

## Genetic diversity analysis in Buckwheat germplasm for nutritional traits

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Feeding the growing global human population is a tough challenge. Researchers look towards lesser known food crops which are nutritionally adequate, if not superior than the common major crops. Buckwheat (*Fagopyrum tartaricum* L. Gaerth), also called 'pseudocereal', is one such multipurpose crop with promising nutritive value. To enhance utilization of buckwheat as supplement food, here, we did genetic diversity analysis for nutritional parameters. Significant genetic diversity was observed in buckwheat germplasm for 24 nutritional parameters including total oil content (0.75-5.45%), oleic acid (36.98-47.87%), linoleic acid (32.75-44.61%), total protein content (5.65-14.17%), lysine (4.19-8.63 g/16 g N), methionine (0.54-2.48 g/16 g N) and half cystine (0.82-3.57 g/16 g N). The entire germplasm was grouped into three clusters. Cluster I comprised accessions promising high oil, stearic acid, oleic acid, least in linoleic acid and essential amino acids. Cluster II had accessions promising quality oil with highest linoleic acid and linolenic acid (PUFA) and highest half-cystine and glycine. Cluster III possessed promising accessions for highest oil percent, total protein, high essential amino acids and highest test weight. The inter-simple sequence repeats (ISSR) analysis using 26 primers indicated significant genetic diversity existed at molecular level. This study includes large number of accessions analyzed for nutriversity in entire spectrum of germplasm collections of the world. Nutritionally rich promising genotypes identified will be potential genetic stocks for making buckwheat, a prospective supplement food crop.

**Keywords:** Amino acid profiling, *Fagopyrum tartaricum*, Fatty acid profiling, Nutraceuticals, Nutriversity, Pseudocereals

Consistent over-reliance on a handful of major staple crops have resulted in less diversified meals and increased risks of health and socioeconomic issues. Despite the enormous progress made through breeding in enhancing crop productivity, malnutrition, poor health, hunger, and even starvation still remain the world's greatest challenges<sup>1-3</sup>. The need to provide food to nine billion people in 2050 is the most challenging task, thus, there has been resurgence in attention being paid to less known food crops which are nutritionally superior to the existing ones.

Buckwheat is an annual herb that belongs to the Polygonaceae family. It is called pseudocereal because of similar uses as of cereals<sup>4</sup>. Nine buckwheat species have agricultural and nutritional value among the many species in the world. Two species are cultivated for human consumption, *Fagopyrum esculentum* or common buckwheat or sweet buckwheat and *Fagopyrum tartaricum* or tartary buckwheat or bitter buckwheat<sup>5</sup>. Buckwheat is a multipurpose crop as the plant and its grains have been used for food, feed, medicine and manure. The flowers are a good

source of honey, and the plant is also used as an ingredient of beer. Buckwheat originated in Asia then introduced into Europe<sup>6</sup>. In India, the crop is widely grown in high mountains of Jammu and Kashmir, Himachal Pradesh, Uttar Pradesh, West Bengal, Sikkim, Meghalaya, Arunachal Pradesh and Manipur<sup>7</sup>.

Buckwheat needs to be explored as it contains gluten-free high quality protein with rich content of essential amino acids, particularly lysine, methionine and tryptophan which are the limiting amino acids in most cereal grains and legumes<sup>8,9</sup>. Consumption of buckwheat has been increasing in many countries because it is nutritionally rich and healthy and substituted for wheat flour for gluten-sensitive patients and the seeds of this herb are healthy and important. Buckwheat grains also contain various nutraceutical compounds<sup>10</sup> and are rich in dietary fiber<sup>11</sup>, macroelements: K, Ca, Na, Mg and microelements, such as: Cu, Mn, Zn, Se, vitamins particularly those of B group<sup>12</sup>, phenolic compounds including rutin, orientin, vitexin, quercetin, isovitexin, kaempferol-3-rutinoside, isorientin, and catechins<sup>13</sup>. Buckwheat reduces the blood cholesterol, regulating the blood sugar, blood lipid and blood pressure in

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human body<sup>14</sup>. Many studies have reported significant genetic diversity in buckwheat germplasm. Over the years more than 1000 accessions of buckwheat germplasm, collected from India and abroad, have been characterized morphologically as well as at molecular level<sup>15-18</sup>. However, information on detailed nutritional diversity analysis on important nutritional parameters *viz.*, protein, amino acids and fatty acid profiling, are scanty.

In the present study, we analyzed the genetic diversity of buckwheat germplasm for nutritional parameters to find out nutritional rich superior genotypes and use them for genetic improvement.

## Materials and Methods

### Plant materials

A total of 456 accessions of buckwheat conserved at NBPGR Regional Station, Shimla were analyzed for total oil content and total protein content at Germplasm Evaluation Division, ICAR\_NBPGR, New Delhi. Out of these, 57 germplasm accessions of buckwheat (indigenous and exotic origin) comprising two species *Fagopyrum esculentum* (29) and *Fagopyrum tataricum* (28) were studied in detail with respect to diversity in oil and protein quality. Out of 57, 47 accessions were indigenous and were collected from five states, namely Himachal Pradesh (28), Sikkim (9), Uttarakhand (6), Jammu & Kashmir (2) and Manipur (2) states, while 10 accessions were exotic and were from USA (4), Japan (4), Germany (1) and Philippines (1). These were analyzed for seed and biochemical traits. All experiments were done in triplicate.

### Test weight (g)

Two hundred grains were counted in three replicates. The test weight was calculated by multiplying with 5 and expressed in grams.

### Oil and Fatty acid analysis (%)

Total oil content of seed was analyzed by a non-destructive method using a Newport NMR analyzer (Model-4000) from Oxford Analytical Instruments Ltd. U. K. and fatty acid analysis by Gas Liquid Chromatograph (GLC, Model-HP6890)<sup>19</sup>.

### Total crude protein content (%)

For estimation of total protein content, nitrogen content of the whole seed of the sample was determined according to AOAC<sup>20</sup>. Nitrogen was determined with a Kjeltac auto analyzer (Tecator, Sweden). Protein was calculated from total nitrogen using a factor of 6.25.

### Amino acid profile of total seed protein by HPLC (g/16g nitrogen)

HPLC based pre-column derivatization technique was used in the present study. Fluorescent active reagent, 6-aminoquinolyl-N-hydroxy succinimidyl carbamate of Millipore Corporation<sup>21</sup> was used for derivatization. Derivatized sample was run in Waters AccQ Tag<sup>TM</sup> column of length  $3.9 \times 150$  mm (Part no.-WAT052885). Mobile phases A containing 10% solution of AccQ Tag<sup>TM</sup> concentrate (Part no. WAT052890) and phase B consisting of 60% HPLC grade. Acetonitrile was used as eluent in gradient conditions. Detection of individual amino acid derivative was made by fluorescence detector with excitation at 250 nm and emission at 395 nm with band width 18 nm. From the area percentage of the individual amino acid of the standard mixtures (PIERCE Product no. NC10180/ vial No. 88122) run under similar condition, the factors were calculated to convert area percentage of the individual amino acid to respective concentration<sup>22</sup>.

### Inter-simple sequence repeats (ISSR) analysis

Molecular characterization of 31 accessions with diverse nutritional parameters was done using inter-simple sequence repeats (ISSR) markers. The leaves of germinated plants were used for DNA extraction. Total genomic DNA was extracted from 1 g of leaves of each accession. A total of 100 ISSR primers were screened for amplification in out of which 26 were found to be suitable for further analysis<sup>23</sup>. To compare the efficacy of ISSR primers used in diversity analysis of the tested accessions, number of polymorphic bands; resolving power; total number of unique banding patterns; Discriminating power, were estimated<sup>24</sup>.

### Genetic diversity analysis

The dissimilarity matrix based on Manhattan coefficient, was used to construct dendrogram by unweighted pair group method for arithmetic mean (UPGMA) based sequential agglomerative hierarchical and nested (SAHN) clustering. The principle component analysis (PCA) was done. Analysis of molecular data was also carried out using SAHN based clustering using Jaccard's similarity coefficient. The multivariate analysis software, NTSYS 2.1 was used for various statistical analyses.

## Results

The range and mean values of seed test weight and 23 nutritional parameters are given in Table 1. Highly

significant differences among the accessions for all nutritional traits were observed.

#### Test weight

Significant differences in test weight were found in two species of buckwheat (Fig. 1). The test weight of 456 buckwheat accessions was (11.79-25.98 g) with a mean value of 19.03 g. Highest test weight was found in *F. esculentum* IC17971 while lowest for IC329192 (*F. tataricum*), which corresponds to the earlier reported value of 14.90-33.0 g<sup>25</sup>.

#### Oil and Fatty acid content

The mean value of oil content in two species (456 accessions) is shown in Fig. 1 and there were no significant differences among the species. The oil content in 57 buckwheat accessions (0.45-5.45%) with a mean value of 2.29%. The oil content was found higher than the previously reported value<sup>25</sup>. Among the accessions studied IC26600, IC16552, IC26600 (*F. tataricum*) were found promising in oil content (>5%). The composition of fatty acids in buckwheat seed oil indicates that buckwheat seeds are rich in unsaturated fatty acids (75-80%). The values of oleic and linoleic acid, were (36.98-47.87%) and (32.75-44.61%), respectively. Oleic acid found in the germplasm is higher than the reported values<sup>26</sup>. It was observed that among the accessions studied, majority of accessions (>70%) had oleic acid higher than the linoleic acid content. Among the accessions investigated, IC16579, IC26584, IC24300, IC17370 and IC18040 (*F. tataricum*) had highest oleic acid (>45%). The quality of oil for industrial purpose depends on the properties of the oil, one of which is the oleic/linoleic acid ratio (O/L ratio). The O/L

ranged from 0.86-1.44. Promising accessions identified for O/L ratio were IC18040, IC17370, IC16579 (*F. tataricum*) (>1.40). While, accessions having high content (>41%) of linoleic acid were IC202268, IC16552, IC108904, IC 109433 and IC 109313 (*F. tataricum*). Linolenic acid ranged from 0.0 to 5.29%. It was found higher than previously reported value<sup>26</sup>.

The values of saturated fatty acids palmitic acid (13.17-18.46%) and stearic acids (1.40-6.30%), respectively, were higher than previous published reports<sup>27</sup>. The results clearly indicate considerable variability for fatty acid profile among the accessions under studied.

#### Protein and amino acids content

Protein is one of the most important nutrients needed by the human body for growth and maintenance. Composition of essential amino acids determines the quality of proteins, which cannot be synthesized and thus must be provided in the diet. The mean value of Protein content in two species (456 accessions) is shown in Fig. 1 and there were no significant differences among the species. The overall range of protein content among 57 accessions (5.65-14.17%) with a mean value of 8.71% was highest protein was found in IC16552 (*F. tataricum*), which is at par with the values, reported previously<sup>28</sup>.

Protein of Buckwheat has excellent composition of amino acids and high biological value and thus, better than many other food crops<sup>8,10</sup> and close to optimum composition suggested by FAO/WHO<sup>29</sup>. This is the first report wherein larger number of germplasm collections were analyzed for their amino acid content.

Buckwheat proteins are rich in lysine, a limiting amino acid in most cereal grains like maize and wheat<sup>8,30</sup>. The lysine (Lys) content (4.19-8.63 g/16 g N) was high (>7.50%) in accessions IC540858, IC109313, IC272442, IC109308, IC37266, EC323723 and EC286521. Similarly, leucine as limiting amino acid with values (2.15-7.62 g/16g N) was high (>6.6 g/16 g N) in accessions EC58322, IC 266447, IC37291, EC276627 and IC37290. These values were higher than recommended by FAO.

Among sulfur containing amino acids i.e., methionine (Met), cystine usually found limiting in legume proteins<sup>31</sup>, were found to be high in buckwheat with values (0.54-2.48 g/16 g N) and (0.82-3.57 g/16 g N) for Met and half cystine (HCys), respectively. Promising accessions IC202268,

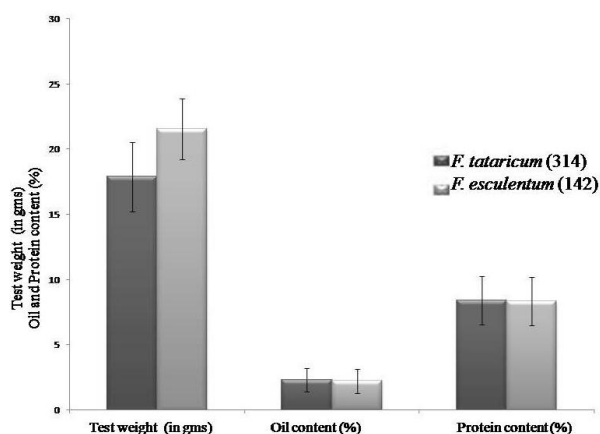


Fig. 1 — Test weight, protein content and oil content in two species of buckwheat (456 Accessions)

IC109211, IC37294, IC26606, IC109316, IC109313 having high contents for Met (>2.40 g/16 g N) and HCys (>3.50 g/16 g N) will be helpful in breeding of nutritionally rich with S-containing amino acids enhanced breeding lines of buckwheat. All the accessions studied had lower Met/Gly index than casein protein (1.57)<sup>32</sup>, a crucial factors for cholesterol-lowering effects and this makes buckwheat a better food crop for Coronary Artery Disease (CAD) patients<sup>33</sup>.

Among other essential amino acids namely, valine (Val), isoleucine (Ile), tyrosine (Tyr), arginine+threonine (Arg+Thr), histidine (His) and phenylalanine (Phe) as well as non-essential amino acids such as alanine (Ala), aspartic acid (Asp), glutamine (Gln), Proline, glycine (Gly) and serine (Ser) were also present in rich amount (Table 1). All amino acids were found higher than the previously reported values<sup>34</sup>.

#### Nutritional diversity analysis

Genetic variability analysis for nutritional parameters was done using Manhattan distance

coefficient and clustered by UPGMA based SAHN clustering method. The values of Manhattan distance coefficient (0.34-2.48) grouped 57 accessions into three clusters i.e. Cluster I, II and III (Fig. 2) at value of 1.50. Each group carrying genotypes being similar to one another and sharing common properties. The mean values of nutritional feature falling in each cluster are presented in Table 2. Cluster III comprised of 33 accessions, Cluster II with 21 and Cluster I with 2 accessions. Cluster II was further subdivided into two subclusters i.e. II A and IIB. Subclusters IIA again divided into IIA1 included 7 accessions and IIA2 included 8 accessions. Subcluster IIB included 6 accessions. Cluster III was further subdivided into three sub clusters i.e. IIIA included 6 accessions; IIIB included 3 accessions and IIIC again divided into IIIC1 included 8 accessions and IIIC2 included 16 accessions. Accession IC109308 did not fit with any group and was very distant from the other accessions.

Table 1 — Average, range of variation and component scores in 57 accessions of buckwheat

Parameter studied	Buckwheat			First four principal components			
	Mean	S.D.	Range	C1	C2	C3	C4
Test weight (g)	20.50	2.34	16.65-25.98	0.0	0.4	0.0	0.6
Oil content (%)	2.53	1.14	0.75-5.45	-0.1	0.0	0.5	-0.4
Fatty acid profile (%)							
Pamintic acid (PA)	15.99	1.38	13.17-18.46	0.1	0.0	0.1	0.3
Stearic acid (SA)	2.99	0.94	1.40-6.30	0.0	-0.8	0.1	0.1
Oleic acid (OA)	41.37	2.64	36.98-47.87	-0.2	-0.8	0.1	0.4
Linoleic acid (LA)	38.51	2.82	32.75-44.61	0.2	0.5	-0.2	-0.6
Linolenic acid (LNA)	1.15	1.08	0.0-5.29	0.0	0.8	0.1	-0.1
Stability index (O/L ratio)	1.08	0.14	0.86-1.44				
Protein content (%)	8.71	1.66	5.65-14.17	-0.1	-0.2	0.0	-0.7
Amino acid profile (g/16 g N)							
Aspartic (Asp)	11.51	1.01	7.58-16.57	0.8	-0.2	0.4	-0.3
Serine (Ser)	6.14	1.80	3.18-8.63	0.2	-0.5	0.3	0.2
Glutamine (Gln)	17.01	2.26	11.88-21.09	0.5	0.0	0.6	0.3
Glycine (Gly)	9.48	2.11	6.21-13.25	0.7	-0.2	-0.7	0.0
Histidine (His)	3.39	0.78	1.84-4.88	0.1	0.3	-0.6	0.4
Arginine (Arg) +Threonine (Thr)	13.93	1.78	8.59-18.32	-0.2	0.7	0.2	0.1
Alanine (Ala)	6.70	1.40	4.62-9.61	0.7	-0.3	-0.5	-0.3
Proline (Pro)	4.22	1.11	2.56-8.83	-0.2	-0.4	-0.3	-0.2
Cystine (Cys)	1.97	0.71	0.82-3.57	0.3	0.4	0.0	0.0
Tyrosine (Tyr)	2.80	1.13	0.58-4.92	-0.8	0.0	-0.2	0.0
Valine (Val)	4.19	0.94	2.33-6.11	-0.9	0.1	0.2	-0.2
Methionine (Met)	1.32	0.41	0.54-2.48	-0.8	0.0	0.0	0.0
Lysine (Lys)	6.10	1.09	4.19-8.63	0.8	0.2	0.3	-0.1
Isoleucine (Ile)	2.50	0.71	1.14-4.14	-0.8	0.1	0.1	-0.1
Leucine (Leu)	5.16	1.06	2.15-7.62	-0.9	0.0	-0.1	0.0
Phenylalanine (Phe)	3.76	1.40	1.28-7.16	-1.0	-0.1	-0.1	-0.1
Eigen value				7.33	3.47	2.39	2.26
%Variance Component				30.54	14.45	9.94	9.42
Cumulative				30.54	45.00	54.93	64.35

Table 2 — Cluster analysis for 24 traits in 57 accessions of buckwheat

Clusters/ Subclusters	No. of accessions	Important features	Remarks
Cluster I	2	Highest in SA (4.41), OA (43.10), Asp (14.95), Ser (8.03), Gln (20.73) and lowest in LA (35.46), Pro (3.2), Tyr (1.16), Val (2.58), Ile (1.25), Leu (3.55), Phe (1.81)	High in total saturated fatty acids and lowest in EAA among the clusters
Sub Sub Cluster IIA1	7	Highest in PA (17.17), HCys (3.05), Lys (7.45) and lower in Asp (5.69), Met (0.93).	High in EAA Cys and Lys.
Sub Sub Cluster IIA2	8	Highest in LA (41.11), LNA (1.48), Ala (8.07) while lowest in SA (2.21) and OA (39.65)	High in total polyunsaturated fatty acids
Sub Cluster IIB	6	Highest in Gly (12.26) and lowest in LNA (0.27), HCys (1.30)	
Sub Cluster IIIA	6	Highest in oil (3.25), Protein (11.78), Pro (4.93), Met (1.66) and lowest in wt (17.59), His (2.56)	
Sub Cluster IIIB	3	Highest in His (4.40), Tyr (4.17) and lowest in oil (1.43), Ser (3.65), Gln (12.96)	Highest in EAA among the clusters
Sub Sub Cluster IIIC1	3	Highest in wt (22.17) Val (5.40), Ile (3.64), Leu (6.70), Phe (5.65) and lowest in protein (7.65), Asp (9.41), Ala (5.06), Lys (4.97),	
Sub Sub Cluster IIIC2	16	Highest in Arg+Thr (15.22)	
IC109308		Highest Pro (8.73), Lys (8.63) and lowest Leu (2.15) and Met (0.54) among all the accessions. Phe (1.77) and Tyr amino acids (0.67) were also present in lower amount.	

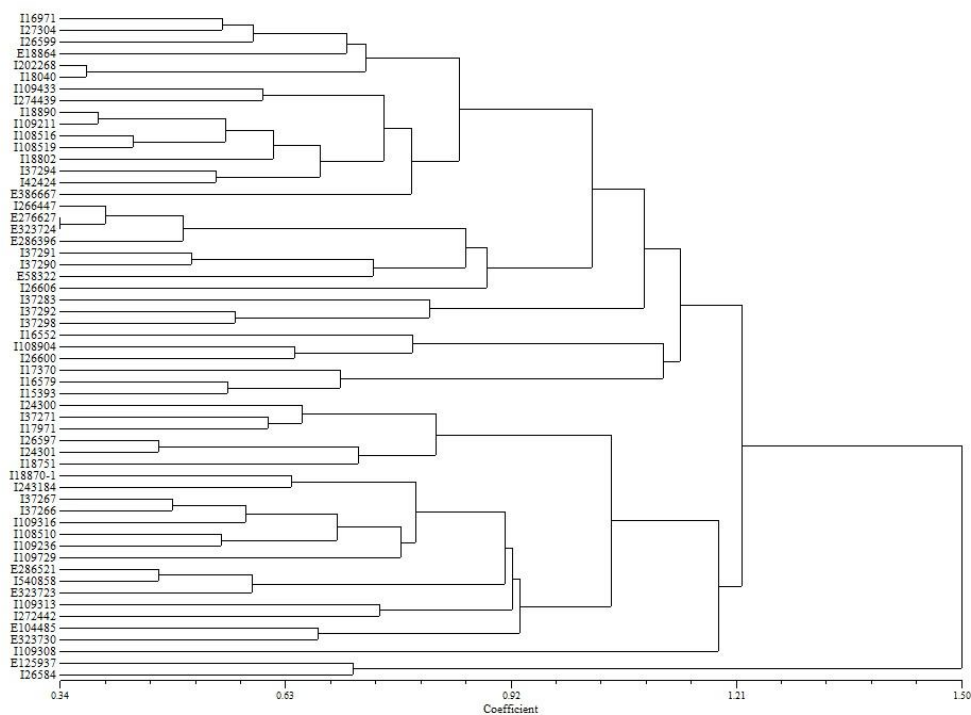


Fig. 2 — Dendrogram based on Manhattan distance co-efficient in 57 accessions of buckwheat

### Principal component analysis

To further ordinate the patterns of variability, principal component analysis (PCA) was done by considering all the 24 variables. PCA identified the minimum number of descriptors, which explained the maximum variation out of the total variance in the study. The outcome of diversity analysis was consistent with the results obtained through PCA,

whereby the major differences between the clusters were attributed to the same traits that contributed most to PC1, PC2 and PC3. The first four principal components contributed 64.35% of the total variability. The contribution of each principal component to the total variance is shown in Table 1, Fig. 3. PC1 contributed for 30.54% of the variation had Asp, Gln, Gly, Ala, Lys, as the variables with the

largest positive coefficient, while Tyr, Val, Met, Ile and Leu content had high negative coefficient. PC2, accounting for an additional 14.45% of the total variation, significantly positively contributed by

linoleic acid, linolenic acid and Arg+Thr, while negatively contributed by stearic acid, oleic acid and Ser. PC3 contributed for 9.94% of total genetic variability significantly contributed by oil content, Gly, Gln, His and Ala content. All variables except oil content and Gln contributed negatively towards PC3. PC4 contributed for 9.42% of total genetic variability had high positive coefficient of variates for seed test weight, while linoleic acid and protein content had high negative coefficients. The pattern of variations illustrated by the PCA was very well described by the correlation coefficients determined for pair-wise association of the traits.

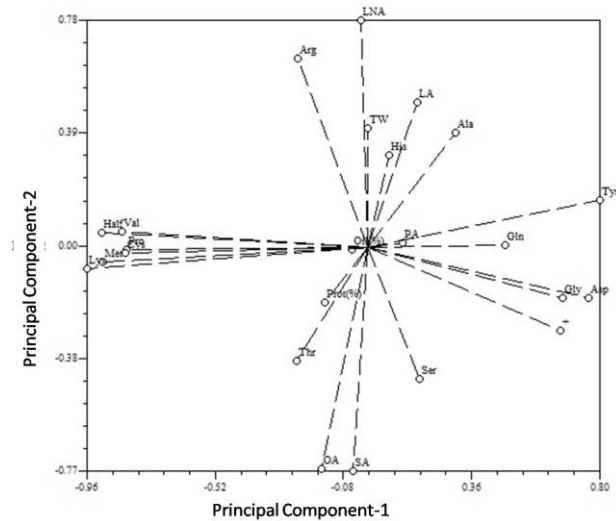


Fig. 3 — Principal Component Analysis in 57 accessions of buckwheat based on 24 nutritional parameters

**ISSR analysis**

Molecular characterization of 31 accessions of Buckwheat was done using inter-simple sequence repeats (ISSR) markers. A total of 100 ISSR primers were screened for amplification, out of which 26 were found to be suitable for further analysis (Table 3). A total of 301 polymorphic amplicons were obtained with an average of 11.5 per primer. All the 26 primers were able to discriminate the accessions (Fig. 4).

Table 3 — Various parameters related to efficiency of 26 ISSR primers used in the present study

ID	Primer sequence 5'-----3'	Ta (°C)	Total no. of bands		Percentage (%)		Resolving power	No. of unique banding pattern	Discriminating power
			Pol	Mono	Poly	Mono			
2903	ACACACACACACACYT	43	12	0	100	0	6.10	24	0.980
2912	GAGAGAGAGAGAGATT	48	9	0	100	0	4.88	14	0.914
2921	AGAGAGAGAGAGAGAGT	48	11	0	100	0	7.10	20	0.955
2922	AGAGAGAGAGAGAGAGC	44	14	1	93.4	6.6	3.54	18	0.877
2923	AGAGAGAGAGAGAGAGG	44	12	0	100	0	4.32	22	0.949
2924	GAGAGAGAGAGAGAGAT	47	9	0	100	0	4.14	14	0.898
2925	GAGAGAGAGAGAGAGAC	50	19	0	100	0	9.94	27	0.981
2930	CACACACACACACAT	43	10	1	90.9	9.1	7.20	24	0.976
2931	CACACACACACACAA	48	15	0	100	0	5.48	25	0.964
2932	CACACACACACACAG	50	11	0	100	0	3.94	21	0.966
2939	ACACACACACACACT	43	14	0	100	0	3.90	19	0.938
2940	ACACACACACACACC	48	9	0	100	0	5.00	22	0.970
2948	AGAGAGAGAGAGAGAYT	51	15	0	100	0	5.48	20	0.965
2949	AGAGAGAGAGAGAGAYC	51	12	1	92.3	7.6	6.64	27	0.989
2950	AGAGAGAGAGAGAGAYA	45	9	1	90	10	1.16	6	0.619
2954	GAGAGAGAGAGAGAYT	45	10	0	100	0	5.60	18	0.956
2955	GAGAGAGAGAGAGAYC	53	10	0	100	0	5.00	16	0.893
2956	GAGAGAGAGAGAGAYG	53	15	0	100	0	3.86	21	0.948
2964	GTGTGTGTGTGTGTGTYC	51	18	0	100	0	5.40	25	0.974
2970	ACACACACACACACYA	44	10	0	100	0	5.38	21	0.956
2982	GAAGAAGAAGAAGAAGAA	39	9	0	100	0	3.80	23	0.968
2983	GTTGTTGTTGTTGTTGTT	45	12	0	100	0	5.72	15	0.923
2994	GGAGAGGAGAGGAGA	43	16	0	100	0	4.75	20	0.958
3004	VHVTGTGTGTGTGTGTGT	48	10	0	100	0	4.58	16	0.948
3005	HVHTGTGTGTGTGTGTGTG	46	11	0	100	0	5.68	22	0.949
3011	CCGACTGGAGNNNNNNATGTG	54	14	0	100	0	4.84	20	0.946

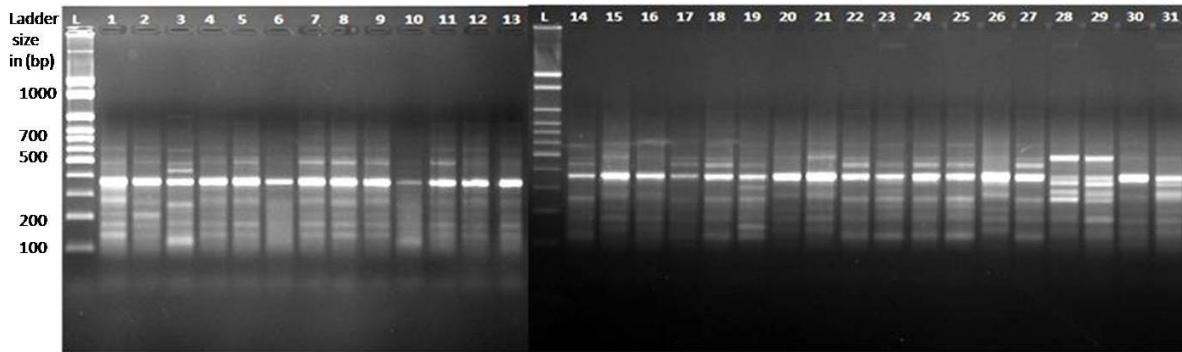


Fig. 4 — ISSR profile of 31 accessions using primer ISSR 2949

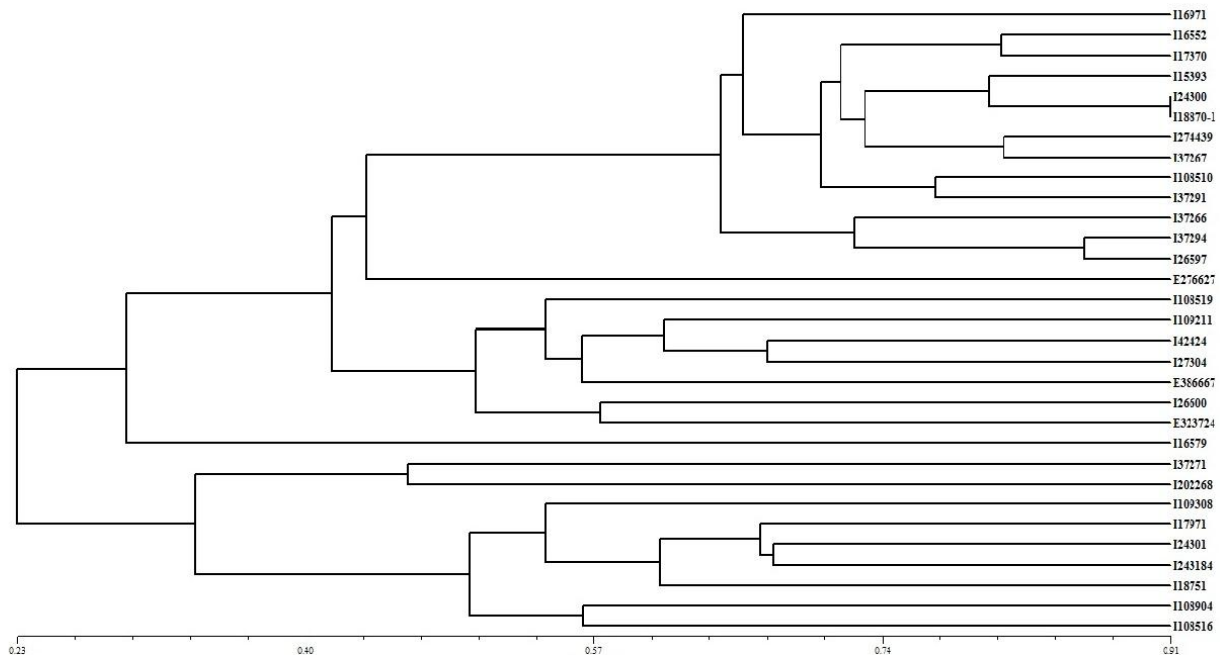


Fig. 5 — Dendrogram based on Jaccard's similarity coefficient

The values of resolving power (1.16-9.94), no. of unique banding patterns (6-27) and discriminating power ranged from (0.619-0.989) indicate the ISSR primers were polymorphic and were able to discriminate the accession. Based on Jaccard's coefficient, all the 31 clusters were grouped into two major groups I and II. Group I contained 22 clusters while group II contained 9 clusters (Fig. 5). The group I could be further divided into two sub groups IA and IB with 13 and 8 accessions, respectively. The present ISSR markers sufficiently distinguish the 31 accessions collected from northwestern and northeastern Himalayan region.

### Discussion

In view of the huge nutritional benefits of the buckwheat, there is a definite need for its genetic

improvement to develop high yielding varieties with high content of desired nutritional traits. In the process of genetic improvement, assessment of genetic diversity and the multivariate statistical analysis among germplasm play a major role for identification of potential breeding lines for nutritional security. It will lead way to determine the most divergent parents based on contribution of different qualitative and quantitative traits for utilization in breeding programs. In the present paper, the nutritional potential of buckwheat germplasm is highlighted and these can be exploited in alleviating nutrition and crop improvement related problems in developing countries. These nutritious underutilized crops can overcome the severe public health problems like obesity and malnutrition, as a result

of overdependence on few staple crops and thus could significantly contribute to improve food security.

In this study, considerable variations were found among 57 buckwheat germplasm in terms of major nutritional compositions. Oil is an essential macronutrient, and vegetable oils are widely used in the food industry and highly represented in the human diet. Buckwheat grain contains oil with good quality of fatty acids and protein content with balanced composition of amino acids. In the present study the test weight of buckwheat accessions corresponds to the earlier reported values<sup>25</sup>. The wide variation in seed size observed in the present study may be valuable to the breeder, especially at the confirmed correlation between seed size and yield reported by Hanbury *et al.*<sup>35</sup>.

Majority of buckwheat accessions had oleic acid as major fatty acid. It has a much greater oxidative stability than polyunsaturated fatty acids<sup>36</sup>. It has been reported that high oleic acid improves the blood lipoprotein profile, suppress tumorigenesis, reduce atherosclerosis, and prevents inflammatory and coronary heart diseases<sup>37,38</sup> and preparation of numerous products including cosmetics and machine lubricants (e.g., high-temperature engine, transmission, hydraulic, gear, and grease applications)<sup>39</sup>. Thus, these mono unsaturated fatty acids levels will not only improve oil stability, but will also support the health of consumers in addition to providing benefits for industrial applications.

Buckwheat oil is rich in polyunsaturated fatty acid especially linoleic acid and also has small amount of linolenic acid; both are nutritionally an essential fatty acid which needs to be supplemented only through diet in humans. High degree of unsaturation is desirable from a nutritional point of view. Linoleic acid has various beneficial effects on cardiovascular disease including improved blood lipid profile, improved insulin sensitivity, lower incidence of type 2 diabetes, and anti-arrhythmic effects. Linolenic acid on the other hand, has been identified as enhancer for transporting bioactive compounds into the skin, and it is converted to arachidonic acid which serves as a precursor for powerful hormone-like compounds<sup>40</sup> and thus, the fatty acids of buckwheat lipid are helpful for health. The available diversity will help the plant breeders to manipulate fatty acid composition, particularly the palmitic, oleic and linoleic acids, to substantially alter the total fat content. Studies have

shown that replacing saturated fat with unsaturated fat in the diet can help lower blood pressure and reducing total and LDL cholesterol<sup>27</sup>.

Protein is one of the most important nutrients needed by the human body for growth and maintenance. Composition of essential amino acids determines the quality of proteins. Buckwheat protein products have preventative effect to some chronic diseases, such as diabetes, hypertension, hypercholesterolemia and many other cardiovascular diseases<sup>10</sup>. They also help in prevention of colon carcinogenesis by reducing cell proliferation, and mammary carcinogenesis by lowering serum estradiol<sup>41</sup>.

The amino acid composition of buckwheat proteins is well balanced, with a rich amount of essential amino acids and is thus superior to that of common cereals like wheat and maize and is close to optimum composition suggested by FAO/WHO<sup>29</sup>. The lysine content is the limiting amino acid in most cereal grains like maize and wheat<sup>30</sup>. Our investigations reported high content of lysine, 2-3 times higher than in wheat and maize. It is higher than the protein reference pattern defined by FAO/WHO, exhibited in egg or milk proteins<sup>42</sup>. It can meet the daily requirement of lysine for infants and adults, including children of school age. Thus, the lysine values present in buckwheat are close to the ideal value and could support its recommendation by food nutritionists.

Leucine (Leu) is reported to be the first limiting amino acid in buckwheat<sup>8</sup>, thus, the accessions found to contain with high Leu content ( $\geq 6.6$  g/16 g N) as required by optimum FAO value, can be used by the breeders to overcome the leu deficiency in buckwheat. The buckwheat has higher essential amino acid content as recommended by FAO. Most of the buckwheat accessions had higher amounts of essential amino acids than the requirements of a 2-5 year old child and comparable amounts as whole egg protein. Relatively higher content of essential amino acids in buckwheat grain predetermines its uses as a substitution of conventional cereals<sup>42</sup>.

It is also important to see underutilized crops as part of a food mix for a particular region, rather than as 'stand-alone' crops and using combinations of underutilized crops as additions to the existing staple. Tartary buckwheat is receiving more and more attention nowadays, owing to its diverse utilities. Besides a higher nutritional value, tartary buckwheat plays an important role in medical care as a pharmaceutical plant, especially with a high



antioxidant activity due to its flavonoid content. For example, its seed rutin content reaches 0.8-1.7%, while common buckwheat contains only 0.01% seed rutin content. Keeping in view prevalence of diseases in children, adults and old aged people, associated with nutrient deficiencies, the accessions having high essential amino acids are of prime importance as genetic stocks for breeding of cultivars, nutritionally rich in essential amino acids. Further, due to more prevalence of celiac disease, particularly at childhood stage, diets with balanced amino acid composition and no gluten, makes buckwheat as an excellent plant nutrient source. Foods prepared with buckwheat had lower GI (glycemic index) properties and high satiety values and thus, have high potential to improve glucose tolerance at the first and second meal (lunch) and are recommended to be introduced in daily diet for healthy and diabetic subjects<sup>43</sup>. These nutritional parameters were also found to be superior in grain amaranth germplasm observed in our earlier study<sup>44</sup>. Thus, buckwheat along with staple foods is emerging as healthy alternatives.

The cluster analysis helped in grouping the accessions into different clusters having specific characteristic traits which may be helpful in selecting parents for future breeding programs for nutritional enhancement. The discriminating technologies of UPGMA clustering and PCA analysis enabled visualization of this complex dataset and underlying relationships among studied samples. Cluster analysis has proved to be an effective method in grouping accessions that may facilitate effective management and utilization in crop breeding programmes. The Accessions of a particular cluster having desirable genes for specific traits, such as cluster IIIC was high in essential amino acids content and sub cluster IIIB had rich amount of polyunsaturated fatty acids, can be hybridized with other promising accessions of different divergent clusters, which can facilitate the accumulation of favorable genes in hybrids. Thus, the obtained hybrids may be fixed following recurrent selections of their selected transgressive segregants in advance generations, which may lead to the development of varieties rich in nutritional components. Accession IC109308 did not fit with any group and showed possibility of getting good recombinants if this accession is crossed with other accessions.

The pairs of genotypes with large Manhattan distance could be used as parents in hybridization program for combination breeding or for generating

variability for selection of superior pure lines in advanced generations. Consistent to the outputs of the PCA, the traits that contributed most for the 1st principal component (Tyr, Val, Met, Ile, Leu and Phe) was negatively correlated with Lys. Moreover, the contrasting relationship of oleic acid with linoleic acid was also depicted which was supported by significant negative correlation coefficient. The environments, the genotypes were grown could be the one of the reason for the inverse relationship between these fatty acids<sup>44</sup>.

This study suggests the need for breeders to tap the genetic diversity for the development of improved varieties with enhanced nutrition. The promising accessions identified in this study from 57 accessions of buckwheat includes IC37290 and EC58322 having high oil, oleic acid and rich in EAA. Moreover, among the accessions studied for 24 nutritional traits, the accessions with superior seed weight (>22 g) Val (>5.30), Ile (>3.60), Leu (>6.60), and Phe (>5.60) nutritional traits were IC266447, IC37291 and EC276627 belonged to group IIIC1 while accessions IC37266 was found to have highest polyunsaturated fatty acids belonging to cluster IIA2. These promising accessions with respect to enhanced nutritional traits will be a potential breeding material for future genetic improvement. Hybridization between genotypes of divergent clusters would generate a broad spectrum of variability for effective selection in the segregating generations for the development of nutritionally superior genotypes.

The evaluation of genetic background of germplasm is essential to plant breeding. The objective of ISSR analysis was to elucidate the genomic diversity existed in nutritionally diverse buckwheat accessions. The parents with diverse genetic base are desirable for development of varieties with broad genetic base. In the present study, ISSR markers were used to find out genetic diversity in 31 accessions, promising for nutritional parameters to find out suitable diverse parents for hybridization programs. Many studies have reported significant genetic diversity in buckwheat germplasm<sup>15-18</sup> at molecular level. Many reliable markers including Simple sequence repeats (SSR), Sequence-related amplified polymorphism (SRAP) have been used in molecular characterization of buckwheat. Inter-simple sequence repeat (ISSR) markers have been successfully used to analyze genetic diversity and relatedness of 15 germplasms of *Fagopyrum tataricum*<sup>18</sup>, but the no. of accessions as well as no. of

primers used were not optimum. In the present study, 31 accessions, selected based on nutritional diversity existed in 456 accessions, were subjected to ISSR analysis using 26 ISSR primers. ISSR markers are random primers hence the coverage of genome is better than more reliable SSR and SRAP markers despite low reproducibility. However, these are able to screen more genomic regions as compared to SSR. Present study indicated a significant genetic diversity existed based on nutritional as well as molecular level. Based on the molecular characterization, broadly two distinct groups corresponding to geographical locations and nutritional parameters were observed. The breeding lines or cultivars developed from these diverse genotypes will have broad genetic base, hence more adaptability and climate resiliency. Present study, therefore, is an exhaustive attempt to analyze the genetic diversity for nutritional parameters in entire spectrum of germplasm collections across the world including its wild relatives. Many accessions with nutritionally values for fatty acid, protein and amino acid parameters, superior to the earlier reported values will be the potential breeding material for nutritional enhancement. Significant genomic diversity existed among these promising accessions will help in broadening the genetic base of development varieties with enhanced adaptability as well as climate resiliency, suitable for growing in new areas. The access to these nutritious foods will address food and nutrition security in large parts of world.

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