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In-depth morphological assessment revealed significant genetic variability in common buckwheat (*Fagopyrum esculentum*) and tartary buckwheat (*Fagopyrum tataricum*) germplasm

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Abstract

Buckwheat (*Fagopyrum* spp.) is an important crop in the high-altitude regions of the Northwest Indian Himalayas. The agro-climatic heterogeneity of this region offers a great deal of diversity in the agro-morphology of buckwheat species. In this study, a total of 61 accessions of *Fagopyrum esculentum* and *Fagopyrum tataricum* were characterized for 17 morphological (8 qualitative and 9 quantitative) traits. Significant differences ($P < 0.0001$) among all the traits were revealed by one-way analysis of variance. Further, significant phenotypic variability in both qualitative as well as quantitative traits was also observed. Both positive and negative correlations were observed between the traits of agronomic relevance. The principal component analysis (PCA) reveals about 69% variability among the first six components. The accessions were divided into two key clusters with numerous subclusters by considering the unweighted pair group method with arithmetic mean dendrogram. A cluster of 19 accessions was formed utilizing a PCA scatter plot indicating accessions with maximum values for important quality traits like plant height, leaf blade width, stem colour (red), primary branches, inflorescence length, flower colour (greenish-yellow), seed anthocyanin colour (green), seed shape (ovate) and seed weight. These accessions can be of vital significance for future buckwheat breeding programmes. The findings from the current study will form a favourable base for genetic resource management, improved cultivation and applications of buckwheat at the commercial level in the northwestern Himalayas of India.

Introduction

Buckwheat (*Fagopyrum* spp.) is an important non-poaceous pseudocereal belonging to the family Polygonaceae (Campbell, 1997). Based on morphological features, the Polygonaceae family which is morphologically assorted and disseminated was divided into two classical subfamilies, *Polygonoideae Eaton* and *Eriogonoideae Arn* (Van Leeuwen *et al.*, 1988) supported by molecular phylogenies with a comprehensive sampling of genera (Wang *et al.*, 2017). Out of the nearly 30 species belonging to the genus *Fagopyrum*, only two species *viz.*, *Fagopyrum esculentum* (common buckwheat) and *Fagopyrum tataricum* (tartary buckwheat) are cultivated (Christa and Soral-Śmietana, 2008). *F. esculentum* is cultivated primarily in the temperate regions of the northern hemisphere, *F. tataricum* is predominantly a crop of higher altitudes (Rana, 2004). In India, buckwheat is cultivated along the Himalayan foothills from Ladakh in the North to Arunachal Pradesh in the East (Ganeshpurkar and Saluja, 2017). It is a traditional food crop in China and has numerous advantages, such as a small growth period, greater adaptability and resistance to punitive environments where the chief food crops find it difficult to endure (Jacquemart *et al.*, 2012; Kumari and Chaudhary, 2020). Tartary buckwheat is an annual self-pollinated species. Seeds of tartary buckwheat have strongly adhering hulls. Despite the self-compatible nature and high rutin content, tartary buckwheat has not received much favour for cultivation because of the tightly adhering hull and bitter taste (Chrungoo and Chettry, 2021), whereas, common buckwheat (*F. esculentum* (L.) Moench) is considered as a nutritionally rich crop with its demand increasing significantly over the years, however, it has a lower yielding capacity as compared to other crops (Ohsawa, 2020) due to its shorter growing period (Sato *et al*., 2001). Buckwheat is also recognized for its excellent protein quality and rich in countless rare constituents such as flavonoids and phytosterols that have been reported to have serious healing benefits in relation to treatment of chronic disorders (Zhang *et al*., 2021) and play a vital role in anti-oxidation pathways. Bioactive compounds, such as rutin, quercetin and many other antioxidants present in buckwheat, help to reduce oxidative stress in the body, leading to better health for consumers. Moreover, buckwheat has a high quantity of soluble fibre, which helps regulate bowel movement and controls hypercholesterolemia (presence of high plasma cholesterol levels), hypertension (high blood pressure) and cardiovascular diseases (Shahbaz *et al*., 2022). Buckwheat also has been paid consideration owing to its feeding worth in contemporary times due to its extraordinary content of starch, microelements, lysine and methionine (Bonafaccia *et al*., 2003). Although difficulty in identification of buckwheat species hampers its operational assessment and application, still in recent times, commercial application of buckwheat has seen a plethora of research attention. Furthermore, the dietary qualities of crops are directly affected by innumerable environmental situations and agricultural practices (Podolska *et al*., 2019; Bellaloui *et al*., 2020).

For plant breeders, the foremost challenge is to improve extraordinary yielding diversities compatible with diverse farming environments thereby, paving a strong foundation for the morphological research in buckwheat. The accessibility of diversity data and population structure in buckwheat germplasm is an imperative genetic reserve to mine the genes that may promote accomplishing food as well as nutritious security (Sabreena *et al*., 2021). As such, evaluation of buckwheat germplasm for beneficial characters is essential to augment their utility in crop advance programmes.

For an assortment of accessions with advanced levels of phenolic constituents, dissimilarities in morphological characters can assist as valued indicative traits (Li *et al*., 2012). The current study was planned to analyse both quantitative and qualitative traits in cultivated buckwheat accessions. Most of the accessions (59) evaluated in the present study have been procured from NBPGR, New Delhi and others (02 accessions) have been collected from dissimilar regions of North West Himalayan regions of Jammu & Kashmir and Ladakh.

Materials and methods

Plant material and growth conditions

Sixty-one accessions of buckwheat mostly from NBPGR, New Delhi and the remaining collected from North Western Himalayas of J&K and Ladakh, India were sown at the experimental field of the SKUAST-Kashmir, Shuhama Campus, on 22 May 2019 and 7 June 2020 as shown in online Supplementary Table S1. Each germplasm line was sowed in three lines of 6 × 1.5 m systematized in a split-plot design. No fertilizers (N, P, and K) or pesticides were used during the cultivation. Buckwheat was cultivated on loamy soil, which characterized the region. Irrigation was given twice (once 5 days after sowing and second 1 month after sowing).

Plant description

The Buckwheat Descriptor of standard DUS guidelines for buckwheat Protection of Plant Varieties and Farmer's Rights Authority, Govt. of India (PPV & FRA, 2012) (https://itestweb.in/ppvf/crop-dus-guidelines) was used to make morphological observations. Seventeen traits (9 quantitative and 8 qualitative) were collected over 2 years (representative pictures of some qualitative traits are provided in the online Supplementary picture). Digital calipers were used to measure leaf length and breadth, petiole length, plant height, and inflorescence length. Similarly, the features including the number of primary and secondary branches on the main stem, number of leaves per plant, petiole length, flower colour, stem colour, leaf colour, leaf margin colour, petiole colour, seed anthocyanin colour, seed colour, and seed shape as shown in online Supplementary Table S2 were measured based on ranking and coding. Furthermore, the seed weight was measured via sensitive balance.

Data analysis

Excel 2013 (Microsoft Office package) was utilized to determine the descriptive statistics (mean, median, minimum value, maximum value and standard deviation) with a coefficient of variation evaluated as a variability indicator. Analysis of variance (ANOVA) and principal component analysis (PCA) were used for both quantitative as well qualitative traits using SPSS v20.0 (IBM SPSS Statistics, 2011). Based on such analysis, a scatter plot was formed employing PAST software (Hammer *et al*., 2001). Furthermore, unweighted pair group method with arithmetic mean based on the Euclidean distance coefficient was utilized for multivariate cluster analysis using PAST software.

Results

Morphological characterization based on quantitative traits

Sixty-one buckwheat accessions were sown at the experimental farm of SKUAST-K Shuhama, Kashmir during 2019 and 2020. Based on one-way ANOVA and Fisher test results (probability level $P < 0.0001$) at the 1% threshold, all nine quantitative characteristics indicated significant differences (online Supplementary Table S3). The descriptive statistics of nine quantitative traits based on UPOV and FRA guidelines are given in Table 1. Plant length ranged from 18.8 to 187.6 cm with an average of 95.99 cm, leaf blade length from 2.02 to 7.18 cm with an average of 4.57 cm, and leaf width from 1.92 to 6.68 cm with an average of 4.60 cm among the 61 accessions. Similarly, the inflorescence length ranged from 0 to 1.8 with an average of 0.75 cm and leaf number/plant varies from 7.6 to 95 with a mean value of 65.00 other than the number of primary and secondary branches ranged from 1.4 to 6.4 with an average of 2.98 cm and 2.2 to 11 with an average of 7.88 cm, respectively. Petiole lengths ranged from 0 to 6.6 cm, with an average of 2.43 cm. Furthermore, seed weights ranged from 1.29 to 3.68 g with an average of 2.19 g. Among the analysed quantitative traits, the highest coefficient of variance (CV%) was shown by petiole length (67.60%) followed by inflorescence length (45.54%), primary branches per plant (37.84%), leaf number per plant (33.27%) and plant height (31.72%). In disparity, quantitative characters such as secondary branches per plant (24.23%), leaf blade width (24.12%) and seed weight (19.25%) indicated the least CV%.

Table 1. Descriptive statistics related to the 14 polymorphic morphological variables among the 62 buckwheat accessions (minimum, maximum and mean values measured for each variable, SD, standard deviation; CV, coefficient of variation)

Trait	Abbreviation	Min	Max	Mean	SD	CV%
Quantitative traits						
Plant: length	PHP	18.8	187.6	95.99	30.45	31.72
Primary braches/plant	PBP	1.4	6.4	2.98	1.13	37.84
Secondary braches/plant	SBP	2.2	11	7.88	1.91	24.23
Leaf number/plant	LNP	7.6	95	65.00	21.62	33.27
Leaf blade: length	LBL	2.02	7.18	4.57	0.97	21.26
Leaf blade: width	LBW	1.92	6.68	4.60	1.11	24.12
Inflorescence: length	ILP	0	1.8	0.75	0.34	45.54
Petiole: length	PLP	0	6.6	2.43	1.64	67.60
Seed weight	SWS	1.29	3.68	2.19	0.42	19.25
Qualitative traits						
Flower colour	FCP	1	3	1.85	0.81	43.90
Stem colour	SCP	1	2	1.16	0.37	32.07
Leaf colour	LCP	1	2	1.05	0.22	20.78
Leaf margin colour	LMC	1	2	1.28	0.45	35.35
Petiole colour	PCP	1	2	1.69	0.47	27.65
Seed anthocyanin colour	SAC	1	2	1.43	0.50	34.96
Seed colour	SCC	1	3	2.00	0.75	37.64
Seed shape	SSP	1	3	1.93	0.65	33.86

Morphological characterization based on qualitative traits

Eight qualitative characters were recorded to characterize the buckwheat accessions. Overall, the maximum CV% among the qualitative characters was revealed by flower colour (43.90%), seed colour (37.64%), leaf margin colour (35.35%), seed anthocyanin colour (37.64%), and seed shape (33.86%). Moreover, the low CV% was shown by leaf colour and seed weight (20.78% and 19.25%), respectively, as shown in Table 1. The majority of the accessions exhibited a prevalence of greenish-yellow flower colour (41%) followed by pink (32.8%) and white (26.2%). The bulk of the accessions showed red stem colour (83.6%), whereas the rest of the accessions revealed green colour (16.4%). Three types of seed colour including grey-green (27.9%), grey-brown (44.3%), and black (27.9%) were detected. Most of the accessions displayed a prevalence of green leaf colour (95.1%) and a few showed red (4.9%). More than half of accessions showed green anthocyanin colouration (57.4%) whereas the rest showed pink colour (42.6%) and petiole colour was differentiated into two colours pink (68.9%) followed by green (31.1%). Of the entire 61 accessions of buckwheat, 27.9% had seeds with elliptic shapes, whereas 57.4% had ovate shapes, and 18% had trullate seed shapes as shown in Table 2.

Correlations between morphological traits

Among the various quantitative traits, significant correlations were observed as described in online Supplementary Table S4. Leaf blade width exhibited a higher significant positive correlation with leaf blade length ($r = 0.73$), and petiole colour with leaf margin colour ($r = 0.60$). Primary branches also exhibited a positive correlation with plant height ($r = 0.54$). There were also negative correlations by the contrast between seed anthocyanin colour with flower colour ($r = -0.52$), and plant height ($r = 0.45$). Furthermore, leaf number showed a significant positive correlation with secondary branches ($r = 0.49$), plant height ($r = 0.41$), and primary branches ($r = 0.33$). In addition, seed shape also indicated a positive correlation with plant height ($r = 0.40$) and primary branches ($r = 0.37$). Stem weight is also significantly correlated with flower colour ($r = 0.51$).

Cluster analysis

The dendrogram created by merging data from quantitative and qualitative morphological features revealed two primary clusters: clusters I and II (Fig. 1). Cluster I was subdivided into IA and IB. Cluster IA is further divided into I-Aa and I-Ab. I-Aa has 13 accessions, I-Ab comprises two accessions and cluster I-B is further divided into two subclusters I-Ba and I-Bb. I-Ba comprises 12 accessions while I-Bb has 33 accessions. Cluster II has only one accession that is the smallest among all accessions and belongs to *tartaricum*. The main cluster I accessions are with large-sized plant, flower and seed dimensions and are more homogeneous while cluster II accessions are with smaller plant, flower and seed dimensions.

PCA based on morphological traits

Greater than 69% of the variability by employing PCA was revealed by the first six components. Of the total variation, the first three PC components exhibited a 45.818% variance

Table 2. Frequency distribution for the qualitative characters in the studied 61 buckwheat accessions

Trait code and frequency	Frequency unit	1	2	3
Flower: colour	No %	Greenish yellow(25) 41.00%	Pink (20) 32.80%	White(16) 26.20%
Stem: colour	No %	Red(51) 83.60%	Green(10) 16.40%	_
Leaf : colour	No %	Green(58) 95.10%	Red(3) 4.90%	_
Leaf margin: colour	No %	Pink(44) 72.10%	Green(17) 27.90%	_
Petiole: colour	No %	Green(19) 31.10%	Pink(42) 68.9%	_
Seed: anthocyanin colour	No %	Green(35) 57.40%	Pink (26) 42.60%	_
Seed: colour	No %	Grey-green(17) 27.90%	Grey-brown(27) 44.30%	Black (17) 27.90%
Seed: shape	No %	Elliptic(15) 24.60%	Ovate(35) 57.40%	Trullate (11) 18.00%

(21.09% on PC1, 13.199% on PC2 and 11.529% on PC3). The components (PC4–PC6) elucidated only 21.79% of the total variance (Table 3). Traits that indicated principal component loading >0.50 were considered significant for each factor. The first PC (21.09%) included plant height, primary branches, flower colour, seed shape and seed weight. The PC2 (13.199%) was correlated with the length of the leaf blade length, leaf blade width, and petiole length. The PC3 (11.529%) was correlated with leaf blade length, leaf margin colour, and petiole colour. Based on the first two components which contribute 34.289% of the total variance, a PCA scatter plot was designed (Fig. 2), where buckwheat accessions based on their phenotypic traits were grouped together. PC1 is greatly linked with plant height, primary branches and flower and seed dimensions. Positive values of PC1 resembled the large plant height with more primary branches with more seed weight, whereas negative values are linked to the small plant height with fewer primary branches. Similarly, PC2 is extremely correlated with leaf blade width and seed colour. In the scatter plot, the development of two distinct groups can be seen; 19 accessions on the right plane resemble most with the highest plant height, primary branches, flower colour, seed shape, seed weight, seed colour, and leaf blade width were positioned more dispersedly. The remaining accessions on the left side of the plane showed less similarity in the above dimensions.

Discussion

Germplasm description based on morphological and agronomic characters is of notable prominence for the commencement of crop breeding programmes and documentation of advanced accessions (Pereira-Lorenzo *et al.*, 2012; Zargar *et al.*, 2021). Sixty-one buckwheat accessions were studied in the present study based on nine quantitative and eight qualitative traits. These traits were selected owing to their expedient research by scientists across the world (Facho *et al.*, 2016; Wang *et al.*, 2017; Misra *et al.*, 2019; Rauf *et al.*, 2020; Aubert *et al.*, 2021). The current study's findings revealed that diversity-based assessment using phenotypic features can be effectively used to document high-quality accessions as well as the genetic links between different types. The present investigation advocates that substantial phenotypic variability occurs within the buckwheat accessions. In terms of quantitative traits, a large variance was recorded for plant height among 61 accessions. The minimum plant height was recorded in BWM-15 (15 cm) and the maximum was recorded in BWM-35 (192 cm). Similarly, the number of primary branches per plant ranged from 1.4 to 6.4 and the minimum was shown by BWM-15 and the maximum by BWM-35. The study conducted by Misra *et al.* (2019) reported the number of primary branches per plant varied from 2.8 to 16.0 in *F. esculentum* germplasm and from 8.2 to 18.2 in *F. tataricum* germplasm. Significant variation was recorded for plant height in both species, while the tartary buckwheat accessions recorded the lowest average plant height. A similar range of variation was also recorded in the north-west Indian Himalayan buckwheat germplasm for primary branches plant, plant height and seed yield plant (Senthilkumaran *et al.*, 2008). Moreover, the recorded range for leaf length and leaf width was 2.02–7.18 and 1.92–6.68, respectively. The 1000-seed weight (TSW) ranged from 1.29 to 3.68 g with a mean of 2.19 g. Among the 17 characters investigated by Yamane and Ohnoshi (2003) in *F. cymosum*, only seed weight has been found as a significant trait to differentiate between diploid and tetraploid cytotypes. Similarly, Misra *et al.* (2019) reported smaller seeds with the lowest average of 1.8 g per TSW among 97 buckwheat landraces. Among the qualitative characters, considerable variations between flower colour, petiole colour, leaf margin colour, and seed shape were detected in almost all of the documented characters. While in north-west Indian buckwheat landraces, major variations have been previously recorded for

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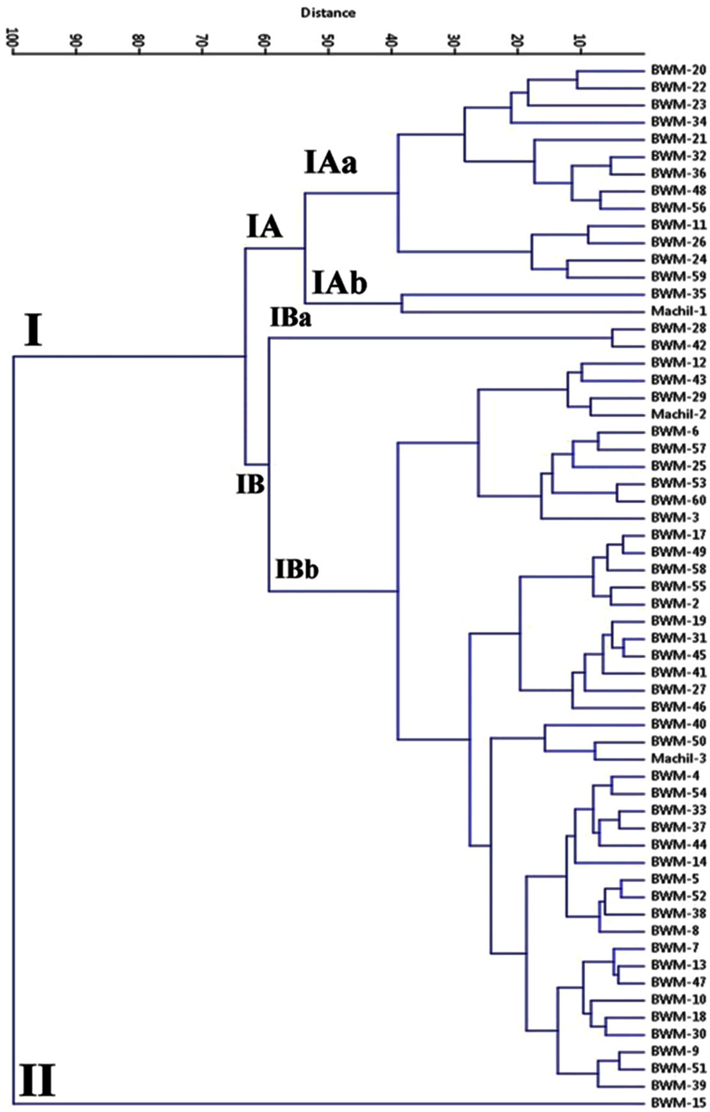

Figure 1. Hierarchical clustering of the 61 buckwheat accessions based on UPGMA method using Euclidian distance.

seed shape and seed colour (Senthilkumaran *et al.*, 2008). In the present experimental investigation, the majority of accessions displayed the prevalence of greenish-yellow colour followed by white, whereas few accessions had pink flower colour. The colour of plant vegetative organs as well as their flavonoid content is affected by different factors (variety, area of collection and ecological conditions) (Fabjan *et al.*, 2003; Podolska, 2016). In the current study, the prominent stem colour was green and red. The results from the study conducted by Kapoor *et al.* (2018) revealed that red and pink stem colour in buckwheat germplasm can be attributed to anthocyanin accumulation which is in line with the assumptions and findings of Fang *et al.* (2019). However, in our study, the seed coat colour varied from grey-green and grey-brown followed by the black group. A study regarding seed coat colour conducted by Rauf *et al.* (2020) revealed that 72.51% (182) accessions exhibited pale-brown colour and 69 of total accessions, i.e. 27.49% displayed dark-brown colour. However, a study conducted by Raina and Gupta (2015) revealed different colours of the seed coat (brownish-yellow, brown, dark-brown, grey-black) in buckwheat germplasm. Furthermore, we observed the seed shape for 61 accessions showed a dominant shape of elliptic 26.4%, ovate (egg shape) 57.4% and trullate 18.0% (triangular-shaped). A similar observation was found by Rauf *et al.* (2020) that revealed ovate seeds in 94.83% (238) of accessions, triangular seeds in 2.79% (7) and winged-form seeds in 2.39% (6). The results obtained from the present study are reliable and consistent with the findings concerning the above-mentioned studies. Similarly, Baniya *et al.* (1995) revealed that 92% of the germplasm evaluated had smooth-type seeds, whereas 8% had winged-form seeds. The observations revealed by the studies indicate that common buckwheat germplasm poses considerable diversity in terms of morphological traits. One possible reason can be the outcrossing, mediated via insect cross-pollination (Iwata *et al.*, 2005; Grahic *et al.*, 2016). Furthermore, the interpretation of the influence of agrometeorological factors on buckwheat yield is complicated due to its low tolerance to temperature limits, its sensitivity to drought and excess rainfall, auto incompatibility, and dependence on pollinators.

The correlation coefficient between the morphological characters can serve as a useful indicator for the genetic improvement of different traits. From the analysis of quantitative and qualitative traits, the present study found interesting correlations and relationships among the studied parameters. For instance, there

Table 3. Eigen vectors of principal component axes from PCA for the morphological traits in the studied genotypes of buckwheat

Trait	Components					
	1	2	3	4	5	6
PHP	0.706*	0.152	−0.346	0.194	−0.039	0.144
PBP	0.637*	0.119	−0.27	0.196	0.085	0.07
SBP	0.264	0.393	0.032	0.599*	−0.288	0.053
LNP	0.399	0.34	−0.148	0.61*	−0.223	−0.224
LBL	0.434	0.454	0.55*	−0.181	0.172	−0.239
LBW	0.056	0.719*	0.579*	−0.13	0	−0.162
ILP	0.386	−0.069	0.442	−0.143	−0.092	0.638
PLP	0.155	0.467	0.331	−0.194	−0.165	0.213
FCP	0.592*	−0.418	0.097	−0.079	0.387	−0.078
SCP	−0.382	0.503*	−0.223	−0.028	0.504*	0.024
LCP	−0.421	0.29	0.129	0.061	0.396	0.095
LMC	−0.077	−0.467	0.542*	0.449	0.172	0.22
PCP	0.374	0.271	−0.527	−0.563	−0.01	0.073
SAC	0.755*	−0.138	−0.012	−0.158	0.237	−0.278
SCC	0.064	0.131	−0.123	0.424	0.638*	0.121
SSP	0.597*	0.063	−0.111	−0.115	0.082	0.403
SWS	0.582*	−0.383	0.357	−0.042	0.005	−0.328
Total	3.586	2.244	1.96	1.663	1.267	1.045
% of Variance	21.097	13.199	11.529	9.784	7.454	6.148
Cumulative %	21.097	34.296	45.825	55.61	63.063	69.211

*Eigen values are significant ≥0.50.
Refer to Table 1 for the code of traits.

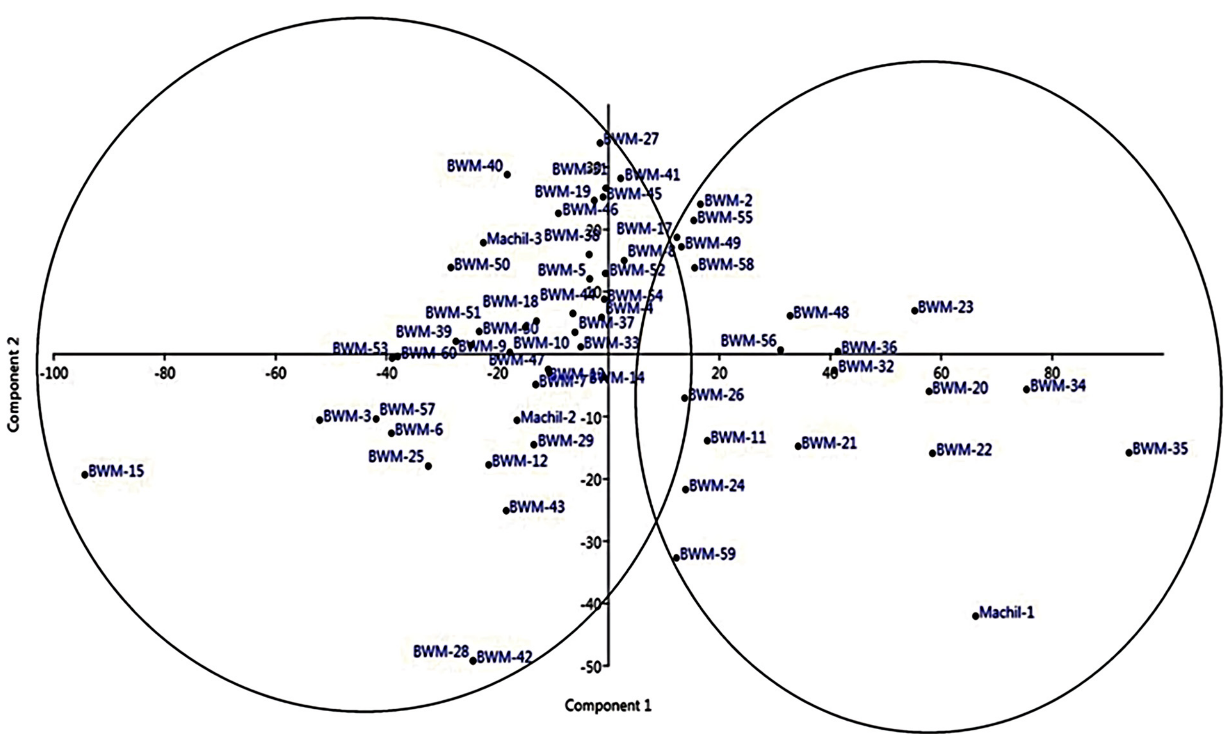

Figure 2. Scatter plot showing the distribution of 61 buckwheat accessions in three clusters based on the first two components PC1 (21.09%) and PC2 (13.199%).

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were positive correlations among different plant traits such as plant height, primary branches, flower colour, seed shape, and seed weight. In the current investigation, PCA analysis reported that the first two components showed 34.289% of the total variance from the different parameters. The early shift from vegetative to reproductive growth, which is considered meager vegetative growth, could be the cause. Moreover, there is also a significant correlation between leaf blade length, leaf margin colour, and petiole colour. Similar observations have been reported in the study conducted by Cepková *et al.* (2009). The dissimilar genetic alignments of a heterogeneous population may be the possible reason for variation in correlation coefficients. The significant positive association between different pairs can be useful for the genetic development of diverse traits. The requirement of both traits and the characters that are negatively correlated cannot be enhanced in a single step. Traits having no significant correlation indicated their independent nature (Chauhan *et al.*, 2020). In the current study, both cluster and PCA revealed similarity among various morphological traits present in the buckwheat germplasm of the north-west Indian Himalayas. The data generated from the present study can serve as useful documentation for plant breeders and will help them to select promising traits and accessions for boosting necessary improvements in buckwheat breeding.

Conclusion

The evaluation of buckwheat accessions revealed a considerable diversity among the morphological traits which varied significantly. Sixty-one accessions were selected for this study, which might be utilized as breeding material to boost grain production, rutin and quercetin content in existing buckwheat cultivars. The design of the species differed mostly in terms of the number of branches, inflorescences per plant, number of flowers per inflorescence, and seed weight. Results obtained from the current study indicate that high management practices enhance the growth of reproductive parts which results in the increment of grain yield of buckwheat. Accessions (BWM-2, BWM-55, BWM-56, BWM-48, BWM-26, BWM-11, BWM-24, BWM-59, BWM-36, BWM-32, BWM-23, BWM-20, BWM-22, BWM-34, BWM-35, BWM-21, Machil-1) reported the highest values in terms of plant height, primary branches, leaf blade width, flower colour (greenish-yellow), seed anthocyanin colour (green), seed weight, seed shape (ovate) and stem colour (red). They can be considered exceptional accessions and employed for direct cultivation as well as future buckwheat breeding programmes to improve quality. Furthermore, the comparison and discussion of the obtained results in the current study with the previously published data will serve as a solid foundation for forthcoming buckwheat breeding and the production of high-yielding spring buckwheat cultivars.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S1479262123000321.

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Conflict of interest. None.

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