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GENETIC DIVERSITY IN STEM AMARANTH BASED ON PRINCIPAL COMPONENT, CLUSTER AND BIPLOT ANALYSES

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Abstract

Genetic diversity is the base line of any breeding program determining the variability of germplasm for crop improvement strategies. Approaches like principal component and cluster analysis measure the amount of genetic diversity in different characters and also assess the relative contribution of different traits to the total variation. This research was carried out at the vegetable research field of Olericulture Division of Horticulture Research Centre (HRC), Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh to study the genetic diversity and select the high yielding stem amaranth genotypes using principal component, cluster and biplot analyses. A total of 27 stem amaranth germplasms were evaluated in RCBD with two replications in consecutive two years 2020 and 2021 in summer. Principal component (PC) analysis depicted that first four PCs with Eigen-values higher than unity contributing 81.012% of total variability for different traits among 27 stem amaranth germplasm. The PC1 accounted for 37.833% variation, showed the positive loading effect of stem fresh weight (yield/plant) (0.864), leaf fresh weight (0.728), shoot fresh weight (yield) (0.968), stem dry weight (0.829), leaf dry weight (0.760) and shoot dry weight (0.980). The PC2 accounted for 24.556% of the variation and the major contributing traits were plant height (0.796), relative water content (RWC) (0.723), SPAD value (0.759) and F_v/F_m (0.694). The PC3 accounted 10.95% of the variation, and traits with high positive loading effects were observed for number of leaves/plant (0.595) and leaf area/plant (0.727). Regarding the first two PCs, germplasms were differentiated into five diverse groups. On biplot, two germplasm i.e. AM65 and AM72, were in positive side and positioned far away from the origin and were considered as diverse from the others. Cluster analysis grouped all germplasms into five clusters. The germplasms in clusters-IV and V exhibited higher stem diameter, stem fresh weight, leaf fresh weight, shoot fresh weight as well as their corresponding dry weight. The four characters *viz.,* stem diameter, stem fresh weight, stem dry weight and shoot dry weight showed maximum contribution to total divergence among 13 characters studied. The D^2 statistics confirmed the maximum inter-cluster distance between cluster-III and V followed by cluster-III and-IV, whereas the smallest inter cluster distance between clusters - I and II. Crossing between the germplasm of Cluster- III with Cluster -V and Cluster-III

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with Cluster-IV could result better genetic recombinant and might exhibit better heterosis in F_1 for hybrid development. Selection of superior germplasm from different clusters could be used in future crop improvement program.

Keywords: Stem amaranth, *Amaranthus* spp, cluster analysis, principal component analysis, eigen value, stem diameter, stem fresh weight.

Introduction

Stem amaranth (*Amaranthu*s spp.), an important vegetable of the family Amaranthaceae is popularly known as 'danta' grown throughout Bangladesh. It is mainly grown during summer and rainy season. This vegetable is very popular in Bangladesh for its quick and vigorous growth and also for higher yield potential. Its tender stem with young leaves is used as vegetables. It is widely cultivated in Bangladesh, India, in tropical and subtropical parts of Asia, Africa and Central America (Hardwood, 1980).

Most amaranths, grain, vegetable and weedy species are diploids with 2n= 2x = 32 or 2n = 2x= 34 chromosomes. *Amaranthus dubius* is the only known naturally occurring tetraploid species in the genus *Amaranthus*, with a chromosome count of 2n=4x=64. *A. dubius* is characterized as an allotetraploid, meaning it likely originated from the hybridization of two different ancestral species followed by chromosome doubling (Greizerstein and Poggio,1994).

Amaranthus is monoecious and self-pollinating crop but outcrossing rate (5-39%) is sufficient to facilitate the gene flow in the population. Amaranth inflorescence consists of both male and female flowers (Sagar *et al*., 2023). Among the 70 species of *Amaranthus*, 17 are cultivated for edible leaves, and 3 are cultivated as food grains (Jensen, 1978). Amaranth leaves and stems are good economic sources of carotenoids, proteins, including the essential amino acids methionine and lysine, dietary fiber and minerals, such as magnesium, calcium, potassium, copper, phosphorus, zinc, iron, and manganese (Sarker *et al.*, 2014; Sarker and Oba, 2019). Amaranth leaves are anti-cancerous, it stops the irregular growth of cancer cells in the breast, colon, and liver, thus are also suitable for cancer patients (Hongyan *et al*., 2015). Besides its nutritional potential, vegetable amaranth is resistant to diseases and tolerant of extreme heat and drought (Rastogi and Shukla, 2013; Barrio and Anon, 2010). Unlike many other green vegetables, it thrives without the need for cold or specific climates, making it cultivable during the summer when other green leafy vegetables are scarce in the market (Rastogi and Shukla, 2013).

As an underutilized and genetically promising orphan crop, limited attention has been dedicated to the genetic improvement of this plant. To develop high-yielding varieties, crop improvement initiatives require genetic within the existing

germplasm. Moreover, the relation of characters with yield, the extent of environmental influences on these traits and the heritability of the characters are essential (Devi *et al*., 2015).

Multivariate analyses like principal component analysis (PCA) and clustering are commonly employed to elucidate genetic variation (Hair *et al*., 1995). Morpho-physiological traits serve as key indicators for identifying genetic diversity within and among populations, as well as for assessing genetic similarities and differences (Hunter, 1993). Addressing diversity in plant breeding is essential, especially considering geographical isolation and genetic barriers (Rauf *et al.*, 2010). The cultivation of appropriate stem amaranth varieties under various agro-climatic conditions is vital for both domestic consumption and exportation. Genetic divergence analysis, utilizing techniques like Euclidean distance and Mahabalonis' D^2 values aids in grouping genotypes to ascertain their degree of variation (Bhatt *et al*., 2015). Cluster analysis categorizes genotypes based on measured variables, grouping similar ones together. Clustering involves segregating genotypes into clusters with strong internal associations and weak associations between different clusters (Crossa and Franco, 2004). Principal component analysis identifies variables and characterizes traits along differentiation axes (Sharma *et al*., 2016), assisting in the examination of genetic divergence, identification of promising cultivars, and evaluation of trait importance in total genotype variation (Jollife, 1986). Besides, variability is essential for any plant breeding program. A new variety as per requirement of the farmers can be developed from an assembled diverse genetic stocks of any crops. So success of any breeding program depends much on the genetic variability available to the breeder and judicious selection of parents. Tomoko (1991) reported that evaluation of genetic diversity is important to know the source of gene for a particular trait within the available germplasm. So, the breeder must have clear understanding about the nature and magnitude of variability among the collected genetic stocks. There is little information regarding divergence study as well as breeding work in stem amaranth in Bangladesh. This study was, therefore, carried out to estimate the genetic diversity among 27 stem (vegetable) amaranth germplasms using cluster analysis through principal component analysis (PCA) based techniques for selection of parents and to identify potential morpho-physiological traits that are better employed as selection criteria for developing high yielding vegetable amaranth germplasm which may help breeders to breed better yielding new stem amaranth varieties.

Materials and Methods

Twenty-seven germplasms of amaranth (Table 2) were studied in a two-replicated trial placed in plastic shed conditions of vegetable research field, Horticulture

Research Centre (HRC), Bangladesh Agricultural Research Institute (BARI) in 2020(16 July 2020 to 28 August 2020) and 2021 (07 July 2021 to 19 August 2021) for 44 days. One hundred sixty- two pots (Height-29 cm, top dia-32 cm and bottom dia-21 cm; capacity 14 L) were used for this experiment. At first, soil collected from Kodda, Kalikoir Upzilla, Gazipur district, was prepared by cleaning all pieces of bricks, stones and other foreign materials and then soil was air dried. Seven kilograms of air-dried soil was mixed with 4 kg cowdung, 5 g Urea, 6 gm TSP, 6 g MoP, 3 g Gypsum and 1 g boric acid for each pot. The ultimate pot capacity was 11.00 kg soil, while moisture was 16.9%. Before sowing each pot, it was irrigated with 2 L water. At the proper moisture condition a little portion of seeds of different germplasms mixed with some fine loose soil was sown in each pot on 16 July 2020 and 13 July 2021. All the germplasms germinated within 6 days. First thinning was done on 25 July 2020 and 16 July 2021 keeping 10 seedlings/pot. The final thinning was done on 30 July 2020and 22 July 2021 keeping 6 plants/pot. Pots with all treatments were irrigated every three days' interval and continued upto experimentation. Each pot was top-dressed in 5 g Urea at 15 days' interval. Data (from 5 plants) was taken on 13 characters given in Table 1. The SPAD value was taken by SPAD meter (SPAD-500 plus, Minolta Konica Inc, Japan). The changes in fluorescence yield reflect changes in photochemical efficiency and heat dissipation. The polyphasic rise of fluorescence transients was measured by an ADC Infrared Gas Analysis Plant Efficiency Analyzer (PEA, Handsatech Instruments Ltd., King's Lynn, UK). The initial fluorescence (F_0) and maximum fluorescence (Fm) were analyzed and quantum efficiency of open photosystem II centers-quantum yield (F_v/F_m) was calculated. The leaf discs were previously adapted to the dark for 20 minutes. The fluorescence data were collected every day at 10.00 am to 12.00 pm from initial to end of the experiment. F_o is the amount of light absorbed initially to raise the fluorescence from a low level to maximum value Fm after dark adaptation. $F_v = F_m - F_0$ which is the variable. Ratio of F_v/F_m is a dark-adapted test used to determine maximum quantum yield. This ratio is also an estimate of the maximum portion of absorbed quanta used in PSII reaction centers. The plants were divided into stems and leaves, dried at 80°C and weighed.

Relative water content (RWC) was measured using leaves (4^{th} no. leaf from the tip of the plant). Immediately after cutting at the base of lamina, leaves were sealed within plastic bags and quickly transferred to the laboratory. Fresh weights were determined within 2 h after excision. Turgid weights were obtained after soaking leaves in distilled water in plastic bowls for 16 to 18 h at room temperature (about 25°C) and under the low light conditions of the laboratory. During soaking bowls are covered with polythene. After soaking, leaves were quickly and carefully blotted dry with tissue paper in preparation for determining turgid weight. Dry

weights were obtained after oven drying the leaf samples for 72 h at 70°C. RWC was calculated from the equation of Schonfeld *et al*. (1988):

$$\% \text{ RWC} = \frac{\text{Fresh weight- dry weight}}{\text{Turgid weight – dry weight}}$$

Leaf area /plant was estimated according to Tongos (2016):

S = aLw, where, a= 0.5, L= length of the leaf blade and W = width of the leaf blade.

Data of 27 genotypes were taken from all pots. Mean values for each data were calculated.Genetic diversity was studied following Mahalanobis' distance (Mahalanobis, 1936) generalized Distance (D^2) extended by Rao (1952). Clustering was done according to Tocher's method (Rao, 1970). Intra and inter cluster distances were calculated by the methods of Singh and Chaudhury (1985).

The effect of levels of the characters determining principal component analysis (PCA) and Correlation Coefficient Analysis, proportion of Eigen values, and the scores of the principal components. PCA and Correlation coefficients were calculated using the XLSTAT statistical analysis program, which is a program that uses the Microsoft Excel infrastructure (XLSTAT, 2020). The dendrogram was built based on agglomerative hierarchical clustering Ward's method using XLSTAT. Principal components with Eigen values greater than one are only considered in the analysis (Chahal and Gosal, 2002).

Table 1. Studied morphological parameters along with SI units

Sl no.	Studied parameters	Abbreviations	SI unit
1.	Plant height	PH	cm
2.	Number of leaves /plant	LN	No.
3.	Leaf area/plant	LA/pl	cm^2
4.	Stem diameter at the base	STDIA	mm
5.	Stem fresh weight	STFW	g
6.	Leaf fresh weight	LFFW	g
7.	Shoot fresh weight	SHFW	g
8.	Stem dry weight	STDW	g
9.	Leaf dry weight	LFDW	g
10.	Shoot dry weight	SHDW	g
11.	Relative water content (leaf)	RWC	%
12.	SPAD value	SPAD	-
13.	Maximum quantum yield of PSII	F_v/F_m	-

Table 2. Studied stem amaranth germplasms along with their seed suppliers

Sl no	Germplasm	Seed supplier	Quantity of seed
1.	AM01	Vegetable division, HRC,	5.0 g
2.	AM02	Vegetable division, HRC, BARI	4.5 g
3.	AM03	Vegetable division, HRC, BARI	5.0 g
4.	BD8278	Plant Genetic Resources Centre, BARI	4.5 g
5.	BD8152	Plant Genetic Resources Centre, BARI	4.5 g
6.	AM51	Vegetable division, HRC, BARI	5.0 g
7.	AM53	Vegetable division, HRC, BARI	5.0 g
8.	AM54	Vegetable division, HRC, BARI	5.0 g
9.	AM55	Vegetable division, HRC, BARI	5.0 g
10.	AM57	Vegetable division, HRC, BARI	5.0 g
11.	AM58	Vegetable division, HRC, BARI	5.0 g
12.	AM61	Vegetable division, HRC, BARI	5.0 g
13.	AM62	Vegetable division, HRC, BARI	5.0 g
14.	AM63	Vegetable division, HRC, BARI	5.0 g
15.	AM64	Vegetable division, HRC, BARI	5.0 g
16.	AM65	Vegetable division, HRC, BARI	5.0 g
17.	AM66	Vegetable division, HRC, BARI	5.0 g
18.	AM67	Vegetable division, HRC, BARI	5.0 g
19.	AM68	Vegetable division, HRC, BARI	5.0 g
20.	AM69	Vegetable division, HRC, BARI	5.0 g
21.	AM71	Vegetable division, HRC, BARI	5.0 g
22.	AM72	Vegetable division, HRC, BARI	5.0 g
23.	AM73	Vegetable division, HRC, BARI	5.0 g
24.	AM75	Vegetable division, HRC, BARI	5.0 g
25.	AM77	Vegetable division, HRC, BARI	5.0 g
26.	AM78	Vegetable division, HRC, BARI	5.0 g
27.	AM79	Vegetable division, HRC, BARI	5.0 g

Note: AM01 = BARI Danta-1 (check); AM = Amaranthus, BD = Bangladesh, HRC = Horticulture Research Centre, BARI = Bangladesh Agricultural Research Institute

Results and Discussions

Summary statistics

Huge variation were observed for plant height, number of leaves/plant, leaf area/plant, stem diameter, stem fresh weight, leaf fresh weight, shoot fresh

weight, stem dry weight, leaf dry weight, shoot dry weight, relative water content, SPAD value and F_v/F_m ranged from 44-76 cm, 13.50-20.50 number, 847.15 – 281.79 cm^2, 10.65 - 18.20mm, 20.70-60.25 g, 11.48-28.28 g, 35.10-80.85 g, 5.30-1.86 g, 1.62-3.96 g, 3.81-8.78 g, 82.17-93%, 29.10-42.80 and 0.70-0.78, respectively (Table 3). The difference between maximum and minimum values was found high in leaf area/pant (565.76 cm^2), shoot fresh weight (45.75 g), stem fresh weight (39.55 g), plant height (32 cm), SPAD value (13.7) and relative water content (11.23%). Standard deviation was observed higher in leaf area/pant (144.13), shoot fresh weight (10.91), plant height (9.77), stem fresh weight (9.24 and leaf fresh weight (4.91). Maximum coefficient of variation (CV) was recorded in leaf area/plant (30.75%), followed by stem dry weight (27.70%), leaf fresh weight (27.40%), leaf dry weight (25.69%), stem fresh weight (24.51%), shoot dry weight (22.08%), plant height (15.89%) and stem diameter (15.19%). The results pertaining to range, standard deviation and CV% indicated that germplasm of amaranth under investigation showed a wide range of variability among themselves in respect of leaf area/plant, stem fresh weight, shoot fresh weight, stem dry weight, stem diameter and plant height. Mandal and Dhangrah (2012) also reported variation exists in plant height (31.00-50.00 cm), stem diameter (9.3 mm-30.7 mm), number of leaves/plant (9.40-29.00) and stem fresh weight (yield)/plant (2.20-34.37 g) at 35 days after sowing while evaluating 17 vegetable amaranth genotypes. Shellikeri *et al.* (2022) obtained ranges in plant height (16.66-32.56 cm), stem diameter (3.24-8.76 mm), number of leaves/plant (6.60-12.10), fresh leaf weight (2.45-11.55 g), fresh stem weight (3.16-10.90 g/plant) and fresh green yield (7.16-24.63 g/plant) at 27.00 - 43.00 days after sowing during *kharif* season in 52 vegetable amaranths. Sharma *et al*. (2024) found the ranges in plant height at vegetative stage (15.95-24.54 cm), stem base diameter (10.2-15.8 mm), number of leaves/plant (11.75-22.08) in 22 vegetable amaranth genotypes during summer season. Rashad and Sarker (2020) obtained the ranges in plant height (19.62-32.65 cm), stem diameter (15.19-29.18 mm), leaf/plant (6.14-13.43), leaf area/plant (795.40-1361.10 cm^2) and shoot weight (66.48-131.30 g/plant) in 20 green amaranth genotypes in summer at 30 days after sowing. Shellikeri *et al.* (2022) got CV%: in plant height (6.70%), stem diameter (8.44%), no of leaves/plant (7.98%), Single leaf area (13.01%), fresh leaf weight (15.94%), fresh stem weight (19.21%), fresh green yield (14.55%). Rashad and Sarker (2020) obtained the CV, 12.51, 13.77, 18.54, 17.95 and 18.19% in plant height, stem diameter, leaf/plant, leaf area/plant and shoot weight, respectively.

Table 3. Range, mean, standard deviation and coefficient of variation (CV) in 27 stem amaranth germplasms

Traits	Range			Mean±SE	Std. deviation	CV%
	Minimum	Maximum	Difference			
PH	44.00	76.00	32	61.52±1.88	9.77	15.89
LN	13.50	20.50	7	17.42±0.31	1.61	9.22
LA/pl	281.79	847.15	565.36	468.67±27.74	144.13	30.75
STDIA	10.65	18.20	7.55	14.16±0.41	2.15	15.19
STFW	20.70	60.25	39.55	37.71±1.78	9.24	24.51
LFFW	11.48	28.28	16.8	17.92±0.95	4.91	27.40
SHFW	35.10	80.85	45.75	55.45±2.10	10.91	19.67
STDW	1.86	5.30	3.44	3.17±0.26	0.88	27.70
LFDW	1.62	3.96	2.34	2.50±0.17	0.64	25.69
SHDW	3.81	8.78	4.97	5.66±0.24	1.25	22.08
RWC	82.17	93.40	11.23	89.03±0.66	3.43	3.85
SPAD	29.10	42.80	13.7	36.16±0.68	3.54	9.80
Fv/Fm	0.70	0.78	0.08	0.73±0.006	0.03	4.23

PH = Plant height at harvesting (40 days after sowing), LN = Number of leaves/plant at harvest, LA/plant = Leaf area per plant, STDIA = Stem Diameter at the base, STFW = Stem fresh weight, LFFW = Leaf fresh weight, SHFW = Shoot Fresh weight/ plant (Yield/plant), STDW = Stem Dry Weight, LFDW = Leaf Dry weight, SHDW = Shoot Dry Weight, RWC = Relative Water Content (%), SPAD = SPAD value (Chlorophyll index), F_v/F_m = Maximum quantum yield of PSII

Patterns of correlation among traits

Simple correlation coefficient values demonstrated significant relationships to design breeding strategy (Table 4). Plant height revealed significant positive correlation with relative water content (RWC) (0.385*), SPAD value (0.711**) and Fv/Fm (Maximum quantum yield of PSII) (0.399*) (Table 4; Fig. 1). Number of leaves/plants had significant correlation with SPAD value (0.374*). However, it had significant negative association with leaf fresh weight (-0.442*) and leaf dry weight (-0.436*). Leaf area/plant showed no significant correlation with any of the characters studied. Stem diameter displayed highly significant and positive correlation with stem fresh weight (0.424**), stem dry weight (0.469**), shoot fresh weight (0.473**), and shoot dry weight (0.457**). Stem fresh weight and leaf fresh weight had significant and positive correlation with shoot fresh weight (0.909** and 0.676**), leaf dry weight (0.387* and 0.995*) and shoot dry weight (0.851** and 0.673**). Stem fresh weight displayed highly significant and positive association with stem dry weight (0.914**), but leaf fresh weight did not show correlation with stem dry weight. Shoot fresh weight demonstrated highly significant and positive correlation with stem dry weight (0.820**), leaf dry weight

(0.699**) and shoot dry weight (0.937**). Stem dry weight and leaf dry weight had highly significant and positive association with shoot dry weight (0.880** and 0.715). RWC displayed significant and positive correlation with SPAD value (0.467*) and Fv/Fm (0.656**). SPAD value showed significant and positive correlation with F_v/F_m (0.306*).

Principal Component Analysis

Principal component analysis (PCA) is one of the multivariate statistical techniques which is a powerful tool for investigating and summarizing underlying trends in complex data structures (Legendre and Legendre, 1998). The results of principal component analysis for thirteen traits of 27 stem amaranth germplasm are presented in Table 5. The variation among 27 germplasms was assessed through principal component analysis based on the physio-morphological traits. Principal components (PCs) with Eigen values greater than unity, and factor loadings greater than ± 0.500were meaningful and valuable. The first four principal components (PCs) accounted 81.012% of the total variation and the first principal component alone explained (37.833% of the total variation having Eigen value of 4.918 (Fig. 1 and Table 5). The variation in principal component (1) (PC1) was mainly due to the positive loading effect of stem fresh weight (0.864), leaf fresh weight (0.728), shoot fresh weight (yield) (0.968), stem dry weight (0.829), leaf dry weight (0.760) and shoot dry weight (0.980). The second principal component had an eigen value of 3.912 and accounted for 24.556% of the variation and the major contributing traits were plant height (0.796), relative water content (RWC) (0.723), SPAD value (0.759) and F_v/F_m (0.694). The third principal component had an Eigen value of 1.371 and accounted for 10.95% of the variation, and traits with high positive loading effects were observed for number of leaves/plant (0.595) and leaf area/plant (0.727). In the fourth principal component, it had an Eigen value of 1.050 and accounted (8.074%) of the variation, traits with high and positive loading effect were F_v/F_m (0.582). Table 6also revealed that the first 4 principal components had most contribution to 27 stem amaranth genotypes. From PC1 it was seen that stem fresh weight (0.746), leaf fresh weight (0.530), shoot fresh weight (0.937), stem dry weight (0.687), leaf dry weight (0.578) and shoot dry weight (0.961), from PC2, plant height (0.634), RWC (%) (0.523), SPAD value(0.575) and F_V/F_m (0.481),from PC3 (leaf no./plant (0.354) and leaf area/plant (0.529) showed higher squared cosine values which also proved that plant height, leaf no./plant, leaf area/plant (cm^2), stem fresh and dry weight, leaf fresh and dry weight, shoot fresh and dry weight, RWC (%), SPAD value and Fv/Fm ratio had greater contribution to genetic divergence. Sharma *et al*. (2024) found 5 principal components when eigen values>1 during evaluating 22 vegetable amaranth genotypes in summer season.

Table 4. Correlation (Pearson) matrix showing correlation among studied traits in 27 stem amaranth germplasms

Variables	PH	LN	LA/pl	STDIA	STFW	LFFW	SHFW	STDW	LFDW	SHDW	RWC	SPAD	F_v/F_m
PH	**1**												
LN	0.314	**1**											
LA/Pl	-0.133	0.142	**1**										
STDIA	0.300	-0.081	0.065	**1**									
STFW	0.248	-0.093	0.213	0.473*	**1**								
LFFW	-0.251	-0.442*	0.197	0.182	0.351	**1**							
SHFW	0.042	-0.244	0.277	0.424**	0.909**	0.676**	**1**						
STDW	0.286	-0.046	0.275	0.469**	0.914**	0.253	0.820**	**1**					
LFDW	-0.200	-0.436*	0.211	0.202	0.387*	0.995**	0.699**	0.305	**1**				
SHDW	0.110	-0.255	0.309	0.457**	0.851**	0.673**	0.937**	0.880**	0.715**	**1**			
RWC	0.385*	0.072	-0.315	0.198	0.093	-0.170	-0.026	0.326	-0.108	0.189	**1**		
SPAD	0.711**	0.374*	-0.322	0.127	0.116	-0.207	-0.049	0.163	-0.164	0.035	0.467*	1	
F_v/F_m	0.399*	0.139	-0.059	0.061	0.043	-0.300	-0.060	0.325	-0.241	0.131	0.656**	0.306*	1

'*'& '**' indicate significant at P<0.01& P<0.05, respectively. Figures with esterix sign are non-significant.PH = Plant height at harvesting (40 days after sowing), LN = Number of leaves/plant at harvest, LA/plant = Leaf area per plant, STDIA = Stem Diameter at the base, LFFW = Leaf fresh weight, SHFW = Shoot Fresh weight/plant (Yield/plant), STFW = Stem Fresh Weight, STDW = Stem Dry weight, LFDW = Leaf Dry weight, SHDW = Shoot Dry Weight, RWC = Relative Water Content (%), SPAD = SPAD value (Chlorophyll index), F_v/F_m = Maximum quantum yield of PSI.

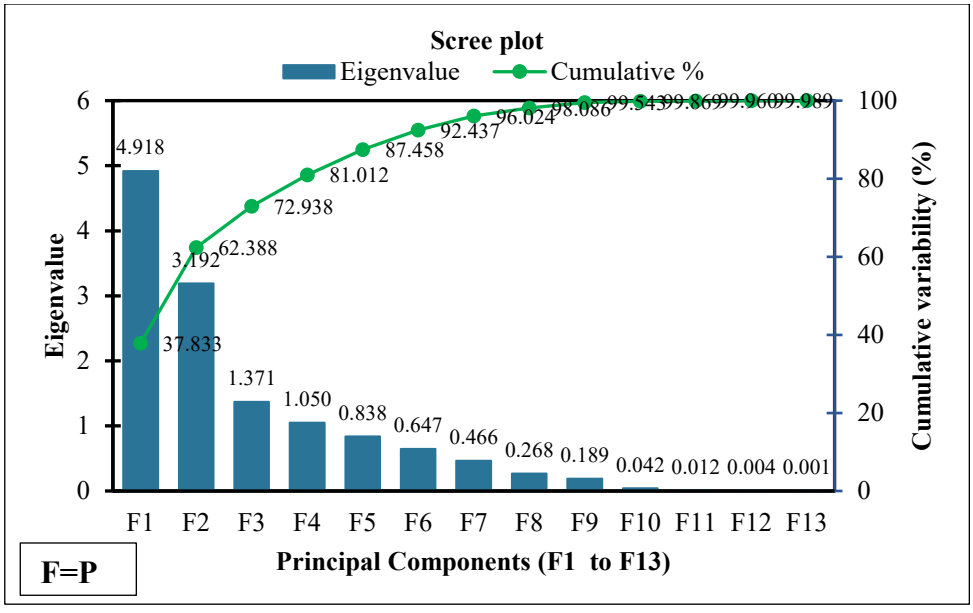

Fig. 1. Graphical representation of Eigenvalues with cumulative variability.

Table 5. Factor loadings, variance explained and Eigen values of thirteen traits and twenty-seven (27) stem amaranth germplasms evaluated at vegetable research field of HRC, BARI

Traits	PC1	PC2	PC3	PC4
Plant height (cm)	0.074	0.796	0.039	-0.358
Leaf No./plant	-0.316	0.436	0.595	-0.240
Leaf area/plant (cm^2)	0.319	-0.241	0.727	0.244
Stem diameter (mm)	0.516	0.295	0.016	-0.168
Stem fresh weight (g)	0.864	0.248	0.212	-0.052
Leaf fresh weight (g)	0.728	-0.477	-0.293	-0.172
Shoot fresh weight (g)	0.968	-0.028	0.079	-0.051
Stem dry weight (g)	0.829	0.415	0.195	0.200
Leaf dry weight (g)	0.760	-0.415	-0.297	-0.148
Shoot dry weight (g)	0.980	0.102	-0.008	0.085
Relative water content (RWC) (%)	0.077	0.723	-0.429	0.323
SPAD value	-0.030	0.759	-0.155	-0.478
Fv/Fm	0.004	0.694	-0.123	0.582
Eigen value	4.918	3.912	1.371	1.050
Variability (%)	37.833	24.556	10.550	8.074
Cumulative variability (%)	37.833	62.388	72.938	81.012

Table 6. Squared cosines of 13 characters by 4 principal components (PC) in 27 stem amaranth germplasms

Traits	PC1	PC2	PC3	PC4
Plant height (cm)	0.005	**0.634**	0.001	0.128
Leaf no./plant	0.100	0.190	**0.354**	0.058
Leaf area/plant (cm^2)	0.102	0.058	**0.529**	0.060
Stem diameter (mm)	0.266	0.087	0.000	0.028
Stem fresh weight (g)	**0.746**	0.062	0.045	0.003
Leaf fresh weight (g)	**0.530**	0.227	0.086	0.030
Shoot fresh weight (g)	**0.937**	0.001	0.006	0.003
Stem dry weight (g)	**0.687**	0.172	0.038	0.040
Leaf dry weight (g)	**0.578**	0.172	0.088	0.022
Shoot dry weight (g)	**0.961**	0.010	0.000	0.007
Relative water content (RWC) (%)	0.006	**0.523**	0.184	0.104
SPAD value	0.001	**0.575**	0.024	0.229
Fv/Fm	0.000	**0.481**	0.015	0.339

Cluster analysis

Cluster analysis was conducted following the agglomerative hierarchical clustering Ward's method, in order to categorize germplasm into different homogeneous groups. Cluster analysis classified the 27-stem amaranth germplasm into five distinct clusters (Table 7 and Figures 2 & 3). These findings indicated the presence of diversity among the germplasms tested. Cluster-III was the largest cluster which consisted of 9 germplasms (33.33%) followed by cluster-II (7 germplasms) (25.92%), cluster-I (5 germplasms) (18.52%) cluster-IV (4 germplasms) (14.81%), respectively. While cluster-V had the lowest number of germplasms that comprised 2 germplasms (7.41%). Similar findings were observed in stem amaranth genotypes by different researchers. Akhter *et al*. (2013) reported that 17 stem amaranth genotypes grouped into four distinct clusters like cluster-IV, cluster-II, cluster-III and Cluster-I having 6, 5, 3 and 1 genotypes, respectively. Ahammed *et al*. (2013) observed 22 genotypes were grouped into four distinct clusters for 13 characters. Joshi and Rana (1995) also reported that 20 grain amaranth genotypes were grouped into nine clusters for 11 characters. Yeshitila *et al.* (2023) obtained five clusters for 120 genotypes using 24 characters and 67amaranth germplasms were grouped into 12 clusters (Rani *et al*., 2020). Twenty-two vegetable amaranth genotypes were clustered into four distinct groups for 14 characters (Sharma *et al*., 2024).

Table. 7. The distribution of 27 stem amaranth germplasm into five clusters based on Euclidean distance

Cluster number	Number of germplasms	List of germplasms in a cluster
1	5 (18.52%)	Am01, AM02, AM03, AM62 and AM63
2	7 (25.92%)	BD8278, BD8152, AM51, AM53, AM54, AM61 and AM64
3	9 (33.33%)	AM55, AM57, AM66, AM67, AM73, AM75, AM77, AM78 and AM79
4	4 (14.81%)	AM58, AM68, AM69 and AM71
5	2 (7.41%)	AM65 and AM72

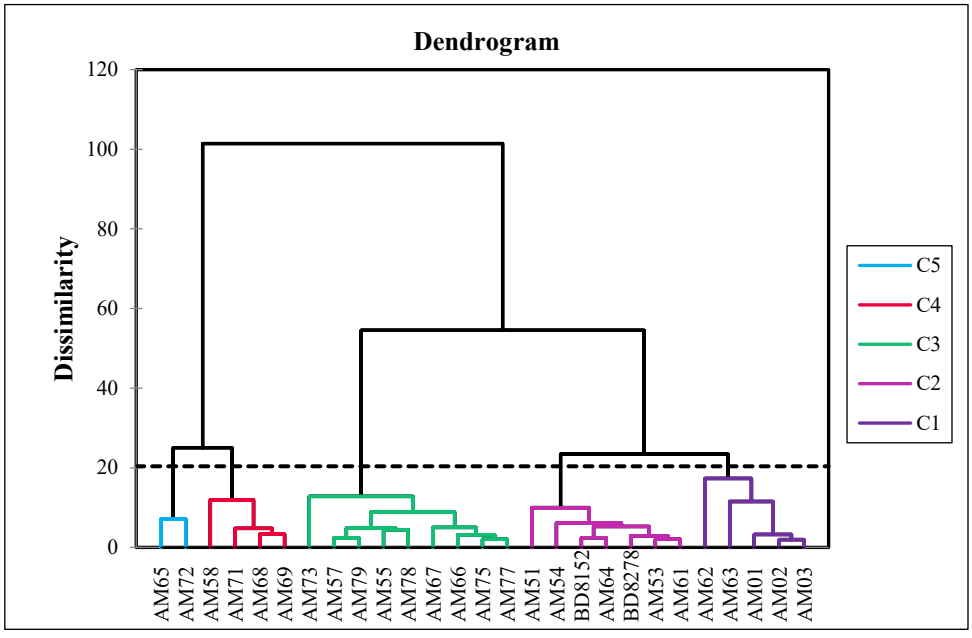

Fig. 2. Dendrogram of 27 germplasms of stem amaranth based on evaluation of 13 traits.

C1 = Cluster-I, C2 = Cluster-II, C3 = Cluster-III, C4 = Cluster-IV and C5 = Cluster-V

Analysis of inter and intra cluster distance

The results of intra and inter-cluster distance analysis are shown in Table 8. The average intra-cluster distance was the highest in cluster-II (D^2 =8.61) followed by Cluster -V (D^2 =4.856) and the lowest in cluster-III (D^2 =0.882). The maximum inter-cluster distance was observed between cluster-III and V (D^2 =59.172) followed by cluster-III and IV (D^2 = 41.197) and Cluster- I and V (D^2=37.655),

whereas, the smallest inter cluster distance was recorded in between clusters-I and II (D^2=9.559) followed by clusters-II and IV (D^2=18.122) and clusters - I and IV (D^2=19.681). It is distinguished that the greater the distance between clusters, the wider the genetic diversity would be between the genotypes. Thus, the largest inter-cluster distance indicates the existence of wide proximity between these clusters as compared with other clusters. Hence, crossing genotypes between these two clusters could result better genetic recombinant. The minimum inter-cluster distances had the lowest degree of divergence between clusters showed that the genotypes were relatively close to each other than genotypes grouped in other clusters. This indicated that the existence of closer proximity between these clusters as compared with other clusters. Consequently, strategic hybridization between these two clusters is now encouraged to exploit heterosis, as increased genetic distance between parental lines is often positively correlated with hybrid vigor and transgressive segregation (Fujimoto *et al*., 2018).

Therefore, the germplasm falling in clusters between Clusters-III and V, between clusters - III and IV were genetically most divergent. The inter-cluster distances were greater than intra-cluster distances, revealing considerable amount of genetic diversity among the genotypes studied (Table 8).

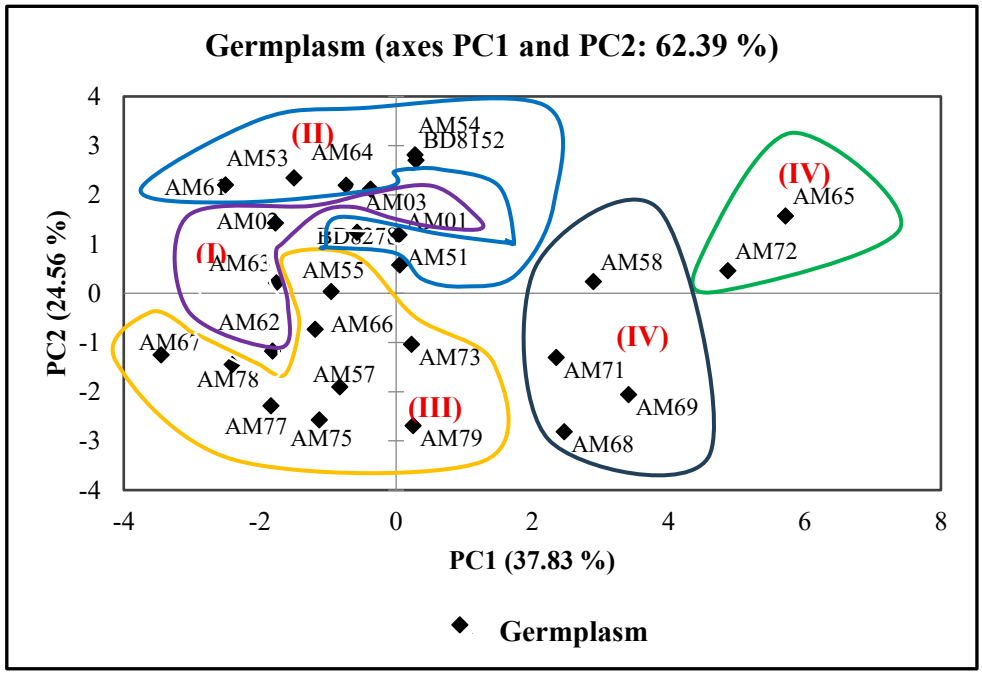

Fig. 3. Scatter distribution of 27 stem amaranth germplasms based on their principal component scores super imposed with clustering.

I = Cluster-I, II = Cluster-II, III = Cluster-III, IV = Cluster-IV and V = Cluster-V.

Table. 8. Intra (bold) and inter-cluster distances (off bold) (D^2) of 27 stem amaranth germplasms

Cluster	I	II	III	IV	V
I	**2.2258**				
II	1.55898	**8.61**			
III	21.5166	23.0756	**0.8822**		
IV	19.6805	18.1215	41.1971	**2.30924**	
V	37.6549	36.096	59.1716	17.9745	**4.85591**

Cluster mean analysis

The mean values of five clusters for 13 different traits revealed considerable differences among the clusters of 27 amaranth germplasms (Table 9). For most traits germplasms grouped in cluster -V had the maximum cluster mean values followed by genotypes assigned in cluster- I, cluster-II and cluster-IV. Minimum cluster mean values was observed in Cluster-III, for leaf area/plant (395.17 cm^2), stem diameter (13.23mm), stem fresh weight (31.74 g), shoot fresh weight (50.13 g), stem dry weight (2.40 g), shoot dry weight (4.41g) and F_v/F_m (0.703). Thus, the cluster mean analysis indicated that, genotypes in cluster-V had the maximum value of stem diameter (17.50 mm), stem fresh weight (58.30 g), leaf fresh weight (25.56 g), shoot fresh weight (79.78 g), stem dry weight (5.06 g), leaf dry weight 3.57 g), shoot dry weight (8.63 g), relative water content (93.31%) and SPAD value (39.10). Whereas, the second largest number for stem diameter (14.42 mm), stem fresh weigh (44.25 g), leaf fresh weight (24.17 g), shoot fresh weight (67.80 g), stem dry weight (3.82 g), leaf dry weight (3.31 g) and shoot dry weigh (7.13 g), the lowest plant height (57.62 g), leaf no./plant (15.20), RWC (87.24%), SPAD Value (33.63) and the highest leaf area/plant (589.30 cm^2) in cluster- -IV. Cluster -I had the highest value for plant height (67.70 cm), leaf no./plant (19.30), 2nd highest for RWC (91.08%) and SPAD value (38.43) and the lowest for leaf fresh weight (13.77 g) and leaf dry weight (1.96 g). Cluster-II had the highest Fv/Fm (0.771).

Table 9. Cluster means for 13 traits of 27 stem amaranth germplasms

Traits	Cluster-I	Cluster-II	Cluster -III	Cluster- IV	Cluster-V
Plant height (cm)	67.70	67.50	63.89	57.62	67.25
Leaf no./plant	19.30	18.24	16.67	15.20	17.75
Leaf area/plant (cm^2)	500.39	454.65	395.17	589.30	528.00
Stem diameter (mm)	14.18	14.25	13.23	14.42	17.50

Traits	Cluster-I	Cluster-II	Cluster -III	Cluster- IV	Cluster-V
Stem fresh weight (g)	38.42	35.27	31.74	44.25	58.30
Leaf fresh weight (g)	13.77	14.87	18.12	24.17	25.56
Shoot fresh weight (g)	52.24	50.56	50.13	67.80	79.78
Stem dry weight (g)	3.02	3.37	2.40	3.82	5.06
Leaf dry weight (g)	1.96	2.15	2.47	3.31	3.57
Shoot dry weight (g)	4.98	5.57	4.81	7.13	8.63
Relative water content (RWC) (%)	91.08	89.25	87.43	87.24	93.31
SPAD value	38.43	37.99	33.96	33.63	39.10
F_v/F_m	0.721	0.771	0.703	0.706	0.736

Biplot analysis

In Biplot analysis, the first two PCs F1 and F2 that had Eigenvalues greater than three (4.918 and 3.192), respectively and contributing 62.39% variability (Fig. 1 and Table 5). Germplasms that are closely located on biplot, perceived as alike when rated on given attributes. More the distance between the point of origin and germplasms, more diverse the genotypes will be from others. Regarding the first two PCs, genotypes were differentiated into five diverse groups (Figure1 and Table 5). Two germplasm i.e. AM65 and AM72 were positioned far away from the origin and were considered as diverse from the others and they fell under group five (Fig. 4). Remaining genotypes were clustered into three groups. Germplasms, namelyAM01, AM02, AM03, AM62 and AM63 were clustered together and closer to origin as well, hence, these germplasms were less diverse and had less breeding value and fell under first group. Genotypes *viz.*, BD8278, BD8152, AM51, AM53, AM54, AM61 and AM64 formed the 2nd group while genotypesAM55, AM57, AM66, AM67, AM73, AM75, AM77, AM78 and AM79 were differentiated from the rest of the genotypes and clustered in third group. The fourth group had only 4germplasms i.e. AM58, AM68, AM69 and AM71. It is shown that Biplot analysis had similar result to dendrogram (Fig. 2).

The graphic in which the biplot of stem amaranth germplasm were combined with the examined morpho-physiological characteristics was given in Figure 5. In this graph, wide angles $>90^0$, had a negative relationship, while there was no correlation between right angles. On the other hand, similarly the Euclidian distances between cultivars were used to calculate in dry beans (Adams, 1977). When the biplot was examined (Fig. 9), there was a positive relationship between the narrow-angled features, for example STDI with STDW, STFW with STDW, SHFW with SHDW, PH with SPAD etc. Right angled features were not related to each other for example SHDW with SPAD value, etc. Wide angle features had negative

relationships with each other for example LFDW with LN, SHDW with LN etc. The above relationships in biplot were proved by correlation matrix of those characters (Table 4). The biplot technique enables the determination of the relationships between the variables as well as the detailed description of a multivariate data set (Yan and Rajcan, 2002).

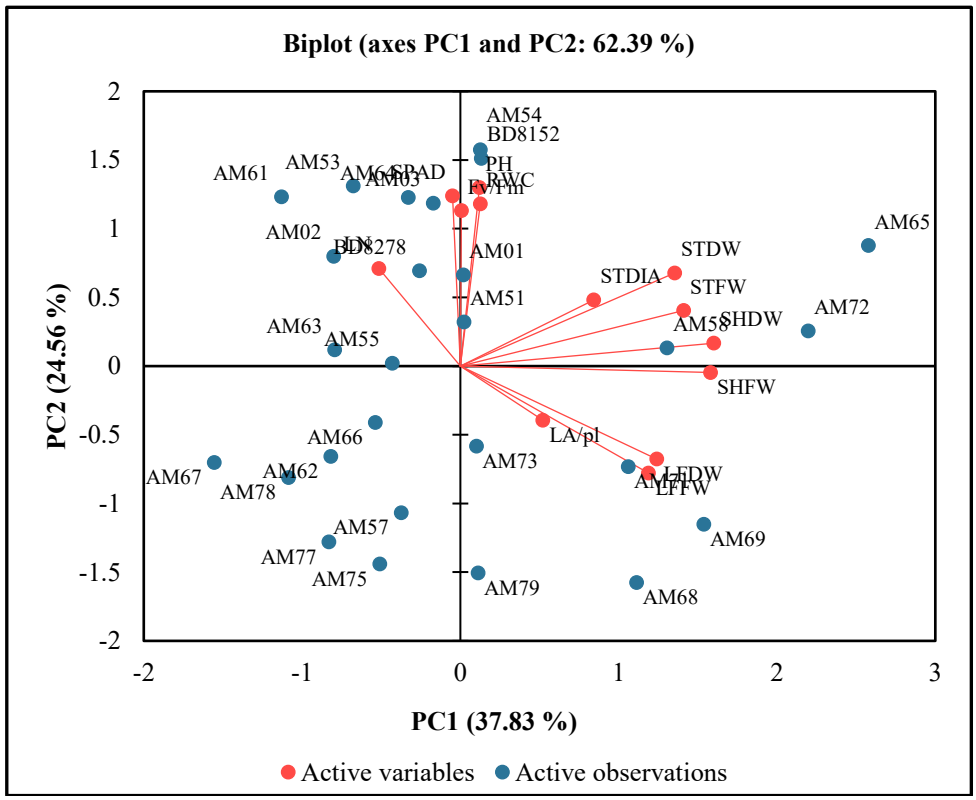

Fig. 4. Principal Component Analysis (PCA) biplot of the morpho-physiological characteristics of 27 stem amaranth germplasms for the first two principal components.

Active variables: Characteristics; Active observations: Stem amaranth germplasm

Contribution of the characters towards divergence of the 27 stem amaranth germplasm

The results of PCA revealed that in vector I the important characters responsible to genetic divergence in the major axis of differentiation were leaf area/plant (0.144), leaf fresh weight (0.328), shoot fresh weight (0.436), leaf dry weight (0.343) having positive vector values (Table 10) which played a major role while rest of the characters played a minor role in the 1st axis of differentiation (Table

10). While in vector II which was second axis of differentiation, plant height (0.446), number of leaves/plant (0.244), RWC (0.405), SPAD value (0.425) and F_v/F_m (0.388) having positive vector values played a major while rest of the characters played a minor role in the 2nd axis of differentiation. The role of stem diameter (0.233 in vector I and 0.165 in vector II), stem fresh weight (0.389 in vector I and 0.139 in vector II), stem dry weight (0.374 in vector I and 0.232 vector II) and shoot dry weight (0.442 in vector I and 0.057 in vector II) in both the vectors indicated that important components of genetic divergence among 27 germplasm of stem amaranth. These results are in partial agreement with the results of Ahammed *et al*., 2013).

Table 10. Latent vectors for 13 principal characters of stem amaranth germplasms

Traits	Vector I	Vector II
Plant height (cm)	0.033	0.446
Leaf no./plant	-0.142	0.244
Leaf area/plant (cm^2)	0.144	-0.135
Stem diameter (mm)	0.233	0.165
Stem fresh weight (g)	0.389	0.139
Leaf fresh weight (g)	0.328	-0.267
Shoot fresh weight (g)	0.436	-0.016
Stem dry weight (g)	0.374	0.232
Leaf dry weight (g)	0.343	-0.232
Shoot dry weight (g)	0.442	0.057
Relative water content (RWC) (%)	0.035	0.405
SPAD value	-0.014	0.425
F_v/F_m	0.002	0.388

Conclusion

The genetic diversity within a crop like stem amaranth is crucial for its improvement in stem yield. By identifying distinct clusters, researchers can identify germplasm with unique traits that could be valuable for breeding programs aimed at enhancing yield. The present study was an attempt to estimate the genetic divergence of 27 stem amaranth germplasms collected from different parts of Bangladesh. The results revealed germplasm had a significant level of genetic divergence. Cluster and PCA analysis were used to divide the stem amaranth germplasm into five groups. Cluster- I and II were determined to be closest, while clusters-III and had the greatest maximum distance followed by clusters -III and IV. These clusters had more divergent germplasms and highly divergent germplasms would provide a wide range of variability, allowing the crop improvement. Therefore, crossing between the germplasm of Cluster-III with those of Cluster -V (Cluster-III × Cluster-V) and Cluster-III with those of Cluster-IV

(Cluster-III × Cluster IV) would exhibit better heterosis and also likely to produce new recombinants with desired characters in stem amaranth.

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