Morpho-physiological characterization of advanced hybrid genotypes of potato

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Bimal Chandra Kundu¹, Md. Abu Kawochar¹, Sauda Naznin¹, Maruf Mostofa^{1*} , Hossain K.M. Delowar², Tuhin Suvra Roy³

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ABSTRACT

Thirteen advanced potato genotypes developed by TCRC were characterized for 24 qualitative and 16 quantitative traits and analyzed for diversity by PCO, PCA, and Cluster analysis. Qualitative traits showed low variability (0.34), which Quantitative traits showed medium-high variation (0.67). The pooling of all traits showed a medium type index (0.51). The genotypes were grouped into four clusters. Cluster I had six genotypes, while cluster II and IV had only one. The highest inter-cluster distance was observed between clusters II and III and the lowest was between clusters III and IV. The highest intracluster distance was found in cluster I, whereas Cluster II (0.00) and cluster IV (0.00) showed the lowest. The highest inter-genotype distance was between genotypes 8.46 and 7.33 (33.79), and the lowest was between 7.86 and 7.48 (5.70). The genotypes of cluster I earned the highest mean values for foliage coverage, plant vigor and tuber numbers/hill. Cluster II produced the highest means for plant vigor, primary stem/hill, leaf length, leaf width, leaflet blade length, blade width, lateral leaflet blade length, width, tuber weight/hill, and tuber yield. The first three PCA counted for 91.75% of the total variation, whereas the first one accounted for 69.47%. From the scree plot, three principal sample components effectively summarized the total variance. Results of PCA showed the reduction of the 16 original variables to three independent linear combinations of PCA. The number of tubers/hill contributed maximum towards divergence.

INTRODUCTION

Potato (*Solanum tuberosum* L.) in Bangladesh is mainly used as a vegetable. The per capita consumption of potato in Bangladesh is about 40.0 kg per annum, which could be increased substantially to reduce the pressure on cereals. So far TCRC (Tuber Crops Research Centre) of BARI (Bangladesh Agricultural Research Institute) has released five varieties from its hybridization program. The first batch of hybrid seed was produced in the year 2000 with only two-gram seeds. Afterward, breeding has been continued

following clonal selection (Rashid and Hoque 2009). The selection of potential varieties in a breeding program is based on the knowledge of genetic diversity amongst them. To realize heterosis, genetically divergent parents are generally considered to be useful. In such crosses, more variability could be expected in the segregating progenies (Joseph et al. 1999). The genetic base of potato in Bangladesh is very narrow (Rashid 1989). An important fact is that the cultivated potato is highly heterogeneous and has a narrow genetic base (Carputo et al. 2011). Precise information about the extent of genetic divergence of the characters used for discrimination among the population is crucial in any crop improvement program (Ananda and Rawat 1984; De et al. 1988). In the on-going breeding program, a number of advanced breeding lines have been added to the germplasm stock. The assessment of genetic diversity among the available genotypes is very important in order to know the magnitude. The quantification of the variability is more important.

¹Tuber Crops Research Centre, Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh

²Department of Environmental Science and Technology, Jashore University of Science and Technology, Jashore-7408, Bangladesh

³Department of Agronomy, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh

* E-mail: marufsau@hotmail.com

The measure of the variability through multivariate analysis based on Mahalanobis (1936) D^2 statistics is a good practice for improving of the characters through breeding and selection. Similarly, cluster analysis is essential in the sense that in order to attain maximum heterosis, distant genotypes should be prioritized during the selection of parents for hybridization. The closely related parents from the groups should be excluded. In order to set the information regarding the extent of genetic divergence among the selected genotypes under this condition, the present study has been undertaken.

MATERIALS AND METHODS

Germplasm Materials

Thirteen promising advanced lines (clones 8.73, 8.46, 8.3, 8.11, 8.102, 8.117, 8.37, 7.33, 7.86, 7.58, 7.95, 7.12 and 7.48), developed at the TCRC through hybridization and selection method, were evaluated during 2012-13 and 2013-14 at BSPC (Breeder Seed Production Centre), Debiganj, Panchagarh. The unit plot size was 3m x 3m. Whole tubers were planted at 60 cm x 25 cm. Planting was done during the second week of November 2012 and 2013. Fertilizers were applied @ 325-220-250-120 kg ha⁻¹ of urea, TSP, MOP and gypsum, respectively (Kundu et al. 2018). Necessary intercultural operations were done as per TCRC recommendation.

Data collection

At particular stages of growth, accessions were characterized and evaluated for 24 qualitative and 16 quantitative morpho-agronomic characters using the Descriptor for the Cultivated Potato by IBPGR, Rome, Italy (Huaman et al. 1977). For each accession, ten randomly chosen plants were used for scoring.

Data analysis

Shannon-Wiener Diversity Index (SWDI)

Quantification of variability for each character was done using the Shannon-Weaver Diversity Index. The estimate of variability for each qualitative character was computed using the standardized Shannon-Weaver Diversity Index, designated as H' and has the formula:

$$H' = -\sum P_i (\log_2 P_i) / \log_2 n$$

Where P_i is the proportion of the total number of genotypes belonging to the i^{th} class.

Following the work of Siopongco et al. (1999), the same formula was applied to the quantitative characters following the construction of frequency classes, with the class boundaries equal to some function of mean and standard deviation. For each quantitative characters, the overall genotype means ($\bar{x}$) and standard deviation (σ) were used to subdivide the population values (x_i) into 10 frequency classes, ranging from class 1 (if $x_i \leq -2\sigma$) to class 10 (if $x_i \leq \bar{x} + 2\sigma$), the class interval being 0.5σ . The lowest and highest values were considered to determine the number of classes construct. The diversity considered high when $H' > 0.75$, moderate when $H' = 0.50 - 0.75$ and low when $H' < 0.50$. The Shannon-Weaver Diversity Index has a value ranging from 0 to 1, where 0 indicates the absence of diversity, and 1 indicates maximum diversity.

Multivariate analysis

Mean data for each character was subjected to multivariate statistical analyses using Principal Component Analysis (PCA), Principals Coordinate Analysis (PCO), and Cluster Analysis using GENSTAT 5:4.1 software (Copyright 1997). The dendrogram was constructed by using the SPSS software.

Principle component analysis

Raw data were first standardized to zero mean unit variance followed by computation of numerical measures of likeness/similarity and construction of distance matrix using variance-covariance co-efficient. Eigenvalues and Eigenvectors of the variance-covariance matrix were then computed.

Cluster Analysis

Using standardized data, numerical measures of likeness/similarity were computed and distance matrix constructed using Euclidean Distance Coefficients. Clustering by UPGMA (Unweighted Pair Group of Arithmetic Mean) method was executed.

RESULTS AND DISCUSSION

Before diversity analysis, range, mean, standard deviation, and coefficient of variation of the different characters of the studied germplasm were measured (Table 1). Higher variation was found in frequency secondary leaflets pairs at midrib (24.41%), Tuber number/hill (21.59%), terminal leaflet blade width (20.73), and other characters also showed considerable variability.

Table 1. Range, mean, standard deviation and coefficient of variation of different characters of the genotypes

Characters	Range	Mean	SD	CV%
Days to 80% emergence	20.00 - 16.00	18.15	1.28	7.06
Foliage coverage	100.00 - 85.00	96.00	4.26	4.44
Plant vigour	10.00 - 8.00	9.08	0.49	5.44
Plant: height at 60 days after planting	100.40 - 68.50	88.62	9.83	11.10
Primary stem per hill	8.25 - 4.80	5.90	0.92	15.66
Leaf length (cm)	32.02 - 23.12	27.80	2.45	8.82
Leaf width (cm)	19.62 - 13.63	16.85	1.65	9.79
Frequency secondary leaflets pairs at midrib	9.80 - 4.50	6.48	1.58	24.41
Terminal leaflet blade length (cm)	9.57 - 5.72	7.91	1.15	14.51
Terminal leaflet blade width (cm)	7.41 - 3.70	5.70	1.18	20.73
Number of primary leaflet pairs	6.30 - 4.00	4.76	0.78	16.30
Lateral leaflet blade length (cm)	8.98 - 6.72	8.00	0.77	9.57
Lateral leaflet blade width (cm)	5.88 - 4.17	5.07	0.55	10.86
Tuber number/hill	14.53 - 5.97	10.56	2.28	21.59
Tuber weight/hill (kg)	0.67 - 0.50	0.60	0.05	7.66
Tuber yield	44.97 - 33.33	40.16	3.07	7.66

Estimate of Variation Using the Shannon-Weaver Diversity Index

The computed diversity indices for qualitative character traits ranged from '0' (stem cross-section, secondary tuber skin color, distribution of secondary tuber color, unusual tuber shape, notes of eyes per tuber, tuber defects: crack, secondary growth) to 0.89 (general tube shape), with a mean diversity value of 0.34. The diversity values showed medium variation in abaxial leaf pubescence (0.62), adaxial leaf pubescence (0.57), pre-dominant tuber flesh color (0.74), depth of tuber eyes (0.63), tuber size (0.66) and uniformity of tuber size (0.63). All other characters exhibited medium variation. The mean diversity index of 0.34 indicated the existence of low variation within the collection, in terms of qualitative characters

(Table 2).

Three quantitative traits exhibited a low diversity values of 0.30 for plant vigor, 0.48 for foliage coverage and 0.19 number of primary leaflet pairs, whereas, leaf length (0.82), leaf width (0.85), frequency of secondary leaflets pairs at the midrib (0.80), lateral leaflet blade length (0.79) and width (0.80), and tuber yield (0.82) showed high diversity values. All the rest gave average diversity values ranging from 0.58 (tuber number/hill) to 0.75 (terminal leaflet blade length). Medium degree of variation exhibited within the quantitative characters, as reflected by the mean diversity value of 0.67 (Table 2).

The pooling of diversity values for the qualitative and quantitative characters gave an

Table 2. Computed diversity indices (H') for the qualitative and quantitative characters of advanced lines of potato

Character	H'	Character	H'
Qualitative			
Branching habit	0.25	Secondary tuber flesh color	0.31
Stem color	0.13	Distribution of secondary tuber flesh color	0.33
Stem cross section	0	General tube shape	0.89
Leaf dissection	0.45	Unusual tuber shape	0
Abaxial leaf Pubescence	0.62	Depth of tuber eyes	0.63
Adaxial leaf pubescence	0.57	Notes of eyes per tuber	0
Type of hairs	0.24	Distributions of tuber eyes	0.39
Predominant tuber skin color	0.39	Tuber size	0.66
Secondary tuber skin color	0	Uniformity of tuber size	0.63
Distribution of secondary tuber color	0	Tuber defects: crack	0
Tuber skin type	0.38	Secondary growth	0
Predominant tuber flesh color	0.74	Lenticels	0.39
Mean Diversity Index= 0.34			
Quantitative			
Days to 80% emergence	0.62	Terminal leaflet : blade length (cm)	0.75
Foliage coverage	0.48	terminal leaflet : blade width (cm)	0.73
Plant vigor	0.30	Number of primary leaflet pairs	0.19
Plant: height at 60 days after planting	0.73	Lateral leaflet blade length (cm)	0.79
Primary stem per hill	0.60	Lateral leaflet blade width (cm)	0.80
Leaf length (cm)	0.82	Tuber number/hill	0.58
Leaf width (cm)	0.85	Tuber weight/hill (kg)	0.72
Frequency secondary leaflets pairs at midrib	0.80	Tuber yield (t/ha)	0.82
Mean Diversity Index = 0.67			
Pooling of Diversity Index = 0.51			

Table 3. Ten highest and ten lowest inter genotypic distance among 13 advanced lines potato

Sl No	Genotypic Combination	Distance (D ²) value	Genotypic Combination	Distance (D ²) value
Higher inter-genotypic distance			Lower inter-genotypic distance	
1	8.46 - 7.33	33.79	7.86 - 7.48	5.70
2	8.46 - 7.86	31.64	8.3 - 8.37	5.94
3	8.46 - 7.48	30.00	8.73 - 8.3	6.30
4	8.46 - 8.11	29.65	8.11 - 7.48	7.24
5	8.46 - 7.58	28.71	8.102 - 8.37	7.28
6	8.3 - 7.33	27.29	8.102 - 8.117	7.70
7	8.73 - 7.33	25.75	7.86 - 7.58	7.79
8	8.46 - 7.95	25.64	8.117 - 8.37	8.26
9	8.37 - 7.33	25.13	7.58 - 7.48	8.29
10	8.46 - 7.12	25.11	7.95 - 7.48	8.96

overall diversity index of 0.51, indicative of medium variability among three collections. Siopongco et al. (1999) reported that the collection exhibited medium variation for the qualitative characters, and high variation was, on the other hand, observed for the quantitative characters, whereas pooling of diversity indices for the qualitative and quantitative characteristics gave medium diversity.

Cluster analysis

Based on cluster analysis, the 13 genotypes were grouped into four clusters (Figure 1). Cluster I contained six genotypes, cluster III having five and clusters II & IV contained one genotype. In many cases, the same cluster included genotypes from different eco-geographic region, indicating the geographic distribution and genetic divergence did not follow the same trend. These findings were in agreement with the findings of other researchers (Haydar et al. 2007). Yahiya et al. (2009) and Huque et al. (2012) reported the non-correspondence of genetic and geographic diversity.

Inter genotypic distance (D²) were obtained from PCO for all possible combination between pairs of genotypes. The highest inter-genotype distance was observed between genotypes 8.46 and 7.33 (33.79) followed by 8.46 and 7.86 (31.64), 8.46 and 7.48 (30.00). The lowest was observed between 7.86 and 7.48 (5.70) followed by 8.3 and 8.37 (Table 3). The difference between the highest and the lowest inter genotypic distance indicated the presence of variability among the genotypes.

The intracluster distance was computed by using the values of inter accession distance from a distance matrix, according to Sing and Chaudhary

(1985). The magnitude of the intracluster distance was not always proportional to the number of genotypes in the clusters (Huque et al. 2012), as cluster I composed of six genotypes showed the highest intracluster distance (6.237) followed by cluster III (5.771). Cluster II (0.00) and cluster IV (0.00) showed zero intracluster distance due to containing only one genotype (Table 4). The results supported by Huque et al. (2012).

The inter distance was calculated by averaging all possible D² values among all genotypes belonging to different clusters, divider being the number of pairs involved (Table 4). Intercluster distance represents the index of genetic diversity among the clusters (Huque et al. 2012). The values of intercluster distances were larger than the intra-cluster distances suggesting wider genetic diversity among the genotypes of the different groups. The results supported by Huque et al. (2012). The cluster II and III were more diverse as indicated by maximum inter-cluster distances between them (33.785) followed by cluster II and IV (27.844), I and III (23.886) and I and IV (15.128). The maximum values of inter-cluster distance indicated that the genotypes belonging to cluster II were far away from those of cluster III. Similarly, the higher intercluster value between cluster II and IV, cluster I and III, and cluster I and IV indicated that the genotypes belonging to each pair of these clusters were far diverse. The minimum distance was observed between cluster III and IV (13.428), indicating that the genotypes of these clusters were genetically close. Higher intracluster and inter-cluster distances indicate closeness among the genotypes of two clusters and within the cluster also.

By applying a non-hierarchical cluster using the covariance matrix, the genotypes were grouped

Table 4. Average intra-(boldface) and inter-cluster distance (D²) for 13 advanced lines of potato

Cluster	I	II	III	IV
I	6.237	-	-	-
II	14.369	0.000	-	-
III	23.886	33.785	5.771	-
IV	15.128	27.844	13.428	0.000

Table 5. Distribution of 13 advanced lines of potato in four different cluster.

Cluster	No. of genotypes	Serial no. of genotypes	Name of genotypes
I	6	4; 9; 10; 11; 12 and 13	8.11; 7.86; 7.58; 7.95; 7.12 and 7.48
II	1	8	7.33
III	5	1; 3; 5; 6; and 7	8.73; 8.3; 8.102; 8.117 and 8.37
IV	1	2	8.46

Table 6. Cluster mean values for various quantitative characters in the genotypes

Characters	Cluster-I	Cluster-II	Cluster-III	Cluster-IV
Days to 80% emergence	17.00	18.00	19.00	18.60
Foliage coverage	97.67	97.33	87.50	97.60
Plant vigour	9.33	9.33	8.50	9.00
Plant height at 60 days after planting	76.07	82.70	96.55	96.52
Primary stem per hill	5.97	6.58	5.40	5.66
Leaf length (cm)	26.06	28.72	27.03	28.61
Leaf width (cm)	16.66	17.93	15.67	16.78
Frequency secondary leaflets pairs at midrib	5.53	5.30	6.30	7.82
Terminal leaflet blade length (cm)	7.37	9.13	7.53	7.65
Terminal leaflet Blade width (cm)	5.77	6.84	4.91	5.28
Number of primary leaflet pairs	4.30	4.03	5.10	5.34
lateral leaflet blade length (cm)	7.55	8.27	7.91	8.14
lateral leaflet blade width (cm)	4.93	5.42	5.00	4.98
Tuber number/hill	13.17	10.28	10.95	9.01
Tuber weight/hill (kg)	0.56	0.62	0.62	0.61
Tuber yield (t/ha)	37.30	41.56	41.29	40.59

into 4 clusters (Table 5). Cluster I comprised of six genotypes, cluster III contained five, while cluster II and cluster IV had only one. The results were confirmed with the cluster pattern of the genotypes obtained through the dendrogram.

The genotypes from cluster I earned the highest cluster mean value for foliage coverage (97.67%), plant vigor (9.33) and tuber number/hill (13.17), but the lowest cluster mean for days to 80% emergence (17.00), plant height at 60 DAP (76.07cm), leaf length (26.06 cm), terminal leaflet: blade length (7.37 cm), lateral leaflet: blade length (7.55 cm), lateral leaflet: blade width (4.93 cm), tuber weight/hill (0.56 kg) and tuber yield (37.30 t ha⁻¹).

On the other hand Cluster II integrated only one genotype produced the highest mean for plant vigor (9.33), primary stem/hill (6.58), leaf length (28.72 cm), leaf width (17.93 cm), terminal leaflet: blade length (9.13cm), terminal leaflet: blade width (6.84 cm), lateral leaflet: blade length (8.27 cm), lateral leaflet: blade width (5.42 cm), tuber

weight/hill (0.62 kg) and tuber yield (41.56 t ha⁻¹) but the lowest mean for frequency of secondary leaflets pairs at midrib (5.30). Cluster III had the highest mean value for days to 80% emergence (19.00), plant height at 60 days after planting (96.55 cm) and tuber weight/hill (0.62 kg) whereas cluster IV produced the highest mean value for frequency of secondary leaflets pairs at midrib (7.82) and number of primary leaflet pairs (5.34) (Table 6). The results supported by Huque et al. (2012).

Principal Component Analysis

Principal Component Analysis is a statistical method which attempts to describe the total variation in the multivariate sample using fewer variables than in the original data set (Bartolome et al. 1999). In the end, the analysis results in the identification of the major attributes that are responsible for the observed variation within a given collection.

The principal component analysis resulted in

Table 7. Computed latent root (Eigenvalues) with corresponding proportion and cumulative variance

Principle Components	Eigenvalue	Proportion %	Cumulative %
01	102.629	69.47	69.47
02	18.551	12.56	82.03
03	14.359	9.72	91.75
04	4.145	2.81	94.56
05	3.626	2.45	97.01
06	1.932	1.31	98.32
07	1.173	0.79	99.11
08	0.745	0.50	99.61
09	0.291	0.20	99.81
10	0.198	0.13	99.94
11	0.056	0.04	99.98
12	0.018	0.01	99.99
13	0.00	0.00	<100.00

Table 8. Factor Loadings for Component characters in Principal Component 1–3

Characters	PC 1	PC 2	PC 3
Days to 80% emergence	-0.0505	-0.0561	-0.1427
Foliage coverage	0.1608	0.9000	0.1098
Plant vigour	0.0260	0.0661	0.0040
Plant: height at 60 days after planting	-0.9681	0.1354	0.0892
Primary Stem per hill	0.0203	-0.0036	0.0475
Leaf length (cm)	-0.0328	0.2294	-0.4888
Leaf width (cm)	0.0514	0.1472	-0.3215
Frequency secondary leaflets pairs at midrib	-0.0834	0.0852	0.0648
Terminal leaflet blade length (cm)	0.0075	0.0879	-0.1499
Terminal leaflet Blade width (cm)	0.0355	0.0938	-0.1159
Number of Primary Leaflet Pairs	-0.0434	0.0109	0.0387
Lateral leaflet blade length (cm)	-0.0153	0.0745	-0.1111
Lateral leaflet blade width (cm)	0.0016	0.0352	-0.0674
Tuber number/hill	0.1203	-0.1945	0.1869
Tuber weight/hill (kg)	-0.0010	-0.0022	-0.0108
Tuber yield (t/ha)	-0.0683	-0.1459	-0.7223

the reduction of the 13 original variables to three independent linear combinations which account for 91.75% of the total variation. The first one accounted for 69.47%, two and three for 12.56%, and 9.72%, respectively (Table 7). A scree plot is a useful visual aid for determining an appropriate number of principal components. The three sample principal components effectively summarized the total sample variance (Figure 2).

Analysis of the factor loading of characters in the three components identified the major characters responsible for maximum variability (Table 8). The first principal component (PC1) can be considered as the component of plant height, foliage coverage and tuber number/hill, indicated by high loadings for plant height at 60 DAP (0.9681), foliage coverage (0.1608) and tuber number/hill (0.1203). Principal component II (PC2) on the other hand, indicated the importance of leaf length, leaf width and tuber yield. High loadings observed for leaf length (0.2294), leaf width (0.1472) and tuber yield (0.1459). High loadings also observed for plant height at 60 days after planting (0.0.1354), foliage coverage (0.9000) and tuber number/hill (0.1945), but they were also important in PC1. The characters associated with

the principal component III (PC3) were days to 80% emergence (0.1427), terminal leaflet blade length (0.150), terminal leaflet blade width (0.1159) and lateral leaflet blade length (0.1111). High loadings were also observed for leaf length (0.4889), leaf width (0.3215) and tuber yield (0.7223), but they were high loadings in PC2.

In summary, through PCA, it was observed that the variation in the seed types was due to several components (three principal components) and several characters in each component. Furthermore, the results of the principal component analysis support the initial findings of variability estimated using the Shannon-Weaver Diversity Index. The collection is medium diverse in terms of quantitative characters and this was reflected by the fact that the first three principal components were loaded by almost all the characters. The results supported by Siopongco et al. (1999).

The contribution of characters towards the divergence of the genotypes is presented in Table 9. The number of tuber/hill was positive for both vectors indicating this trait contributed maximum towards divergence.

Table 9. Latent Vectors for various principal component characters of advanced lines of potato

Characters	Vector I (Z ₁)	Vector II (Z ₂)
Days to 80% emergence	0.0295	-0.3467
Foliage coverage	-0.1606	0.1774
Plant vigour	-0.2466	0.2366
Plant: height at 60 days after planting	0.1221	-0.3230
Primary Stem per hill	-0.0856	0.2217
Leaf length (cm)	-0.2590	-0.3078
Leaf width (cm)	-0.3137	-0.0998
Frequency secondary leaflets pairs at midrib	0.2441	-0.2366
Terminal leaflet blade length (cm)	-0.3906	-0.0384
Terminal leaflet Blade width (cm)	-0.4004	0.0821
Number of Primary Leaflet Pairs	0.2549	-0.2355
Lateral leaflet blade length (cm)	-0.3416	-0.1883
Lateral leaflet blade width (cm)	-0.3804	-0.0500
Tuber number/hill	0.0391	0.2658
Tuber weight/hill (kg)	-0.1101	-0.3912
Tuber yield (t/ha)	-0.1109	-0.3910

Figure 1. Dendrogram produced from cluster analysis of locally developed advanced lines of potato using morphological data and executed by UPGMA.

Figure 2. Scree plot constructed for 12 principal components

CONCLUSIONS

The mean diversity index was 0.51, which indicated the presence of a medium-range of variation within the population. As the high variation is an important key for selection, more variation should be created through the collection of germplasm of various backgrounds. The population is medium diverse for quantitative characters but low for qualitative characters. In the future, the selection of materials should be focused on these characters, which showed low variability. From the distance (D^2) matrix, genotypes from clusters II and III can be selected for hybridization programs, while clone 8.46 and 7.33 can subtly be chosen to get the maximum heterosis from the existing collection. The character, number of tubers/hill, is the most important because it made a maximum contribution towards divergence.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

ORCID

Maruf Mostofa: <https://orcid.org/0000-0002-0213-6812>

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