analyzer (Hitachi High- Technologies Corporation, Tokyo, Japan) with a 4.6(ID) × 60mm ion exchange column). Quantification was done with reference to the peak area of the corresponding amino acid standard through automatic amino acid analyzer, Biochrome using cation exchange chromatography. Amino acid concentrations were calculated in g/100g.

STATISTICAL ANALYSES

The data were subjected to statistical analyses, including descriptive statistics, cluster analysis, and correlation coefficients. To evaluate the genetic relationships among genotypes, the similarity coefficients were calculated using a standard method (24). Cluster analysis was performed using the unweighted pair-group method with arithmetic mean (UPGMA). Additionally, Pearson correlation coefficients among genotypes were computed (25). All statistical analyses were conducted using the software packages STATISTICA (StatSoft Inc., version 5.5) and Statistix (version 8.1, USA).

RESULTSs

Quantitative analysis of rutin by HPLC among local buckwheat genotypes showed wide variation in results (Fig. 1).

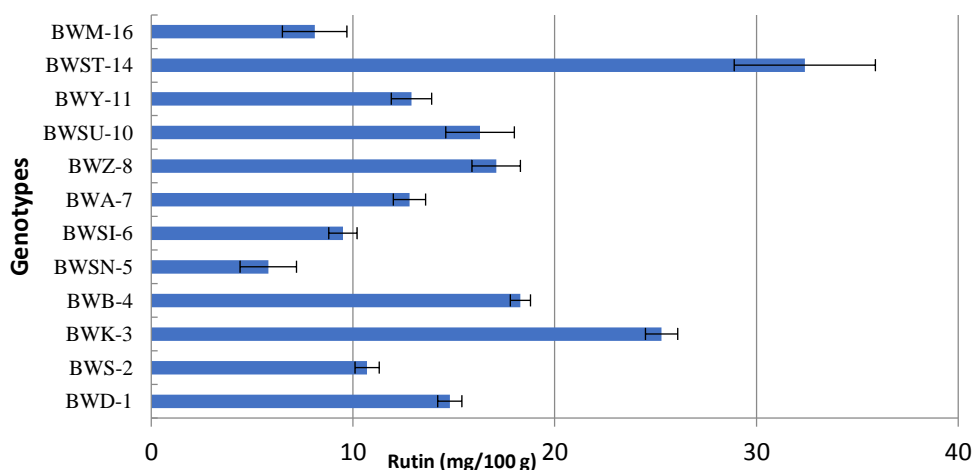


Fig. 1. Rutin content in grains of local buckwheat genotypes

For quantification of rutin by HPLC DAD system showed linear graph for standard ($R^2 = 0.99$). The highest rutin content (32.4 mg/100g) was noticed in genotype BWST-14 followed by BWK-3 (25.3 mg/100g).

Whereas, the genotypes BWSN-5 and BWM-16 were found to be a poor source of rutin contents. Correlation results among genotypes showed highly significant at $P < 0.05$ between BWB-4 and BWSI-6 ($p=0.002$) whereas negative significant correlation was observed between genotypes BWK-3 and BWM-16 ($p=0.036$) (Table I).

Table I. Correlation among local genotypes for amino acid

Genotypes	BWD-1	BWS-2	BWK-3	BWB-4	BWSN-5	BWSI-6	BWA-7	BWZ-8	BWSU-10	BWY-11	BWST-14	BWM-16
BWD-1	1.00											
BWS-2	0.51	1.00										
BWK-3	0.94	0.41	1.00									
BWB-4	0.84	0.42	0.89	1.00								
BWSN-5	0.93	0.53	0.95	0.91	1.00							
BWSI-6	0.92	0.37	0.91	0.85	0.93	1.00						
BWA-7	0.95	0.39	0.90	0.86	0.90	0.90	1.00					
BWZ-8	0.98	0.41	0.97	0.87	0.95	0.95	0.95	1.00				
BWSU-10	0.42	0.18	0.44	0.51	0.43	0.40	0.51	0.41	1.00			
BWY-11	0.36	0.16	0.38	0.34	0.32	0.29	0.33	0.36	0.01	1.00		
BWST-14	0.65	0.95	0.57	0.52	0.64	0.47	0.53	0.56	0.14	0.26	1.00	
BWM-16	0.86	0.49	0.82	0.81	0.89	0.89	0.85	0.86	0.61	0.32	0.51	1.00

*Marked correlations are significant at $p < .050$

A strong but negative correlation was also found between genotypes BWK-3 and BWY-11 ($r=-0.0385$, $p < 0.05$). The UPGMA cluster analysis by Euclidean distances for 16 amino acids in all genotypes separated 07 genotypes in group-I and the remaining 05 genotypes in the group-II showing the maximum number of genotypes in the group-I. Genotypes of cluster group II (BWSU-10, BWY-11, BWM-16) have higher proline, aspartic acid, glycine and lysine content whereas cluster group I (BWK-3, BWB-4, BWA-7, BWST-14) have higher histidine, arginine, glutamic acid and other amino acid as well as higher rutin content (Fig. 2).

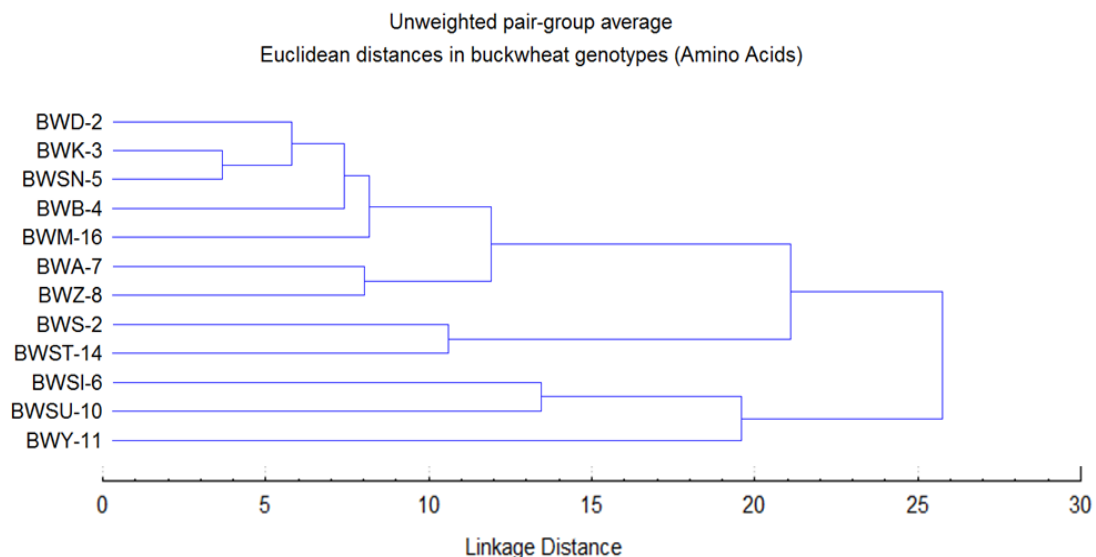


Fig. 2. Dendrogram (UPGMA) by clusters analysis for buckwheat genotypes based on amino acid content

Variations were observed for amino acids among these buckwheat genotypes. Aspartic acid content of the genotypes ranged from 0.5 to 2.4 (g/100 g protein). Aspartic acid content was higher in BWM-16 followed by BWB-4, BWA-7 and BWSU-10. Threonine content of the genotypes ranged from 0.5 to 4.1 (g/100 g protein) and was higher in BWY-11. Serine content of the genotypes ranged from 0.9 to 10 (g/100 g protein) with mean value 3.71 ± 0.90 (g/100 g protein) and 6.65 ± 0.96 (g/100 g protein) for glutamic acid ranging from 4 to 14 (g/100 g protein) across all genotypes. Similarly, mean proline, glycine, alanine, valine, isoleucine and leucine content in all the genotypes was 0.76 ± 0.10 , 3.24 ± 0.29 , 0.24 ± 0.07 , 2.34 ± 0.16 , 3.3 ± 0.22 and 0.97 ± 0.18 g/100 g protein respectively (Table II). Maximum proline content was recorded in BWY-11, Glycine in BWA-7 and

methionine in BWSI-6 cxd (Table III). The average value for phenylalanine and histidine was 0.44 ± 0.10 and 0.64 ± 0.10 (g/100 g protein) respectively (g/100 g protein). The range of NH_3 (ammonia) in all genotypes of buckwheat was 1 to 3.2 with mean value 1.79 ± 0.19 (g/100 g protein). Histidine in all genotypes was ranged from 0.1 to 1.2 (g/100 g protein). The highest content of histidine was found in BWST-14 followed by BWA-7 (Table 2). For lysine it was 5.10 ± 0.25 ranging from 3.3-6.3 (g/100 g protein). The highest lysine content was found in BWSU-10 (6.32 g/100g protein) whereas BWM-16 exhibited the lowest lysine content (3.3 g/100 g protein). Arginine content in all genotypes was ranged from 6.8 to 11.5 (g/100 g protein) with mean value 9.0 ± 0.47 (g/100 g protein). Minimum arginine content was recorded in BWD-1 and maximum arginine was found in BWA-7 followed by BWSU-10.

Table II. Descriptive statistics of amino acid contents (g/100g protein) across all genotypes

Amino acid	Min.	Max.	Mean \pm SE	Variance	SD
Aspartic acid (Asp)	0.5	2.4	1.45 \pm 0.18	0.38	0.61
Threonine (Thr)	0.5	4.1	1.425 \pm 0.27	0.89	0.95
Serine (Ser)	0.9	10.0	3.71 \pm 0.90	9.72	3.12
Glutamic acid (Glu)	4.0	14.0	6.65 \pm 0.96	10.97	3.31
Proline (Pro)	0.3	1.4	0.76 \pm 0.10	0.13	0.36
Glycine (Gly)	2.4	5.7	3.24 \pm 0.29	0.98	0.99
Alanine (Ala)	0.2	0.9	0.42 \pm 0.07	0.05	0.23
Valine (Val)	1.6	3.4	2.34 \pm 0.16	0.29	0.54
Methionine (Met)	0.39	1.5	1.18 \pm 0.13	0.20	0.45
Isoleucine (Ile)	2.1	4.4	3.3 \pm 0.22	0.57	0.76
Lecine (Leu)	0.3	2.1	0.975 \pm 0.18	0.37	0.61
Phenylalanine (Phe)	0.1	1.3	0.44 \pm 0.10	0.11	0.33
Histidine (His)	0.1	1.2	0.64 \pm 0.10	0.11	0.34
Lysine (Lys)	3.3	6.3	5.10 \pm 0.25	0.77	0.88
Ammonia (NH_3)	1.0	3.2	1.79 \pm 0.19	0.42	0.64
Arginine (Arg)	6.8	11.5	9.0 \pm 0.47	2.67	1.63

*Min =Minimum, Max = Maximum, SE = Standard error, SD = Standard deviation

Table III. Quantities of sixteen amino acids (g/100g protein) across nine local buckwheat genotypes

	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Val	Met	Ile	Leu	Phe	His	Lys	NH_3	Arg
BWD-1	1.7	1.8	2.5	4.7	0.5	2.4	0.3	2.8	1.5	4.4	0.7	0.4	0.7	4.9	1.4	6.84
BWS-2	0.8	0.6	9.3	4.0	0.3	4.3	0.2	1.9	1.41	2.6	0.6	0.1	0.1	5.5	1.7	9.0
BWK-3	1.4	1.4	1.5	5.2	0.6	3.6	0.2	2.3	0.46	2.8	1.5	0.2	0.7	4.72	1.0	7.0
BWB-4	2.0	1.4	1.6	5.9	0.5	3.2	0.3	1.7	1.35	2.1	0.3	0.1	0.1	4.32	2.7	9.5
BWSN-5	1.2	1.0	2.1	5.5	0.8	2.5	0.4	2.1	0.39	3.2	1.1	0.5	0.8	6.12	1.4	9.2
BWSI-6	1.0	1.1	0.9	12	0.4	2.7	0.5	2.5	1.54	3.6	0.4	0.3	0.8	4.44	1.8	8.88
BWA-7	2.1	1.5	5.9	4.1	0.9	5.7	0.7	1.6	1.4	4.2	0.7	0.5	1.0	4.59	2.2	11.5
BWZ-8	1.4	0.5	4.8	5.2	1.3	2.7	0.2	2.8	0.5	4.4	2.1	1.3	0.7	6.1	1.4	9.4
BWSU-10	2.1	0.7	2.1	14	0.5	2.9	0.2	3.4	1.4	2.6	1.5	0.3	0.8	6.32	1.9	11.32
BWY-11	0.8	4.1	2.1	9.5	1.4	3.9	0.9	2.3	1.39	2.8	1.8	0.3	0.5	5.2	3.2	10.48
BWST-14	0.5	1.8	10	5.0	0.8	2.5	0.6	1.9	1.5	3.5	0.5	0.8	1.2	4.43	1.1	7.2
BWM-16	2.4	1.2	1.8	4.7	1.1	2.5	0.5	2.8	1.4	3.4	0.5	0.4	0.3	3.39	1.7	7.21
Mean	1.45	1.425	3.72	6.65	0.75	3.24	0.42	2.34	1.18	3.3	0.97	0.43	0.64	5.10	1.79	8.96

*Aspartic acid (Asp), Threonine (Thr), Serine (Ser), Glutamic acid (Glu), Proline (Pro), Glycine (Gly), Alanine (Ala), Valine (Val), Methionine (Met), Isoleucine (Ile), Lecine (Leu), Phenylalanine (Phe), Histidine (His), Lysine (Lys), Ammonia (NH_3), Arginine (Arg)

DISCUSSION

Buckwheat is considered to be a major of rutin as well as a minor source of other flavonoids acting as antioxidants (6, 13, 14). Cultivars having higher level of bioflavonoids and amino acid are considered the



best source of nutritional requirements and nutraceutical products. Determination of superior lines is also considered as better source of breeding materials for crop improvement. In the present study highest rutin content was found in local buckwheat genotype BWST-14 and BWK-3. Varietal differences and heritability of rutin content in common buckwheat was reported earlier (26, 27) who represented the variation due to geographic origin, environmental conditions. Such differences are also due to agronomic practices and soil water conditions (6). The variation in low rutin contents in some of the local genotypes of Baltistan may also be linked to environmental condition. Previous studies also discussed that the accumulation of rutin was mainly affected by varietal and environmental condition including location and growing season (28). Some of the essential amino acid were detected at different growth stages, especially methionine at sprouting and high leucine content was reported (25-29 g.kg⁻¹) in leaves (29). As compared to other amino acid, histidine was much lower across all genotypes. Local genotypes BWSU-10 and BWZ-8 were found to be a good sources of lysine. Similar amino acid composition results were reported earlier for common buckwheat (11). The presence of histidine, lysine and tryptophan in buckwheat seeds were reported higher than other major grains including barley and wheat (30). The physiological and biochemical processes including stress adaptation of plants affect biosynthesis of amino acid in common buckwheat (31, 32). All these genotypes showed diversity in metabolites and such varietal differences showed its worth for conservation of native germplasms in local and national gene bank. Further studies focusing metabolomics would be beneficial for future breeding programs.

CONCLUSION

The results concluded that these local genotype especially BWST-14 and BWK-3 showed highest rutin content as best sources of rutin and potential breeding material. Local genotype BWSU-10 and BW-7 showed the appreciable amount of lysine and arginine content to improve increasing amino acid in buckwheat cultivars. The diversity in metabolites and varietal differences showed its worth for conservation of native germplasms in local and national gene bank.

References:

- Gupta A, Gupta HS, Chaudhari B, Singh DD. Increasing production and productivity of buckwheat in Uttaranchal. Proceeding of the 9th International Symposium on Buckwheat, Prague. 2004.
- Ohnishi O. Buckwheat in Karakoram and the Hindkush. *Fagopyrum* 1994;14: 17-25.
- Hussain I, Bano A, Ullah F. Traditional drug therapies from various medicinal plants of central karakoram national park, Gilgit-Baltistan Pakistan. *Pak J Bot.* 2011;43:79-84.
- Tahir I. and Farooq S. Growth and yield characteristics of some buckwheats from Kashmir. *Geobios* 1983;10:193-208.
- Park CH, Kim YB, Choi YS, Heo K, Kim SL, Lee KC, Chang KJ, Lee HB. Rutin content in food products processed from groats, leaves and flowers of buckwheat. *Fagopyrum*, 2000;17: 63-66.
- Zamaratskaia G, Gerhardt K, Knicky M, Wendin K. Buckwheat: an underutilized crop with attractive sensory qualities and health benefits. *Critical reviews in food science and nutrition.* 2024 Dec 16;64(33):12303-18.
- Siracusa L, Gresta F, Sperlinga E, Ruberto G. Effect of sowing time and soil water content on grain yield and phenolic profile of four buckwheat (*Fagopyrum esculentum* Moench.) varieties in a Mediterranean environment. *Journal of food composition and Analysis.* 2017;62:1-7.
- Facho ZH, Tullah F, Khalil IH, Khan NU and Ali S. Morphological characterization and estimation of genotype × environment interaction of indigenous buckwheat germplasm collected from Gilgit Baltistan Pakistan. *Pak J Bot.* 2016;48(6): 2391–2398.
- Peng L, Zou L, Wang J, Zhao J, Xiang D, Zhao G. Flavonoids, antioxidant activity and aroma compounds analysis from different kinds of Tartary Buckwheat Tea. *Ind J Pharma Sci.* 2015;77(6), 661.
- Hussain I, Bano A, Faizanullah, Nosheen A. Multivariate analysis for elemental composition among indigenous common buckwheat genotypes of Baltistan. *JAPS: Journal of Animal & Plant Sciences.* 2016;26(6), 1725-1731.
- Pomeranz Y, HG Marshall, Robbins GS, Gilbertson JT. Protein content and amino acid composition of maturing buckwheat (*Fagopyrum esculentum* Moench). *Cereal Chem.* 1975; (4):479-485.
- Steadman KJ, Burgoon MS, Lewis BA, Edwardson SE, Obendorf RL. Minerals, phytic acid, tannin and rutin in buckwheat seed milling fractions. *J Sci Food Agri*, 2001;81(11):1094-1100.



13. Liu X, Ma Q, Sun Y, Ju W, Kumrungsee T, Zhou Z, Wang L, Cao R. Effects of processings and complexation on solubility, antioxidant and antibacterial properties of buckwheat polyphenols. *Food and Bioprocess Technology*. 2024;17(6):1583-95.#
14. Qaiser H, Abdullah R, Kaleem A, Iqtedar M, Sajer BH. Buckwheat. In *Essentials of Medicinal and Aromatic Crops 2023* Oct 10 (pp. 811-829). Cham: Springer International Publishing.
15. Qin, P, Wang Q, Shan F, Hou Z, G Ren. Nutritional composition and flavonoids content of flour from different buckwheat cultivars. *Int J Food Sci Technol*. 2010;45: 951-958.
16. Zheng GH, Sosulski FW, Tyler RT. Wet-milling, composition and functional properties of starch and protein isolated from buckwheat groats. *Food Res. Intl*. 1998;30: 493-502.
17. Kreft I, Fabjan N, Yasumoto K. Rutin content in buckwheat (*Fagopyrum esculentum* Moench) food materials and products. *Food Chem*. 2006;98(3): 508-512.
18. Zielińska D, Turemko M, Kwiatkowski J, Zieliński H. Evaluation of Flavonoid Contents and Antioxidant Capacity of the Aerial Parts of Common and Tartary Buckwheat Plants. *Molecule*. 2012;17: 9668-9682.
19. Morishita T, Yamaguchi H, Degi K. The contribution of polyphenols to antioxidative activity in common buckwheat and tartary buckwheat grain. *Plant Prod Sci*. 2007;10:99–104.
20. Gupta N, Sharma SK, Rana JC, Chauhan RS. Expression of flavonoid biosynthesis genes vis-à-vis rutin content variation in different growth stages of *Fagopyrum* species. *J Plant Physiol*. 2011;168(17): 2117-2123.
21. Vojtišková P, Švec P, Kubáň V, Krejzová E, Bittová M, Kráčmar S, Svobodová B. Chemical composition of buckwheat plant parts and selected buckwheat products. *Potravinarstvo*, 2014;8(1): 247-253.
22. Fabjan RJ, Kosir IJ, Wang ZH, Zhang Z and Kreft I. Tartary buckwheat as a source of dietary rutin and quercitrin. *J Agri Food Chem*. 2003;51: 6452-6455.
23. Tkachuk and Irvine, amino acid composition of cereals and oilseed meals, *cereal chemistry*, 1969:46.
24. Nei M, Li WH. Mathematical-model for studying genetic variation in terms of restriction endonucleases. *Proc Natl Acad Sci. USA*1979;76(10):5269–5273.
25. Steel RGD, Torrie JH. *Procedures of statistics. A biometrical Approach* 2nd edn McGraw Hall Book Co Inc. New York, 1980; 633.
26. Kitabayashi H, Ujihara A, Hirose T, Minami M. Varietal differences and heritability for rutin content in common buckwheat, *Fagopyrum esculentum* Moench. *Breed Sci*. 1995;45: 75–79.
27. Gupta N, Sharma SK, Rana JC, Chauhan RS. AFLP fingerprinting of tartary buckwheat accessions (*Fagopyrum tataricum*) displaying rutin content variation. *Fitoterapia*, 2012;83(6): 1131-1137.
28. Jiang P, Burczynski F, Campbell C, Pierce G, Austria JA, Briggs CJ. Rutin and flavonoid contents in three buckwheat species *Fagopyrum esculentum*, *F. tataricum*, *F. homotropicum* and their protective effects against lipid peroxidation. *Food Res Intl*. 2007;40(3): 356-364.
29. Sytar O, Chrenkova M, Ferencova J, POLAČIKOVÁ M, Rajský M, BRESTIČ MA. Nutrient capacity of amino acids from buckwheat seeds and sprouts. *Journal of Food & Nutrition Research*. 2018;57(1).
30. Pirzadah TB, Malik B, Tahir I, Ul Rehman R. Buckwheat journey to functional food sector. *Current Nutrition & Food Science*. 2020;16(2):134-41.
31. Winter G, Todd CD, Trovato M, Forlani G, Funck D. Physiological implications of arginine metabolism in plants. *Frontiers in plant science*. 2015; 6:534.
32. Wan C, Gao L, Wang J, Lei X, Wu Y, Gao J. Proteomics characterization nitrogen fertilizer promotes the starch synthesis and metabolism and amino acid biosynthesis in common buckwheat. *International Journal of Biological Macromolecules*. 2021;192:342-9.