Genetic diversity of tartary buckwheat (Fagopyrum tataricum Gaertn.) genotypes based on cluster and principal component analyses in Organic Agriculture

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Manuscript Received:01.03.2024; Accepted: 21.03.2024

Abstract

Atotal of 24 accessions of tartary buckwheat with some local germplasm were analyzed by cluster and principal component using 17 quantitative characters to group them on the similarity at phenotypic level, and to identify the most relevant characters in explaining the variation. The genotypic variance of all the traits was lower than the phenotypic variance. Highest GCV and PCV were recorded for seed yield per plant, seed per plant and protein content. The non-hierarchical Euclidean cluster analysis using Mahalanobis statistic, grouped the genotypes into five distinct clusters among which two clusters were polygenotypic and three clusters were monogenotypic. Cluster I was the largest group comprising of 15 genotypes characterized with highest primary branch per plant, inflorescence length, leaf per plant, seed per plant, seed yield per plant, magnesium content, iron content and zinc content. Most of the high yielding germplasm along with Himpriya were grouped under this cluster. Cluster II composed of the genotypes having high amount of protein, calcium and phosphorus that can be used as a source for introgression of genes responsible for high nutrition. The results of PCA confirmed the findings of cluster analysis. Six principal components having eigen value greater than one, accounted for nearly 78.58% of the total variation. According to eigen vector analysis, the observed variation for first, second, third, fourth, fifth and sixth principal component were about 26%, 15%, 12%, 10%, 8% and 6%, respectively. In the first principal component, number of seed per plant and seed yield per plant were the most contributing traits whereas days to maturity, calcium and iron were the principal traits of the second principal component.

Key words: Fagopyrum, variation, multivariateanalysis, micronutrients

Buckwheat (Fagopyrum spp.) contains more than 18 species and among them, two species *i.e.*, Common buckwheat (Fagopyrum esculentum Moench, 2n = 2x = 16) and Tartary buckwheat (Fagopyrum tataricum Gaertn., 2n = 2x = 16) are being utilized for food and feed (Campbell 2003). Grain yield of tartary buckwheat is high and stable due to its self-compatibility, low seed abortion and tolerance to stresses such as frost (Campbell 1995). In addition to higher grain yield of tartary buckwheat, it is nutritionally and medicinally considered superior to common buckwheat and other cereals. Due to its short

growing period and cold tolerance, it is suitable to cultivate in high hills, where summer season is short. However, most of the landraces of tartary buckwheat have a tightly adhering husk, low grain yield and bitter components. In mountain areas of Himachal, farmers are maintaining different landraces of tartary buckwheat (also called mountain buckwheat), some of which are unique e.g., *Bhate Phaper* (rice Tartary buckwheat) which has a non-adhering hull (Bhardwaj and Kaur 2020).

Despite its importance, area under its cultivation has decreased over years, primarily due to its

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replacement with more remunerative cash crops likeapple, green pea, hops and potato apart from non-availability of improved varieties having high yield potential, quality flour and resistance to biotic and abiotic stresses (Joshi and Rana, 1997). Therefore, breeding work is necessary to increase their productivity. To initiate the breeding work, it is important to group the large number of diverse accessions based on the multiple characters. Characterization, systematic documentation and conservation of germplasm are important for its utilization in crop improvement programmes (Sekhon *et al.* 2019; Singh *et al.* 2024).

Therefore, under the present investigation, local germplasm of Tartary buckwheat (*F. tataricum*) from higher northern hilly region of the Himalayas along with a few important exotic and other indigenous accessions were characterized for 17 important agromorphological and biochemical traits to assess the extent of genetic diversity. The information generated can be useful for identifying groups of accessions that have desirable characters for crossing, planning efficient germplasm collecting expeditions, revealing the patterns of variation in germplasm collections and investigating some aspects of crop evolution (Sharma *et al.* 2020).

Among the different methods of multivariate analysis, cluster analysis and principal component

analysis (PCA) are commonly used. Cluster analysis used to group accessions according to similarity in certain characteristics. PCA is a technique for analyzing relationships among several quantitative variables measured on a number of accessions. It provides information about the relative importance of each variable in characterizing the genotypes and to identify the most relevant characters in explaining the variation (Sharma *et al.* 2022).

Materials and methods

Plant Materials

A total of 24 accessions of tartary buckwheat were characterized and evaluated in 2023 (Table 1). These accessions were selected from the Reginal Station, ICAR-National Bureau of Plant Genetic Resources, Shimla.

Experimental Design

The field experiment was conducted in Organic Agriculture and Natural Farming Farm, Holta, CSKHPKV, Palampur in the *Rabi* season of 2023. Farm is located at an elevation of 1,290.8 metres above mean sea level, with latitude of 32° 6' N and longitude of 76° 3' E.

The experimental material was sown in Randomized Complete Block Design (RCBD) with two checks namely Shimla B1 and Himpriya. The plot design was two rows, 1 m long with a 25 cm row to row

Table 1. List of 24 buckwheat genotypes

Table 1. East of E. S. Marri, many Benefit bes							
Genotypes	Code	Source	Genotypes	Code	Source		
IC 26755	3	ICAR-NBPGR, Shimla	IC 37288	15	ICAR-NBPGR, Shimla		
Sangla B 444	4	CSKHPKV, Palampur	IC 341667	16	ICAR-NBPGR, Shimla		
Sangla B 214	5	CSKHPKV, Palampur	IC 345059	17	ICAR-NBPGR, Shimla		
Sangla B 129	6	CSKHPKV, Palampur	IC 323723	18	ICAR-NBPGR, Shimla		
Sangla B 5	7	CSKHPKV, Palampur	IC 341674	19	ICAR-NBPGR, Shimla		
IC 46160	8	ICAR-NBPGR, Shimla	IC 341683	20	ICAR-NBPGR, Shimla		
Himgiri 109728	9	ICAR-NBPGR, Shimla	IC 371665	21	ICAR-NBPGR, Shimla		
IC 318859	10	ICAR-NBPGR, Shimla	IC 42430	22	ICAR-NBPGR, Shimla		
IC 109729	11	ICAR-NBPGR, Shimla	EC 286377	23	ICAR-NBPGR, Shimla		
IC 47929	12	ICAR-NBPGR, Shimla	Chitkul	24	Local landrace		
IC 341589	13	ICAR-NBPGR, Shimla	Shimla B1 (C)	1	ICAR-NBPGR, Shimla		
IC 356112	14	ICAR-NBPGR, Shimla	Himpriya (C)	2	ICAR-NBPGR, Shimla		

Where, ICAR-NBPGR - Indian Council of Agricultural Research, National Bureau of Plant Genetic Resources CSKHPKV - Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur

distance and 75 cm block to block. Block was little raised for proper drainage because, stagnate water could damage buckwheat completely. Under organic input conditions soil was treated with *jeevamrit* (10%), *beejamrit* was used for seed treatment, vermicompost was added @ 5 t/hafollowed by sprays of organic liquid manure (vermiwash @ 10%) at 15 days interval.

Data Collection and Statistical Analysis

The observations for 17 agro-morphological and biochemical traits viz., Days to 50% flowering (DTF), Days to 80% maturity (DTM), Inflorescence length (IL), Inflorescence per plant (IPP), Leaves per plant (LPP), Primary branches per plant (PB), Plant height (PH), Seed index (SI), Seed per plant (SPP), Straw yield per plant (SYPP), Seed yield per plant (YPP), Protein, Calcium content (Ca), Phosphorus content (P), Magnesium content (Mg), Iron content (Fe) and Zinc content (Zn) were made on individual plants as well as on a plot basis depending on the nature of the characters based on Descriptors for buckwheat (IPGRI 1994). For measurement on an individual plant basis, ten individual plants per plot or plant's parts were considered and the averaged data was used for analysis. Thoroughly washed plant samples were dried in oven at 70° C for 48 hr, ground in a stainlesssteel Wiley mill, and digested in a di-acid mixture of HNO₃ and HClO₄ (Jackson 1973). Micronutrient was determined in aqueous extracts of the digested plant material by atomic absorption spectrophotometer (AAS). Protein fractionation was done by dissolving different fractions of proteins in double distilled water, 1M NaCl, 1% NaOH and 90% ethanol. The total crude protein estimation was done using the standard micro-Kjeldahl method and the amount of total nitrogen in the raw materials were multiplied with the traditional conversion factor of 6.25.

Based on this averaged data, descriptive statistics were estimated, and cluster and PC analyses were applied. The genetic divergence among genotypes was computed following Mahalanobis D² technique (1936) and the genotypes were grouped into different clusters following Tocher's method as described by Rao

(1952) using Windostat 8.0 software package developed by Indostat Services, Hyderabad, India. The grouping pattern of the 24 buckwheat genotypes was computed by principal component analysis (PCA) in PAST (Paleontological Statistics) software v.4.03 (Hammer *et al.* 2001) using Eigen procedure based on correlation coefficient between two genotypes and the principal components with eigenvalues >1.0 were selected.

Results and discussion

Determination of Genetic Variations

The experimental results revealed a wide range of variation among 24 buckwheat genotypes for both morphological and biochemical traits (Table 2). The genotypic variance (σ^2G) of all the traits was lower than the phenotypic variance ($\sigma^2 P$). Genotypic variance ranged from 0.08 (Zn) to 640.02 (P). GCV values ranged from 4.71% for days to 80% maturity to 31.05% for seed yield per plant. Similarly, the PCV values ranged from 4.95% for days to 80% maturity to 31.12% for yield per plant. PCV values were higher than the GCV values for all the traits which indicates the environmental role in trait expression (Soharu et al. 2022). Deshmukh et al. (1986) suggested that PCV and GCV values greater than 20% are regarded as high, values between 10% to 20% as medium, whereas values less than 10% are considered to be low. Accordingly, highest GCV was recorded for seed yield per plant (31.12%), seed per plant (20.74%) and protein (20.12%) whereas medium GCV was recorded for inflorescence length (20.34%), inflorescence per plant (11.94%), leaf per plant (19.16%), primary branches per plant (14.68%), plant height (10.02) and seed index (14.04%) and lowest GCV was observed for days to 50% flowering (9.53%), days to 80% maturity (4.95%), straw yield per plant (8.40%), calcium content (9.40%), phosphorus content (7.15%), magnesium content (7.85%), iron content (8.40%) and zinc content (9.02%). Similarly, the highest PCV was also recorded for seed yield per plant (31.05%), seed per plant (20.70%) and protein (20.06%). Medium PCV was recorded for inflorescence length (18.61%), inflorescence per plant

Table 2. Assessment of genetic variability of eleven morphological and six biochemical traits of buckwheat

Duckwiicat										
Traits	Mean	MSG ¹	MSE ²	Genotypic	Phenotypic	GCV^3	PCV ⁴	Heritability	Genetic	GAM ⁵
				variance	variance	(%)	(%)	(%)	Advance	<u> </u>
DTF	52.10	73.93*	2.03	23.96	24.64	9.40	9.53	97.25	9.94	19.09
DTM	89.20	58.34*	5.42	17.65	19.45	4.71	4.95	90.70	8.24	9.24
IL	3.21	1.28*	0.21	0.36	0.43	18.61	20.34	83.69	1.13	35.06
IPP	19.14	15.66*	0.23	5.14	5.22	11.85	11.94	98.53	4.64	24.23
LPP	27.09	80.86*	0.36	26.83	26.95	19.12	19.16	99.50	10.65	39.30
PB	2.94	0.56*	0.01	0.18	0.19	14.50	14.68	97.52	0.87	29.50
PH	80.71	196.30*	4.42	63.96	65.43	9.91	10.02	97.75	16.29	20.19
SI	2.30	0.31*	0.01	0.10	0.10	13.97	14.04	99.04	0.66	28.64
SPP	120.26	1867.04*	8.37	619.56	622.35	20.70	20.74	99.55	51.16	42.54
SYPP	7.30	1.13*	0.04	0.36	0.38	8.24	8.40	96.20	1.22	16.65
YPP	2.68	2.09*	0.01	0.69	0.70	31.05	31.12	99.57	1.71	63.82
Protein	13.04	20.66*	0.12	6.84	6.89	20.06	20.12	99.41	5.38	41.20
Ca	58.27	90.10*	2.40	29.24	30.03	9.28	9.40	97.35	10.99	18.86
P	361.78	2005.04*	85.00	640.02	668.35	6.99	7.15	95.76	51.00	14.10
Mg	207.63	796.64*	29.77	255.62	265.55	7.70	7.85	96.26	32.32	15.56
Fe	3.94	0.33*	0.02	0.11	0.11	8.24	8.40	96.36	0.66	16.66
Zn	3.08	0.23*	0.01	0.08	0.08	8.93	9.02	98.06	0.56	18.22

DTF= Days to 50% flowering, DTM= Days to 80% maturity, IL= Inflorescence length, IPP= Inflorescence per plant, LPP= Leaves per plant, PB= Primary branches per plant, PH= Plant height, SI= Seed index, SPP= Seed per plant, SYPP= Straw yield per plant, YPP= Seed yield per plant, Ca= Calcium, P= Phosphorus, Mg= Magnesium, Fe= Iron, Zn= Zinc

¹MSG- Mean square of genotypes; ²MSE- Mean square of error; ³GCV- Genotypic coefficient of variation; ⁴PCV- Phenotypiccoefficient of variation; ⁵GAM-Genetic advance as percent of mean

(11.85%), leaf per plant (19.12%), primary branches per plant (14.50%) and seed index (13.97%) while low PCV was recorded for days to 50% flowering (9.40%), days to 80% maturity (4.71%), plant height (9.91), straw yield per plant (8.24%), calcium content (9.28%), phosphorus content (6.99%), magnesium content (7.70%), iron content (8.24%) and zinc content (8.93%). Dutta *et al.* (2008) also observed high PCV and high GCV for the number of secondary branches, number of leaves and seed yield per plant in buckwheat. Hiremath *et al.* (2017) observed high genotypic and phenotypic coefficients of variation for seed yield. Similar results were obtained by Bisht *et al.* (2018) hence the selection for these traits could be effective.

The difference between PCV and GCV was less (<1) in all the traits except for inflorescence length which indicates higher contribution of genotypic effect towards phenotypic expression of such traits. For inflorescence length, the gap was high indicating

that environmental factors are playing an important role in addition to the genotype for expression of this trait. The genotypic coefficient of variance offers insight into the genetic variability of quantitative traits. However, it does not directly indicate the proportion of variation that is heritable.

To gain a comprehensive understanding of the potential advancement through selection, it's crucial to complement the genotypic coefficient of variance with heritability estimates. The genetic coefficient of variance, combined with these heritability values, provides the most accurate depiction of the anticipated progress achievable through selection. Heritability values play a pivotal role in forecasting the expected gains attainable via selection processes (Chauhan and Sharma 2021). Estimation of heritability in broad sense ranged from 99.57% for seed yield per plant to 83.69% for inflorescence length. All traits showed very high heritability with values greater than 80%. In similar case, Rana *et al.* (2023) found high heritability for days

to 50% flowering while moderate and low heritability for rest of the traits in wheat.

According to Fehr (1987), the heritability of a trait is influenced by factors such as the studied population, environmental conditions and the methodology employed. However, heritability alone does not reveal the extent of genetic improvement achievable through individual genotype selection. A more comprehensive understanding is obtained by considering both heritability and genetic advance. Genetic advance (GA) in selection context refers to the enhancement of traits in genotypic value within a new population compared to the base population after one selection cycle at a specified selection intensity (Hamdi et al. 2003). This combined knowledge of heritability and genetic advance provides a more practical insight into the potential improvements achievable through selection processes.

Genetic advance as percent of the mean (GAM), in the present study, ranged from 9.24% to 63.82% for days to 80% maturity and seed yield per plant, respectively. Johnson et al. (1955) categorized genetic advance as percent of mean as low (<10%), moderate (10-20%) and high (>20%). Using this classification as the basis, inflorescence length, inflorescence per plant, leaf per plant, primary branches per plant, plant height, seed index, seed per plant, seed yield per plant and protein had the highest genetic advance as percent of mean followed by moderate in days to 50% flowering, straw yield per plant, calcium content, phosphorus content, magnesium content, iron content and zinc content whereas low for days to 80% maturity. High heritability along with high genetic advance as percent mean is more helpful in predicting gain under selection than heritability alone. Accordingly, high heritability along with high genetic advance was observed in inflorescence length, inflorescence per plant, leaf per plant, primary branches per plant, plant height, seed index, seed per plant, seed yield per plant and protein indicating the presence of additive gene action for the expression of these traits and selection in next population based on these traits would be ideal. Traits with high values of heritability coupled with moderate genetic advance as

per plant, calcium content, phosphorus content, magnesium content, iron content and zinc contents uggesting that selection for improvement of these characters may be rewarding. It also indicates the greater role of non-additive gene action in their inheritance.

Cluster analysis

The non-hierarchical Euclidean cluster analysis using Mahalanobis statistic, grouped the genotypes into five distinct clusters among which two clusters were polygenotypic and three clusters were monogenotypic (Fig 1). Cluster I was the largest group comprising of 15 genotypes *viz.*, Himpriya, IC 323723, IC 341674, Sangla B 5, IC 345059, Sangla B 444, IC 341589, IC 109729, IC 318859, IC 371665, IC 42430, Himgiri 109728, IC 37288, IC 341667 and IC 47929. This cluster was characterized with highest primary branch per plant, inflorescence length, leaf per plant, seed per plant, seed yield per plant, magnesium content, iron content and zinc content.

Most of the high yielding germplasm along with widely grown variety Himpriya were grouped under this cluster. Hence this group may be used for selection of genotypes with high grain and foliage yield. Cluster II had six genotypes viz., Sangla B 214, EC 286377, IC 356112, IC 46160, IC 341683 and Chitkul while Cluster III, IV and V comprised of single genotype each viz., Sangla B 129, IC 26755 and Shimla B1, respectively indicating towards the diverse origin of these genotypes. Cluster II is composed of genotypes having high amount of protein, calcium content and phosphorus content that can be used as a source for introgression of genes responsible for high nutrition. The intra-cluster distance varied from 32.82 (cluster II) to 31.00 (cluster I), whereas it was zero for the monogenotypic clusters (Table 3). The inter-cluster distance ranged from 35.11 (clusters IV and V) to 92.24 (clusters II and V). Rana and Sharma (2000) also found that inter-cluster distances were greater than intra-cluster distances in forty-three buckwheat genotypes; similar results were also obtained by Deng et al. (2011) in tartary buckwheat and by Rana et al. (2023) in cauliflower.

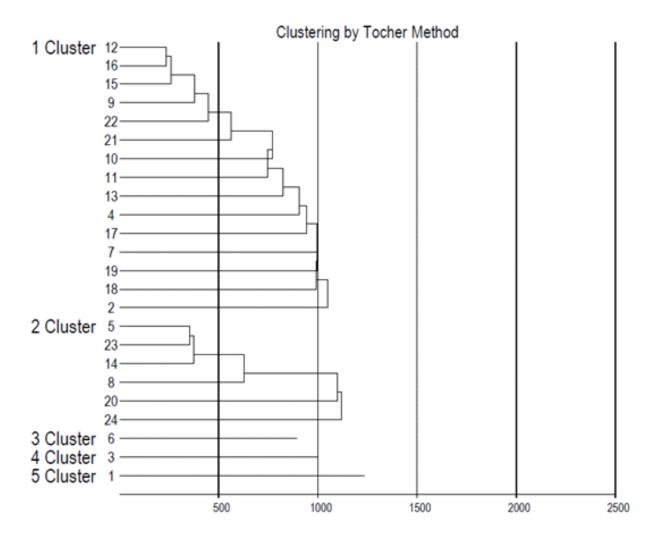


Fig. 1 Dendrogram of 24 buckwheat genotypes constructed using Tocher's method

 $1= Shimla \ BI \ 2= Himpriya, \ 3=IC \ 26755, \ 4= Sangla \ B \ 444, \ 5= Sangla \ B \ 214, \ 6= Sangla \ B \ 129, \ 7= Sangla \ B \ 5, \ 8=IC \ 46160, \ 9= Himgiri \ 109728, \ 10=IC \ 318859, \ 11=IC \ 109729, \ 12=IC \ 47929, \ 13=IC \ 341589, \ 14=IC \ 356112, \ 15=IC \ 37288, \ 16=IC \ 341667, \ 17=IC \ 345059, \ 18=IC \ 323723, \ 19=IC \ 341674, \ 20=IC \ 341683, \ 21=IC \ 371665, \ 22=IC42430, \ 23=EC \ 286377, \ 24=Chitkul$

Table 3. Average intra and inter-cluster distances of D² among clusters

Cluster	I	II	III	IV	V
I	31.00	39.50	38.30	49.28	69.63
II		32.82	41.09	71.36	92.24
III			0.00	61.82	79.38
IV				0.00	35.11
V					0.00

Principal Component Analysis (PCA)

PCA reduces the dimensionality of data by converting the original variables into a smaller set of variables while retaining the essential information from the initial variables. The results of PCA confirmed the findings of cluster analysis. Six principal components, PC1 to PC6, which were extracted from the original data and having eigenvalue greater than one, accounted for nearly 78.58% of the total variation.

According to eigen vector analysis, the observed

variation for first, second, third, fourth, fifth and sixth principal component were about 26%, 15.44%, 11.95%, 10.44%, 8.35% and 6.40%, respectively (Table 4).

In the first principal component, number of seed per plant (0.40) and seed yield per plant (0.40) were the most contributing traits whereas days to 80%

maturity (0.42), calcium (0.41) and iron (0.38) were the principal traits of the second principal component. Genotypes no. 1, 2, 3, 10, and 24 in the PCA biplot stood out from the other genotypes, which may be the result of their unique ancestry and extensive variance in morphological traits (Fig 2). Rana and Sharma (2000) revealed direct positive effects of 100-seed

Table 4. Eigenvectors for the first six components of 17 morphological and biochemical traits in buckwheat genotypes

Schot	Schotypes								
	PC1	PC2	PC3	PC4	PC5	PC6			
Eigenvalue	4.42	2.63	2.03	1.77	1.42	1.09			
% variance	26.00	15.44	11.95	10.44	8.35	6.40			
Cumulative	26.00	41.44	53.39	63.83	72.18	78.58			
DTF	-0.05	0.37	0.02	0.44	-0.08	-0.18			
DTM	-0.17	0.42	-0.08	0.29	-0.12	-0.31			
LPP	0.23	-0.04	-0.14	0.30	0.55	-0.04			
PH	0.25	0.27	-0.01	-0.01	0.48	0.09			
PB	-0.05	0.18	-0.17	0.03	0.08	0.77			
IL	0.34	-0.08	0.09	-0.07	-0.06	0.10			
IPP	0.34	-0.03	0.18	0.03	-0.21	-0.09			
SI	-0.16	0.10	0.30	0.44	-0.11	0.34			
SPP	0.40	-0.06	0.22	-0.08	-0.17	0.06			
SYPP	0.20	0.09	0.33	-0.05	0.41	-0.17			
YPP	0.40	0.03	0.19	0.07	-0.22	0.18			
Ca	-0.13	0.41	0.17	-0.33	0.03	0.10			
Fe	0.27	0.38	-0.01	0.07	-0.30	0.04			
Mg	0.21	0.25	-0.34	-0.31	-0.14	-0.19			
P	-0.07	0.25	0.51	-0.14	0.13	-0.09			
Zn	0.27	0.18	-0.46	0.09	0.03	0.03			
Protein	-0.17	0.28	-0.04	-0.43	0.04	0.09			

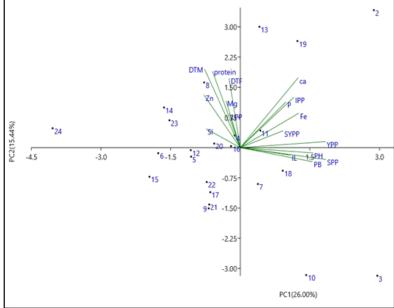


Fig 2. Principal component analysis of 24 buckwheat genotypes using morphological and biochemical traits

weight on seed yield. Joshi (2005), Dutta *et al.* (2008) and Bisht *et al.* (2018) also observed similar results.

Conclusion

In the resent study, PCV values were higher than the GCV values for all the traits which indicates the environmental role in trait expression. Highest GCV and PCV were recorded for seed yield per plant, seed per plant and protein which shows that these traits can be improved through these genotypes. Cluster analysis using Mahalanob is statistic, grouped the genotypes based on Jaccard's coefficient, into five distinct clusters shows that there are 3 groups intercrossed for heterosis breeding. First cluster was characterized with highest primary branch per plant, inflorescence length, leaf per plant, seed per plant, seed yield per plant, magnesium content, iron content and zinc contents how this cluster carries genotypes that can be utilized for these traits. The results of PCA confirmed

the findings of cluster analysis. Six principal components showed eigen value more than one where first principal component showed maximum number of seed per plant and seed yield per plant genotypes in this group which could be exploited for their direct release as a variety(s) after testing under wide range of environments. Moreover, these genotypes can also be used as parents in hybridization programs to develop high yielding buckwheat varieties.

Acknowledgement: The authors would like to express their gratitude to the Heads, Department of Genetics and Plant Breeding, CSK HPKV, Palampur (H.P.) and Department of OrganicAgriculture and Natural Farming, CSK HPKV, Palampur (H.P.) for providing financial supportand research facilities and NBPGR for providing the required germplasm.

Conflicts of Interest: The authors declare that there is no conflict of interest.

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