GENETIC DIVERGENCE IN BUCKWHEAT (*Fagopyrum esculentum* Moench.)

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ABSTRACT

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An experiment was conducted with 21local genotypes of buckwheat at the experimental field of Department of Genetics and Plant Breeding of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Salna, Gazipur to study the variability and their interrelationship and diversity pattern based on quantitative and qualitative characters during the November 2004 to March 2005. Multivariate techniques were used to classify 21 buckwheat genotypes. All the genotypes were grouped into five different clusters. Cluster IV had the maximum (six) and cluster II had the minimum (one) genotype. The highest inter cluster distance was observed between I and II. The lowest inter cluster distance was found between the cluster III and V. Days to first germination, days to first flowering, first node distance from the soil level, plant height, branches per plant, inflorescence per plant, grains per raceme and total grains per plant were found to contribute maximum towards genetic divergence among the buckwheat genotypes. The clustering pattern of genotypes revealed that genotypes collected from the same places did not form a single cluster. Considering diversity pattern and other agronomic performance the genotypes $G_{21,}$ G_9, G_8 and G_{10} might be selected as promising genotypes for future hybridization program.

Key words: Genetic variability, multivariate technique, buckwheat

INTRODUCTION

Buckwheat is one of the minor crops grown in Bangladesh belonging the family Polygonaceae. Buckwheat is believed to be cultivated first in the Himalayan region of India, from where it spreaded to China, Middle Asia and the Caucasus and later on to other European areas (Krotov, 1976). Common buckwheat, the principal species *Fagopyrum esculentum* appears to have been derived from *F. cymosum*, a wild species of Asia.

Buckwheat is called 'Poor man's food' in Danish. In Bangladesh, buckwheat is cultivated in the north-west region especially in Thakurgaon, Panchagar and parts of Dinajpur and Rangpur districts during the rabi (October to March) season. Most of the farmers in east and south are not familiar with the principle product of buckwheat, i. e. flour.

The buckwheat plant is an annual. Buckwheat requires only a short growing season of 10-12 weeks in the temperate zone (Martin, *et.al.*, 1976) and is highly cross-pollinated. The grain contains 10.3% protein. 2.4% fat, 2.4% mineral matter, 6.8% fibre and 65.0% carbohydrate (chiefly starch). Besides, it also contains calcium (0.07%) phosphorus (0.03%), iron (13.2 mg) and vitamin B (Narain, 1979 and Ram *et al.*, 1979).

It is well suited to light and well-drained soils such as sandy loam or silt loams and it grows satisfactorily on soil, too acid for other grain crops. It produces a better crop on relatively infertile, poorly tilled land than other grain crops when the climate is favorable. It, however, does not produce enough biomass to lodge badly on rich soil with high nitrogen content (White *et al*., 1941, Sando, 1956, Narain 1979 and Ram *et al.*, 1979). For this reason, buckwheat can be called as 'poor land's crop'.

Though it is a minor crop it has a great importance. But it may be mentioned that until to date there is no released variety of buckwheat with high yield potential and better quality. Further a very few limited attempt had been made for genetic improvement of this crop.

Knowledge of genetic diversity among existing cultivars of any crop is essential for long term success of breeding program and maximizes the exploitation of the germplasm resources (Belaj *et al.*, 2002 and Rasul and Okubo, 2002).

Genetically diverse parents are able to produce considerable variability, which can enhance the scope of selection. More diverse the parents, greater are the chances of obtaining high heterotic F_1 and broad spectrums of variability in segregating generations (Arunachalam, 1981). Such a study also permits to select the genetically divergent parents to obtain the desirable recombinant in the segregating generations. Hence, in the present investigation, an effort has been made to find out the genetic variability among the existing genotypes for their further utilization in the breeding program.

MATERIALS AND METHODS

The investigation was carried out at the Experimental Field of Department of Genetics and Plant Breeding of the Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Salna, Gazipur during November

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2004 to March 2005. Twenty-one indigenous buckwheat genotypes included in this study were collected from the northern part of Bangladesh. The experiment was laid out in a randomized complete block design (RCBD) with three replications. The field was divided into three blocks and each of the blocks was subdivided into 21 plots where genotypes were randomly assigned. The unit plot size was 4m X 1.25m consisting of five rows. Row to row distance was 25 cm. Ten plants were selected at random from each plot for recording observations on various characters. Data were recorded on days to first germination, days to 100% germination, days to first flowering, first node distance from soil level (cm), plant height (cm), branches per plant, inflorescence per plant, flowers per inflorescence, grain setting raceme per plant, grains per raceme, length of raceme (cm), grain number per plant, seed yield per plant (g), 100-seed weight (g) and seed yield (kg/m^2)

Statistical Analysis of Data

Replicated and mean data for each quantitative character were subjected to univariate and multivariate analysis, respectively.

Univariate analysis

For univariate analysis, analysis of variance was done individually and test of significance was done by F-test (Pense and Shukhatme, 1978). Mean, range, standard error (SE) and co-efficient of variation (CV %) were estimated using MSTATAC computer program.

For calculating the genotypic and phenotypic correlation co-efficient for all possible combination the formula suggested by Miller *et al,* (1958). Hanson *et al,* (1956), Johanson *et al,*(1955) were adopted.

Multivariate analysis (D^2 analysis)

Multivariate analysis viz., genetic diversity was analyzed using GENSTAT 5.13 software program (copyright 1987, Lawes agricultural Trust, Rothamasted Experimental Station,UK). Genetic diversity analysis involves several steps, i.e., estimation of distance between the varieties clustering and analysis of inter-cluster distance. Therefore, more than one multivariate technique are required to represent the results more clearly and it is obvious from the results of many researches (Basher, 2002; Uddin, 2001; Juned *et al* , 1988; Ariyo, 1987; Patil *et al.,* 1987).

Principal Component Analysis

Principal component analysis (PCA), one of the multivariate techniques, is used to examine the inter-relationships among several characters and can be done from the sum of squares and products matrix for the characters. Thus, PCA finds linear combinations of a set variate that maximize the variation contained within them, thereby displaying most of the original variability in a smaller number of dimensions. Therefore, principal components were computed from the correlation matrix and genotype i.e. scores obtained for the first components (Which has the property of accounting for maximum variance) and succeeding components with latent roots greater than unity (Jeger *et al*., 1983). Contributions of the different morphological characters towards divergence are discussed from the latent vectors of the first two principal components.

Principal Coordinate Analysis

Principal Coordinate (PCO) analysis is used to calculate inter unit distances. Though the use of all dimensions of PCA it gives the minimum distance between each pair of the N points using similarity matrix (Digby *et al*., 1989).

Canonical Variate Analysis

Canonical Vector Analysis (CVA) finds linear combination of original variability that maximizes the ratio of between groups to within group's variation, thereby giving functions of the original variables that can be used to discriminate between the groups. Thus in this analysis, a series of orthogonal transformations sequentially maximizing the ratio of among groups to within group variations.

RESULTS AND DISCUSSION

Variability among buckwheat genotypes

Range, mean, standard error (SE) and coefficient of variation (CV) of fifteen characters of buckwheat genotypes are presented in the Table 1. The genotypes showed that considerable variability existed among the genotypes. Analysis of variance showed that the genotypes varied significantly (1% level of probability) for all the characters (Table 1).

Table 1. Range, mean, standard error (SE) with coefficient of variation (CV) for fifteen characters of 21 local buckwheat genotypes

Characters	Maximum	Minimum	Mean ± SE	CV%
Days to first germination	3.80	3.20	3.49±0.025	4.04
Days to 100% germination	7.93	7.00	7.47±0.034	2.65
Days to first flowering	31.53	25.87	28.35±0.198	3.23
Distance from first node to soil level (cm)	7.07	3.27	4.58±0.162	26.34
Plant height (cm)	84.57	66.29	77.22±0.785	5.75
Branches per plant (no.)	27.47	13.53	23.463±0.434	6.78
Inflorescence per plant (no.)	112.53	84.47	98.6±1.008	5.06
Flowers per inflorescence (no.)	45.80	24.87	30.25±0.721	6.63
Grain setting raceme per plant (no.)	95.87	68.20	82.8±1.220	9.38
Grains per raceme (no.)	11.67	5.40	8.05±0.204	9.73
Raceme length (cm)	1.86	1.29	1.61±0.029	8.99
Total grains per plant	864.20	317.20	557.98±21.156	18.4
Seed yield per plant (g)	10.71	2.89	6.79±0.369	33.11
100 seed weight (g)	1.49	1.03	1.22±0.018	6.71
Seed yield (kg/m^2)	9.35	3.25	6.32±9.995	31.57
Characters	Maximum	Minimum	Mean ± SE	CV%
Flowers per inflorescence (no.)	45.80	24.87	30.25±0.721	6.63
Grain setting raceme per plant (no.)	95.87	68.20	82.8±1.220	9.38
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Seed yield (kg/m2)	9.35	3.25	6.32±9.995	31.57

Table 2. Analysis of variance for 15 characters in 21 local genotypes of buckwheat

Source of variation	df	Mean sum of squares				
		DFG	DHG	DF	PH	BP
Replication	2	0.109	0.012	1.679	14.31**	1.07
Genotype	20	0.068**	0.155**	5.79**	2.68**	12.44**
Error	40	0.02	0.037	0.841	19.689	2.53

Source of variation	df	Mean sum of squares				
		IP	FI	GSRP	GR	RL
Replication	2	1.48	1.48	213.76	0.94	0.096
Genotype	20	5.84**	93.33**	148.86**	6.79**	0.108**
Error	40	24.86	4.021	60.27	0.613	0.021

Source of Variation	df	Mean sum of squares			
		GR	SYP	100SW	SY
Replication	2	40006.16	31.48*	0.016	8594.04
Genotype	20	62333.69**	13.3**	0.049**	10622.14**
Error	40	10538.164	5.055	0.007	4014.048

DFG=Days to first germination, DHG= Days to hundred percent germination, DF= Days to first flowering, DN= Distance of first node from the soil level, PH= Plant height, BP= Branches per plant, IP= Inflorescence per plant, FI= Flowers per inflorescence, GSRP=Grain setting raceme per plant, GR= Grains per raceme, RL= Raceme length, GP= Grains per plant, SYP= Seed yield per plant, 100SW= 100-seed weight, Y=Seed yield (kg/m^2**).**

Diversity of the Buckwheat genotypes

Principal component analysis

The principal component analysis yielded eigen values of each principal component axes of ordination of genotypes with the first axes totally accounted for the variation among the genotypes, while three of these with eigen values above unity accounted for 81.71%. The first two principal axes accounted for 70.58% of the total variation among the 15 characters describing 21 Buckwheat genotypes (Table 3).

Table 3. Eigen values and percentage of variation for corresponding 15 components characters in 21 buckwheat genotypes.

Principal component characters	Eigen values	Percentage of total variation accounted for	Cumulative percentage
Days to first germination	3.748	49.97	49.97
Days to100% germination	1.545	20.61	70.58
Days to first flowering	0.834	11.13	81.71
Distance from first node to soil level (cm)	0.425	5.67	87.38
Plant height (cm)	0.40	4.53	91.91
Branches per plant (no.)	0.249	3.32	95.23
Inflorescence per plant (no.)	0.173	2.30	97.53
Flowers per inflorescence (no.)	0.062	0.82	98.35
Grain setting raceme per plant (no.)	0.0472	0.63	98.98
Grains per raceme (no.)	0.0267	0.36	99.34
Raceme length (cm)	0.0205	0.27	99.61
Total grain per plant	0.0134	0.18	99.79
Seed yield per plant (g)	0.0105	0.14	99.93
100 seed weight (g)	0.0033	0.04	99.97
Seed yield (kg/m^2)	0.0023	0.03	100

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Raceme length (cm)	0.0205	0.27	99.61
Total grain per plant	0.0134	0.18	99.79
Seed yield per plant (g)	0.0105	0.14	99.93
100 seed weight (g)	0.0033	0.04	99.97
Seed yield (kg/m^2)	0.0023	0.03	100

Construction of scattered diagram

Based on the values of principal component scores I and II obtained from the principal component analysis, a two-dimensional scattered diagram (Z_1-Z_2) using component score 1 as X-axis and component score 2 as Y-axis was constructed, which has been presented in the Figure 1. The position of the genotypes in the scattered diagram was apparently distributed into five groups, which indicated that there exists considerable diversity among the genotypes. The scattered diagram for the Buckwheat genotypes of different clusters revealed that the genotype number 9 and the genotypes of cluster V were distantly located which suggesting more diverged from rest of the genotypes.

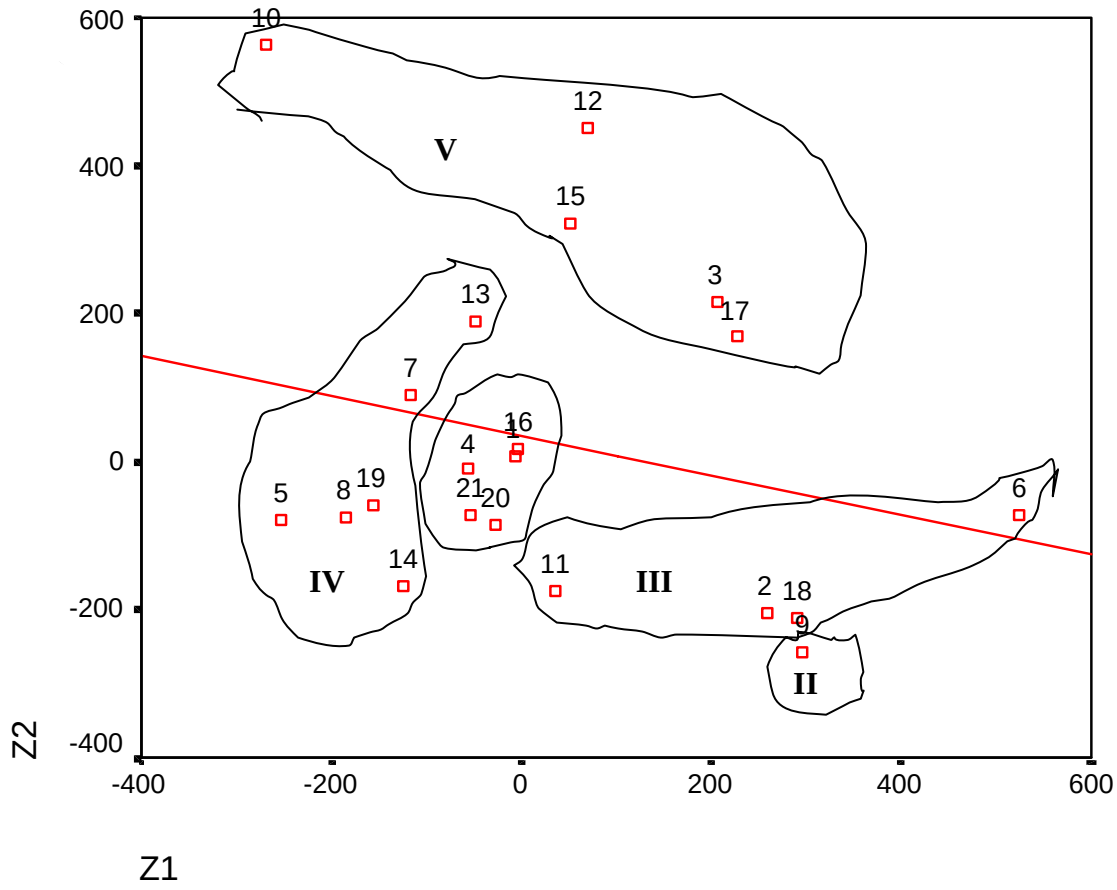

Figure 1. Scatter distribution of 21 buckwheat genotypes on the basis of principal component scores

Principal Coordinate Analysis

Inter genotypic distances as obtained by principal coordinate (PCO) analysis for selective combination showed that the highest distance was 1.743 observed between the genotypes G_9 and G_{21} and the lowest distance was observed between G_{13} and G_{14} (0.217) (Table 4).

The intra-cluster distances were computed by the values of inter-genotypic distance matrix of PCO according to Singh and Chaudhary (1985). There were not marked variation in intra-cluster distances, which ranged from 0 to 0.852 (Table 5). The magnitudes of the intra-cluster distances were not always proportion to the number of genotypes in the clusters. In the present study it was found that although cluster IV composed of the largest number of genotypes, (6) but its intra-cluster distances was moderate (0.737) among the five clusters (Table 5). The highest intra-cluster distance was computed for the cluster I (0.852) composed of five genotypes followed by the cluster IV (0.737) composed of six genotypes.

The intra-cluster distance in cluster III and V were 0.477 and 0.427 consisting of four and five genotypes, respectively. The intra-cluster distance was 0 for cluster II that composed of only one genotype. However, the highest value (0.852) of intra-cluster distance in cluster I indicated the genotypes (5) constituted this cluster might have diverged characters, which contributed to the formation of this cluster (Table 5).

Canonical variate analysis

Canonical variate analysis was performed to obtain the inter-cluster distances (Mahalanobis's D^2 values). These values of inter-cluster distance (D^2) are presented in the Table 5.

The inter-cluster distance was maximum between cluster I and II (69.62), while the distance was minimum between the cluster III and V (12.42), followed by the distance between I and III (15.50). The maximum values of inter-cluster distance indicated that the genotypes belonging to cluster I was far diverged from those o f

cluster II. Similarly the highest inter-cluster distance values between clusters II and III, cluster II and V, clusters I and IV indicated the genotypes belonging to each pair of clusters were far diverse.

Table 4. Highest and lowest four inter-genotypic distances (D^2) of different clusters (from PCO analysis)

Category	Between genotypes	Distances(D^2)
Highest four inter genotypic distances	G_9-G_{21}	1.743
	G_8-G_{21}	1.675
	G_{13}-G_{21}	1.649
	G_{14}-G_{21}	1.629
Lowest four inter genotypic distances	G_9-G_{14}	0.334
	G_{10}-G_{15}	0.320
	G_{10}-G_{17}	0.289
	G_{13}-G_{14}	0.217

Table 5. Average intra (Bold) and intercluster distance (D^2) for 21 buckwheat genotypes

Clusters	I	II	III	IV	V
I	**0.852**				
II	69.62	**0.00**			
III	15.50	56.89	**0.48**		
IV	45.02	28.12	31.33	**0.737**	
V	25.76	48.85	12.42	21.71	**0.427**

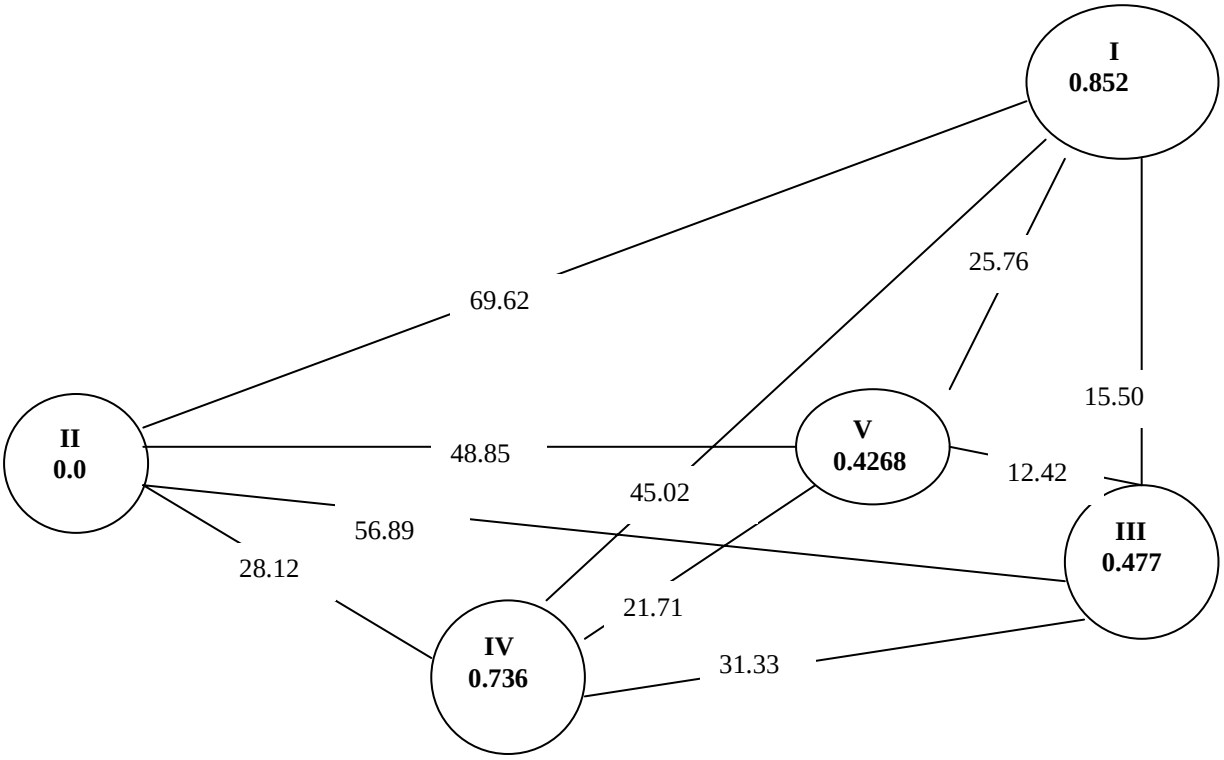

Figure 2. Diagram showing inter cluster (outside the circle) and intra cluster (inside the circle) distances of 21 local buckwheat genotypes.

Non- hierarchical clustering

By application of non-hierarchical clustering using co-variance matrix, 21 buckwheat genotypes were grouped into five different clusters. These results confirmed the clustering pattern of the genotypes according to the principal component analysis.

Composition of different clusters with their corresponding genotypes and collection site included in each cluster are presented in Table 6.

Cluster IV had maximum six genotypes followed by clusters I, V, III and II which had five, five, four and one genotypes respectively. Cluster I composed of five genotypes, namely, G_1, G_4, G_{16}, G_{20} and G_{21} collected from Ranigonj, Ranigonj, Goraya, Thakurgaon sadar and Patikadangi.

From the clustering mean values (Table 7), it was observed that cluster I produced the highest mean for days to first germination (3.53), first node distance from the soil level (5.04 cm), total grains per raceme (8.43) but produced the lowest mean for plant height (77.00 cm), branch number per plant (22.48), total grain number per plant (385.53), seed yield per plant (4.75g).

Cluster II was composed of a single genotype, namely G_9 collected from Bhulli. This genotype produced the highest mean value for plant height (82.23 cm), branches per plant (24.73), inflorescence per plant (112.53), grain setting raceme per plant (92.67), raceme length (1.70 cm), total number of grains per plant (864.20), seed yield per plant (9.91g). This produced the lowest mean value for days to first germination (3.33), first node distance from the soil level (4.00 cm), flower number per inflorescence (26.67), 100-seed weight (1.07).

Cluster III was constituted of four genotypes such as G_2, G_6, G_{11} and G_{18} collected from Ranigonj, Ranigonj, Bhulli and Chaudury Hat, respectively. These genotypes produced the highest mean value for days to first flowering (28.58), flower number per inflorescence (31.90), 100-seed weight (1.34) but produced the lowest mean value for days to 100% germination (7.37), number of inflorescence per plant (92.62), grain setting raceme per plant (75.93), seed yield kg/m^2 (3.68).

Cluster IV constituted of six genotypes, namely, G_5, G_7, G_8, G_{13}, G_{14}, and G_{19} collected from Ranigonj, Ranigonj, Ranigonj, Board Bazar, Goraya and Chaudury Hat area. These genotypes produced the highest mean value for seed yield kg/m^2 (7.66) but the lowest for grains per raceme (7.65), raceme length (1.58cm). For rest of the characters this cluster contained the moderate mean value.

Cluster V composed of five genotypes, namely, G_3, G_{10}, G_{12}, G_{15} and G_{17} collected from Ranigonj, Bhulli, Board bazar, Goraya and Chaudury Hut. The highest mean value was found for days to first germination (3.53) and days to100% germination (7.63) but produced the lowest values for days to first flowering (28) and raceme length (1.58 cm).

From the class mean values it was found that all the cluster mean values for days to first germination, days to 100% germination, days to first flowering, first node distance from the soil level, raceme length, 100-seed weight were more or less similar. The maximum range of variability were recorded for total number of grains per plant (385.53-864.20) and incase of seed yield kg/ m^2 (3.68-7.66) among all the characters in five clusters. Genotype of cluster II was important in respect of highest plant height, branches per plant, inflorescence per plant and grain setting raceme per plant. Cluster IV was important for seed yield kg/m^2 while cluster V was important for the early flowering

Table 6. Distribution of 21 genotypes in five different clusters

clusters	Number of genotypes	Genotypes with place of collection
I	5	G_1 (Ranigonj), G_4 (Ranigonj), G_{16} (Goraya),G_{20} (Thakurgaon sadar), G_{21} (Patikadangi)
II	1	G_9 (Bhulli)
III	4	G_2 (Ranigonj),G_6 (Ranigonj),G_{11} (Bhulli),G_{18} (Chaudury Hat)
IV	6	G_5 (Ranigonj), G_7 (Ranigonj), G_8 (Ranigonj), G_{13} (Board Bazar), G_{14} (Goraya), G_{19} (Chaudury Hut)
V	5	G_3 (Ranigonj), G_{10} (Bhulli), G_{12} (Board Bazar), G_{15} (Goraya), G_{17} (Chaudury Hat)

Table 7. Cluster means of 15 characters of local buckwheat genotypes.

Characters	I	II	III	IV	V
Days to first germination	3.53 **(H)**	3.33	3.40	3.50	3.53
Days to 100% germination	7.36	7.40	7.37	7.52	7.63 **(H)**
Days to first flowering	28.31	28.53	28.58 **(H)**	28.48	28.00
Distance from first node to soil level (cm)	5.04 **(H)**	4.00	4.63	4.45	4.35
Plant height (cm)	77.00	82.23 **(H)**	72.91	78.57	78.25
Branches per plant (no.)	22.48	24.73 **(H)**	22.57	23.70	24.63
Inflorescence per plant (no.)	97.03	112.53 **(H)**	92.62	101.18	99.03
Flowers per inflorescence (no.)	31.40	26.67	31.90 **(H)**	27.72	31.47
Grain setting raceme per plant (no.)	80.81	92.67 **(H)**	75.93	85.09	85.56
Grains per raceme (no.)	8.43 **(H)**	8.13	7.85	7.65	8.28
Raceme length (cm)	1.61	1.70 **(H)**	1.60	1.58	1.58
Total grains per plant (no.)	385.53	864.20 **(H)**	481.98	703.89	554.88
Seed yield per plant (g)	4.75	9.91 **(H)**	6.25	8.65	6.41
100-seed weight (g)	1.24	1.07	1.34 **(H)**	1.23	1.10
Seed yield (kg/m^2)	6.52	6.93	3.68	7.66**(H)**	6.51

Contribution of characters towards divergence of the genotypes

The PCA revealed from Table 8 that in Vector 1 (Z_1) the important characters responsible for genetic divergence in the major axis of differentiation were days to first germination (19.259), number of grains per raceme (2.241), branches per plant (1.851), first node distance from the soil level (1.429), plant height (1.028), days to first flowering (0.878), inflorescence per plant (0.228) and total number of grains per plant (0.181). In vector II (Z_2), which was the second axis of differentiation were days to first germination (13.951), days to 100% germination (12.745), number of grains per raceme (1.893) and number of branches per plant (1.770). The role of days to first germination, node distance, branches per plant, number of grains per raceme and number of grains per plant for both the vectors was positive across two axis indicating the important components of genetic divergence in these materials.

From the above results it appeared that contribution of days to first germination was the highest followed by number of grains per raceme, branches per plant, first node distance from the soil level, plant height in the buckwheat genotypes

Table 8. Latent vectors for 15 characters of local buckwheat genotypes

Characters	Vector1	Vector2
Days to first germination	19.259	13.951
Days to100% germination	-5.496	12.745
Days to first flowering	0.878	-0.846
Distance from first node to soil level (cm)	1.429	0.614
Plant height (cm)	1.028	-0.455
Branches per plant (no.)	1.851	1.770
Inflorescence per plant (no.)	0.228	-1.150
Flowers per inflorescence (no.)	-0.991	-0.115
Grain setting raceme per plant (no.)	-1.103	-0.225
Grains per raceme (no.)	2.241	1.893
Raceme length (cm)	-10.944	-17.356
Total grain per plant (no.)	0.181	0.046
Seed yield per plant (g)	-2.882	-1.800
100-seed weight (g)	-0.584	-10.326
Seed yield (kg/m^2)	-0.054	0.016

Selection of genotypes for future hybridization program

The genotypes of cluster I could be selected for earliest germination, higher number of grain per raceme and the shorter plant stature and lesser 100-seed weight. The genotypes of cluster II could be selected for the highest plant height, highest number of branches per plant, inflorescence per plant, grain setting raceme per plant, raceme length, total number of grains per plant, seed yield per plant and the lowest for earliest germination, flower number per inflorescence, 100-seed weight. The genotypes of cluster III for the earliest flowering, the highest flower number per inflorescence, 100-seed weight and for the lowest 100% germination, number of

inflorescence per plant, grain setting raceme per plant and seed yield (kg/m^2). The genotypes of cluster IV for the highest seed yield (kg/m^2) and lowest for grains per raceme, raceme length. The genotypes of cluster V for the earliest first germination and 100% germination and the lowest flowering and raceme length.

CONCLUSION

The highest inter cluster distance was observed between I and II (69.62) followed by II and III (56.89). The lowest inter cluster distance was found between the cluster III and V (12.42) followed by I and III (15.50). The clustering pattern of genotypes revealed that genotypes collected from the same places did not form a single cluster. The genotypes included in cluster I were important for earliest germination, higher number of grain per raceme and the lowest plant height, cluster II for the highest plant height, highest number of branches per plant, inflorescence per plant, grain setting raceme per plant, raceme length, total number of grains per plant, seed yield per plant. The genotypes of cluster III were important for the earliest flowering, the highest flowers number per inflorescence, 100-seed weight. The genotypes of cluster IV were important for the highest seed yield. The genotypes of cluster V were important for the earliest first germination and late flowering. Considering the genetic diversity of $G_{21,}$ G_9, G_8 and G_{10} might be selected as promising genotypes for future hybridization. Divergent genotypes (G_9 and G_{10}) are recommended to use as parent in future hybridization program, which may produce desirable segregants.

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