**Genetic Diversity and Trait Association in Tartary Buckwheat (*Fagopyrum tataricum*) Using Mahalanobis D² Analysis in the Cold-Arid Region of Kinnaur, Himachal Pradesh, India**

**Abstract**

Buckwheat, a member of the genus Fagopyrum Mill. under the family Polygonaceae, is widely distributed across Asia and Europe. A total of 22 accessions of Tartary buckwheat with some local germplasm were analysed by cluster analysis using 6 quantitative characters to group them based on the similarity at the phenotypic level and to identify the most relevant characters. The study aims to successfully reveal significant genetic diversity among the evaluated genotypes of Tartary buckwheat (*Fagopyrum tataricum*) collected from the cold-arid region of Kinnaur, Himachal Pradesh, India, using Mahalanobis D² Analysis. The analysis of variance (ANOVA) showed significant genetic variability among the evaluated genotypes across all traits studied. Among the studied genotypes, Shimla B1 was found to have maximum leaves per plant, plant height, biological yield and straw yield per plant. The genotypic variance for all the traits was lower than the phenotypic variance. High phenotypic and genotypic variation, heritability, and genetic advance were observed for leaf per plant and harvest index. Straw yield per plant showed a strong and positive correlation with leaves per plant, plant height, biological yield and harvest index. The nonhierarchical Euclidean cluster analysis using Mahalanobis's statistic grouped the genotypes into two distinct clusters, among which one was polygenotypic, and one was monogenotypic. Among all the genotypes studied, Shimla B1 and IC 341589 were found to be the best and recommended for further testing and evaluation. This research emphasises the importance of conserving and utilising local landraces of buckwheat for sustainable crop improvement, especially in ecologically fragile hill regions like Kinnaur.

**Keywords**: Tartary Buckwheat, Mahalanobis D² Analysis, Genetic Diversity, Himachal Pradesh, Germplasm, Asia

**Introduction**

In the present era, food scientists are concerned about exploiting functional crops with nutraceutical properties. Buckwheat is one of the functional pseudocereals with nutraceutical components used in the treatment of health-related diseases, malnutrition, and celiac diseases. As a preferred diet as a gluten-free product for celiac diseases, buckwheat is a good source of nutrients, bioactive components, phytochemicals, and antioxidants (Sofi et al., 2023). Buckwheat, a member of the genus *Fagopyrum* Mill. under the family Polygonaceae, is widely distributed across Asia and Europe. Of the many species in the genus, only two—*Fagopyrum esculentum* (common buckwheat) and *Fagopyrum tataricum* (Tartary buckwheat) are cultivated on a significant scale. Common buckwheat is a self-incompatible species, primarily grown in countries such as China, the Russian Federation, Ukraine, and Kazakhstan (Campbell, 1995). Major exporters include China, Brazil, France, the USA, and Canada, while Japan is the leading global importer. Buckwheat can become an important crop in the feeding of mankind and domestic animals to meet the ever-increasing demands of a rapidly expanding population (Ashoka et al., 2023).

Tartary buckwheat, in contrast, is self-pollinating and is typically grown in various regions worldwide. However, it is mostly used or marketed locally. Owing to its strong tolerance to frost, *F. tataricum* is mainly cultivated in the high-altitude mountainous regions of Asia and, to a lesser extent, in other areas. Tartary buckwheat cultivation takes place at high altitudes around the Himalayas, in China, Nepal, Bhutan, India, and Pakistan, as well as in some other countries: Korea, Japan, Kazakhstan, Russia, Ukraine, Belarus, Sweden, Poland, Italy, Luxembourg, and Slovenia (Kreft et al., 2023). In many such regions, common buckwheat, which is less frost-tolerant and has lower yields, is gradually being replaced by Tartary buckwheat. This trend is particularly noticeable in parts of India, Nepal, and China, where common buckwheat cultivation is decreasing while Tartary buckwheat remains stable or is expanding. In the hill regions of Himachal Pradesh, farmers continue to grow various landraces of Tartary buckwheat, including some unique types such as Bhate Phaper (rice Tartary buckwheat), which is notable for its non-adhering hull (Bhardwaj and Kaur, 2020).

Despite its value, the cultivation area of buckwheat has declined over time. This reduction is mainly due to its replacement by more profitable crops like apple, green pea, hops, and potato, as well as the limited availability of high-yielding, stress-resistant varieties with good grain quality (Joshi and Rana, 1997). Tartary buckwheat contains a wide range of bioactive compounds, such as flavonoids, phenolic acids, triterpenoids, phenylpropanoid glycosides, bioactive polysaccharides, and bioactive proteins and peptides, as well as D-chiro-inositol and its derivatives. Consumption of Tartary buckwheat and Tartary buckwheat-enriched products is linked to multiple health benefits, e.g., antioxidant, anti-inflammatory, antihyperlipidemic, anticancer, antidiabetic, antiobesity, antihypertensive, and hepatoprotective activities (Zou et al., 2023). Therefore, breeding efforts are essential to improve its productivity. A foundational step in buckwheat breeding involves classifying and evaluating a wide range of germplasm based on multiple traits. Proper characterisation, documentation, and conservation of genetic resources are critical to support breeding programs (Singh et al., 2024).

In this context, the present study focused on characterising local germplasm of Tartary buckwheat (*F. tataricum*) collected from the high-altitude regions of the northern Himalayas, along with selected indigenous and exotic accessions. Six key agro-morphological traits were used to evaluate genetic diversity. The findings from this study can help in identifying promising groups of accessions for future hybridisation, organising efficient germplasm collection strategies, understanding variation patterns, and gaining insights into crop evolution.

**Materials and Methods**

**Plant Materials**

A total of 22 accessions of tartary buckwheat were used in the present investigation (Table 1). These were characterised and evaluated in 2023. The accessions were collected from the Regional Station, ICAR-National Bureau of Plant Genetic Resources, Shimla (ICAR-NBPGR) and Chaudhary Sarwan Kumar Krishi Vishvavidyalaya, Palampur (CSKHPKV).

Table 1. List of 22 buckwheat genotypes used in the present study

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **S. No.** | **Genotype** | **Source** | **S. No.** | **Genotype** | **Source** |
| 1 | Shimla B1 | ICAR-NBPGR, Shimla | 12 | IC 47929 | ICAR-NBPGR, Shimla |
| 2 | Himpriya | ICAR-NBPGR, Shimla | 13 | IC 341589 | ICAR-NBPGR, Shimla |
| 3 | IC 26755 | ICAR-NBPGR, Shimla | 14 | IC 356112 | ICAR-NBPGR, Shimla |
| 4 | Sangla B 444 | CSKHPKV, Palampur | 15 | IC 37288 | ICAR-NBPGR, Shimla |
| 5 | Sangla B 214 | CSKHPKV, Palampur | 16 | IC 341667 | ICAR-NBPGR, Shimla |
| 6 | Sangla B 129 | CSKHPKV, Palampur | 17 | IC 345059 | ICAR-NBPGR, Shimla |
| 7 | Sangla B 5 | CSKHPKV, Palampur | 18 | IC 323723 | ICAR-NBPGR, Shimla |
| 8 | IC 46160 | ICAR-NBPGR, Shimla | 19 | IC 341674 | ICAR-NBPGR, Shimla |
| 9 | Himgiri 109728 | ICAR-NBPGR, Shimla | 20 | IC 341683 | ICAR-NBPGR, Shimla |
| 10 | IC 318859 | ICAR-NBPGR, Shimla | 21 | IC 371665 | ICAR-NBPGR, Shimla |
| 11 | IC 109729 | ICAR-NBPGR, Shimla | 22 | IC 42430 | ICAR-NBPGR, Shimla |



**Experimental Design and Location**

The experimental material was evaluated at the Experimental Farm, Mountain Agricultural Research & Extension Centre (MAREC), Sangla, Kinnaur. The farm is located at 31° 29' N latitude and 78° 60' E longitude at a height of 2,621 meters above mean sea level (amsl). The experimental material was sown in a well-prepared field in Randomised Complete Block Design (RCBD) with two checks, viz., Shimla B1 and Himpriya. In a block, two 1m long lines were there with a spacing of 25 cm row to row and 75 cm block to block. For proper drainage, the blocks were raised a little to avoid water stagnation. The recommended cultural practices were followed to grow vigorous plants.

**Data collection and Statistical analysis**

The accessions were characterised on the basis of six agro-morphological traits viz., leaves per plant (LPP), primary branches per plant (PBPP), plant height (PH), biological yield (BY), harvest index (HI) and straw yield per plant (SYPP). Five plants of each genotype in each replication were selected at random (excluding border plants), and phenotypic data were collected for these traits.

Based on the averaged data, descriptive statistics were estimated. Statistical analysis, including analysis of variance (ANOVA), variability parameters, correlation coefficients (both genotypic as well as phenotypic), and path coefficients based on six quantitative, was conducted using Windostat 8.0 software package developed by Indostat Services, Hyderabad, India. The replicated dataset was analysed for variance as per Panse and Sukhatme (1984). Genotypic and phenotypic coefficients of variation were computed as per the method described by Burton and DeVane (1953). Broad-sense heritability and genetic advance as a percentage of the mean were estimated using the approaches of Burton and DeVane (1953) and Johnson et al. (1955). Correlation coefficients at both genotypic and phenotypic levels were calculated according to Al-Jibouri et al. (1958). The genetic divergence amongst genotypes was computed following Mahalanobis' D2 technique (1936), and the genotypes were grouped into different clusters following Tocher’s method described by Rao (1952).

**Results** **and** **Discussion**

**3.1. Analysis of variance (ANOVA)**

Table 2 summarises the ANOVA results for six quantitative traits of tartary buckwheat, evaluated under RCBD. The findings revealed statistically significant differences (p<0.05) among the genotypes for each of the evaluated traits.

Table 2. Analysis of variance for six morphological traits evaluated for 22 buckwheat genotypes

|  |  |
| --- | --- |
|  | **Mean sum of squares** |
| **Traits** | **Replication**  | **Treatments** | **Error** |
| **LPP** | 0.00 | 105.00\* | 0.53 |
| **PBPP** | 0.03 | 0.59\* | 0.01 |
| **PH** | 5.28 | 229.26\* | 5.34 |
| **BY** | 0.05 | 6.46\* | 0.06 |
| **HI** | 0.07 | 123.16\* | 0.82 |
| **SYPP** | 0.02 | 2.80\* | 0.06 |

LPP= leaves per plant, PBPP= primary branches per plant, PH= plant height, BY= biological yield, HI=harvest index, SYPP=straw yield per plant, \* represent significance at 5 % level according to F test

**Mean performance**

The mean values of all genotypes exhibited distinct patterns of variation across all evaluated traits (Table 3). Amongst the evaluated genotypes, Shimla B1 (42.40) exhibited maximum number of leaves per plant followed by IC 341589 (34.53) and IC 37288 (32.93) similarly maximum primary branches were found in Sangla B214 (3.93) followed by IC 341674 (3.67), IC 341589 (3.53) and IC 37288 (3.53). Maximum plant height and biological yield were exhibited by Shimla B1 (112.35 cm and 16.00 g, respectively), followed by Himpriya (99.69 cm and 13.53 g, respectively). For harvest index, IC 341589 exhibited maximum harvest index (35.26), followed by IC 318859 (34.91) and Shimla B1 (34.19). The maximum straw yield was observed in Shimla B1 (10.53 g), followed by IC 46160 (9.73 g) and IC 42430 (9.73). These results corroborate with the previous findings of Auber et al. (2021) and Singh et al. (2024).

Table 3. Mean values of 22 buckwheat genotypes for different traits under the study

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Genotypes** | **LPP** | **PB** | **PH** | **BIO YIELD** | **HI** | **SYPP** |
| **1** | 42.40 | 2.47 | 112.35 | 16.00 | 34.19 | 10.53 |
| **2** | 30.60 | 2.87 | 99.69 | 13.53 | 29.56 | 9.53 |
| **3** | 25.47 | 2.73 | 88.35 | 13.53 | 32.04 | 9.20 |
| **4** | 30.87 | 3.13 | 89.95 | 11.07 | 27.11 | 8.07 |
| **5** | 31.13 | 3.93 | 76.63 | 10.87 | 21.47 | 8.53 |
| **6** | 26.87 | 3.27 | 85.83 | 9.13 | 16.80 | 7.60 |
| **7** | 22.27 | 2.93 | 81.73 | 9.73 | 22.60 | 7.53 |
| **8** | 19.93 | 2.27 | 89.48 | 12.33 | 21.09 | 9.73 |
| **9** | 20.00 | 2.47 | 76.97 | 11.60 | 21.26 | 9.13 |
| **10** | 25.07 | 2.93 | 79.38 | 11.27 | 34.91 | 7.33 |
| **11** | 31.13 | 3.47 | 91.92 | 12.07 | 31.51 | 8.27 |
| **12** | 27.07 | 2.47 | 90.28 | 11.00 | 21.82 | 8.60 |
| **13** | 34.53 | 3.53 | 94.65 | 11.53 | 35.26 | 7.47 |
| **14** | 29.73 | 2.67 | 85.82 | 9.93 | 16.78 | 8.27 |
| **15** | 32.93 | 3.53 | 85.47 | 11.67 | 16.58 | 9.73 |
| **16** | 30.47 | 2.93 | 95.06 | 12.00 | 23.89 | 9.13 |
| **17** | 18.87 | 2.67 | 83.61 | 9.80 | 30.62 | 6.80 |
| **18** | 21.00 | 3.07 | 73.20 | 12.07 | 29.29 | 8.53 |
| **19** | 24.80 | 3.67 | 81.96 | 11.20 | 32.15 | 7.60 |
| **20** | 18.00 | 2.73 | 78.22 | 11.47 | 31.40 | 7.87 |
| **21** | 24.93 | 3.33 | 83.25 | 11.13 | 21.56 | 8.73 |
| **22** | 29.27 | 3.33 | 89.93 | 11.80 | 17.53 | 9.73 |
| **Mean** | 27.15 | 3.02 | 86.99 | 11.58 | 25.88 | 8.54 |
| **CD** | 1.20 | 0.18 | 3.81 | 0.40 | 1.49 | 0.42 |
| **SE(m)** | 0.42 | 0.06 | 1.34 | 0.14 | 0.52 | 0.15 |
| **CV** | 2.69 | 3.69 | 2.66 | 2.12 | 3.50 | 2.98 |

**3.2 Genetic variability estimations**

The estimates of parameters of genetic variability, including range, mean, standard error, phenotypic and genotypic coefficients of variation (PCV and GCV, respectively), broad-sense heritability, and genetic advance as a percentage of the mean of six quantitative traits of tartary buckwheat have been presented in Table 4.

Table 4. Estimates of parameters of genetic variability for six quantitative traits of buckwheat

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Traits** | **Range** | **PCV** | **GCV** | **H2** | **GA** | **GAM** |
| **LPP** | 18.00-42.40 | 21.79 | 21.73 | 99.49 | 12.13 | 44.66 |
| **PBPP** | 2.27-3.93 | 14.81 | 14.66 | 97.94 | 0.90 | 29.88 |
| **PH** | 73.20-112.35 | 10.05 | 9.93 | 97.67 | 17.58 | 20.22 |
| **BY** | 9.13-16.00 | 12.68 | 12.62 | 99.07 | 2.99 | 25.88 |
| **HI** | 16.58-34.91 | 24.75 | 24.67 | 99.33 | 13.11 | 50.66 |
| **SYPP** | 6.80-10.53 | 11.33 | 11.19 | 97.69 | 1.94 | 22.79 |

The results showed that the genotypic coefficient of variances was minutely lower than the phenotypic coefficient of variances, which highlights the role of environment in the expression of characters. The classification of PCV and GCV estimates followed the standard thresholds: low (<10%), moderate (10-20%), and high (>20%) as per Deshmukh et al (1986). Notably high PCV and GCV were observed for HI (24.75% and 24.67%, respectively) and LPP (21.79% and 21.73, respectively), whereas moderate PCV and GCV estimated were found for PBPP (14.81% and 14.66%, respectively), BY (12.68% and 12.62%, respectively), and SYPP (11.33% and 11.19%, respectively). On the other hand, PH exhibited moderate PCV (10.05%) and low GCV (9.93%). The characters displaying high PCV and GCV correspond to their high inherent variability and provide huge scope for selection and genetic enhancement. Dutta et al. (2008) and Singh et al. (2024) also observed that high PCV and high GCV for the number of secondary branches and, number of leaves in buckwheat.

The GCV offers insight into the genetic variability of the quantitative traits but cannot alone be indicative of the heritable proportion of variation. To gain a comprehensive understanding of advancement through selection, it is crucial to complement the genotypic coefficient of variance with heritability estimates (Sood et al., 2025). The GCV, combined with heritability values, offers the most precise depiction of the anticipated progress achievable through selection. Heritability values play a pivotal role in the forecast of expected gains attainable via selection processes (Singh et al., 2024).

The estimates of broad-sense heritability were categorised into low (<50%), moderate (50-80%), and high (>80%) categories. All the traits under the study exhibited high heritability with values greater than 90%. This signifies that the traits are under strong genic control and are less affected by environmental influences, and hence, they are highly responsive to phenotypic selection. In a similar case, Aubert et al. (2021) for different yield-related traits in buckwheat.

However, heritability alone cannot reveal the amount of genetic improvement achievable through individual genotype selection. Factors such as the studied population, environmental conditions, and the methodology used in the study influence the heritability of a trait (Fehr, 1987). To achieve a more practical insight into the potential improvements achievable through selection processes, a more comprehensive understanding and knowledge are required using both heritability and genetic advance. The genetic advance as per cent of the mean (GAM) was classified into three categories, viz., low (<10%), moderate (10-20%), and high (>20%) as per Johnson et al. (1955). Based on this classification, all the traits under the study exhibited high GAM, indicating the predominance of additive gene action and the possibility of significant gains through direct selection. All the traits exhibited high heritability coupled with high GAM, suggesting that the selection of these traits for improvement will be effective and rewarding. Also, it suggests the role of non-additive gene action in their inheritance. Similar results in buckwheat have been reported by Aubert et al. (2021), Sowmya et al. (2021) and Singh et al. (2024) in their respective materials.

**Correlation** **studies**

Correlation analysis is a valuable tool in the determination of ideal plant types and the optimisation of selection efficiency in breeding schemes (Prasad, 2022), with genotypic correlations considered more precise than phenotypic ones due to less environmental influence (Kumar and Hazarika, 2020). Table 3 presents the estimates of phenotypic and genotypic correlation coefficients among the studied traits of tartary buckwheat. Notably, certain traits displayed higher genotypic coefficients of correlation than their corresponding phenotypic values, attesting to the genetic basis of trait associations (Table 5). Straw yield per plant (SYPP) exhibited a significant and positive association with PH (0.473 and 0.513) and LPP (0.402 and 0.421) at phenotypic and genotypic levels, respectively. Whereas with BY significantly higher and positive phenotypic coefficient (0.745) was observed than that of the genotypic coefficient (0.740). Additionally, it also exhibited a significant and negative association with HI (-0.273 and -0.256) at the phenotypic and genotypic level, respectively. These associations suggest that genotypes with tall plants, a greater number of leaves per plant and high biological yield tend to produce more straw yield. Similar results were observed by Bisht et al. (2018) and Papaiahgari et al. (2023) in their studies.

Table 5. Correlation matrix of six quantitative traits of buckwheat

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  | **PBPP** | **PH** | **BY** | **HI** | **SYPP** |
| **LPP** | P | 0.288\* | 0.717\*\* | 0.460\*\* | 0.073NS | 0.402\*\* |
| G | 0.305\* | 0.752\*\* | 0.471\*\* | 0.073NS | 0.421\*\* |
| **PBPP** | P |  | -0.209NS | -0.254\* | -0.031NS | -0.240NS |
| G |  | -0.229NS | -0.258\* | -0.040NS | -0.241NS |
| **PH** | P |  |  | 0.615\*\* | 0.210NS | 0.473\*\* |
| G |  |  | 0.649\*\* | 0.217NS | 0.513\*\* |
| **BY** | P |  |  |  | 0.433\*\* | 0.745\*\* |
| G |  |  |  | 0.455\*\* | 0.740\*\* |
| **HI** | P |  |  |  |  | -0.273\* |
| G |  |  |  |  | -0.256\* |

**Cluster** **analysis**

The multivariate analysis using Mahalanobis D2 statistic grouped the 22 genotypes of tartary buckwheat into two clusters (Fig. 1) using Tocher’s method, wherein cluster II was monogenotypic, having Shimla B1and cluster I was polygenotypic, consisting of the remaining 21 genotypes. The same method, when used by Sood et al. (2023), Singh et al. (2024) and Kapoor et al. (2024), yielded different results, which might be due to the differences in the genotypes used in the study. The intracluster distance varied from 0.00 (Cluster II) to 20.33 (Cluster I). Whereas the intercluster distance was found to be 43.61 between cluster I and II (Table 6). The inter-cluster distance was found to be higher than the intra-cluster distance, suggesting a wide genetic diversity among the genotypes of the two clusters. The highest intra-cluster distance was observed for cluster one, indicating that the genotype within this cluster was quite diverse, implying that selecting parents within this cluster would be effective. Inter-cluster distance between cluster I and II indicated that genotypes from these divergent clusters can be intercrossed to achieve a high heterotic response while also recovering desirable transgressive segregants. The cluster means for different traits showed considerable differences among the clusters for each trait (Table 7). Cluster II was found to be most important with desirable cluster means for five traits viz., LPP, PH, BY, HI and SYPP, while cluster I was found to be superior for PB. Similar results were also obtained by LinQiong et al. (2011) in tartary buckwheat.

Fig 1 Dendrogram of 22 buckwheat genotypes constructed using Tocher’s method



1= Shimla B1, 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=IC 42430

Table 6. Average intra and inter cluster distances of D2 among clusters

|  |  |  |
| --- | --- | --- |
|  | **Group 1** | **Group 2** |
| **Group 1** | 20.33 | 43.61 |
| **Group 2** | 43.61 | 0.00 |

Table 7. Mean cluster values for different traits of buckwheat

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **LPP** | **PBPP** | **PH** | **BY** | **HI** | **SYPP** |
| **Group 1** | 26.43 | 3.04 | 85.75 | 11.37 | 25.49 | 8.45 |
| **Group 2** | 42.40 | 2.47 | 112.35 | 16.00 | 34.19 | 10.53 |

**Conclusions:**

The present study successfully revealed significant genetic diversity among the evaluated genotypes of Tartary buckwheat (*Fagopyrum tataricum*) collected from the cold-arid region of Kinnaur, Himachal Pradesh. Using Mahalanobis D² statistics, the genotypes were grouped into distinct clusters, highlighting the existence of substantial genetic variability, which can be effectively utilised in future breeding programs. The traits contributing most to the genetic divergence were plant height, grain yield per plant, and 1000-seed weight, indicating their importance for selection.

Trait association analysis suggested that grain yield was positively correlated with key agronomic traits, which can be strategically targeted to enhance overall productivity. The results underline the potential of specific diverse genotypes as parents for hybridisation to exploit heterosis and develop high-yielding cultivars suitable for the unique agro-climatic conditions of the region.

This research emphasises the importance of conserving and utilising local landraces of buckwheat for sustainable crop improvement, especially in ecologically fragile hill regions like Kinnaur.

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