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Principal component analysis in putative mutant lines of snake gourd (*Trichosanthes anguina* l.)

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ABSTRACT

Snake gourd, a monoecious, warmth-loving underutilized cucurbit, is a good source of proteins, carbohydrates, minerals, fibre and several other phyto-nutrients, making it a wholesome and healthy food. In a populous country like India, the present challenge and dire need is to enhance food productivity as well as the crop biomass potential per unit area in the farmers' fields to ensure nutritional and income security. Fifteen advanced mutant lines and a parental control of snake gourd were evaluated for yield performances in 2023 to assess genetic diversity through principal component analysis (PCA) and D^2 analysis.PCA showed that the first Eigen root had a maximum of 39.491% variation of the total variation, while the first four principal component axes together explained 83.054% of the variation. Clustering through D^2 analysis revealed maximum inter-cluster distance of 200.489 between clusters I and III followed by cluster III and V (187.754), thus, the genotypes grouped under cluster I, III and V may contribute maximum to heterosis with desirable agronomic traits having wide variability , including transgressive segregants in selfed generations in future hybridization programmes. Principal components reduced the yield related traits into four PCs, which explain 83.054% of the total variation. Length and girth of fruits, number of seeds per fruit and ascorbic acid content contributed the most towards divergence. Based on divergence, all the mutant lines along with the control were grouped into five clusters and selecting the parents from widely separated clusters are the most probable to give desirable recombinants. BCSG-2, BCSG-9, BCSG-12 and BCSG- 14 have been found to be significantly potent lines. It is quite evident that the genotypes from among the geographically different populations and the places of collection may not be genetically diverse and hence, it should be taken into consideration for future snake gourd improvement programmes.

Keywords: *Snake gourd, Underutilized cucurbit, Putative mutants, Genetic divergence, PCA, D2 analysis, Cluster distance.*

Introduction

Snake gourd (*Trichosanthes anguina* L.) belongs to the family of Cucurbitaceae with a somatic chromosome number of 2n=2x=22. It is an annual, hardy and monoecious herbaceous climbing type plant which is cultivated widely in the warm and humid regions of India. *Trichosanthes* is the largest genus of Cucurbitaceae with 91 species, of which 24 have been reported from India [7] [4], out of which, only two are cultivated, namely, *Trichosanthes anguina* L. and *Trichosanthes dioica* Roxb., having considerable variability and the rest are wild. Irradiation through gamma rays may significantly induce variability in characters like plant height, disease resistance, yield and nutritional qualities [14] [10]. Appropriate parents selection is essential to exploit the genetic recombination potential to increase yield. It is a prerequisite for choosing promising genetically diverse lines for desirable traits [20] [16]. Based on the genetic divergence study, the genotypes are assigned to specific heterotic groups to create segregating progenies with maximum genetic variability for further breeding purposes. Genetic diversity analysis is well exploited for transferring desirable genes from diverse genetic stocks available in the gene pool for broadening the genetic base in crops with narrow ones. Cluster analysis and PC (principal component) analysis are the important genetic diversity measuring tools employed for exhibiting relative genetic differences among the genotypes. Therefore, the present study was undertaken to assess and determine the nature and magnitude of genetic diversity in advanced snake gourd mutant lines.

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Materials and Methods

The present investigation was carried out at the Horticulture Research Station, Monduri, Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal, during March to August of 2023. The experimental materials comprised of 15 advanced snake gourd mutant lines (BCSG-1, BCSG-2, BCSG-3, BCSG-4, BCSG-5, BCSG-6, BCSG-7, BCSG-8, BCSG-9, BCSG-10, BCSG-11, BCSG-12, BCSG-13, BCSG-14 and BCSG-15),which were selected from the M_4 generation obtained through gamma ray irradiated *Bongaon Desi* seeds (parental control). These lines have been maintained in the Department of Vegetable Science, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia and were planted in a randomized block design with three replications. The crop was planted in 6 m long rows, spaced 1.0 m apart with 75 cm plant to plant spacing. All the recommended agronomic practices and plant protective measures were followed to raise a good crop. Observations on twenty eight qualitative and quantitative characters from each mutant lines were recorded on growth habit, stem pubescence, stem colour, stem shape, petiole pubescence, lamina type, lamina colour, tendrils type,

flower colour, fruit rind colour, fruit surface, rind thickness, fruit shape, seed colour, length of main stem (cm), number of primary branches, number of nodes on the main stem, number of fruiting nodes on main stem, days to 50 % flowering, sex ratio (F:M), length of fruit (cm), girth of fruit (cm), average fruit weight (g), number of fruits per plant, fruit yield per plant, number of seeds per fruit, total soluble solids (^{0}Brix) and ascorbic acid (mg/100g). The recorded observations were averaged to get the mean values. Principal component analysis was done to summarize the data in a reduced number of factors that helps in selection of the superior genotypes. Genetic divergence was assessed through D^2 statistic [15] for quantitative traits. Grouping of genotypes was done using Torcher's method [19]. Hierarchical cluster analysis was done to observe the degree of association based on characteristics expressed in dendrogram following Ward's method [21].

Results and Discussion

Qualitative characterization of sixteen putative mutant lines of snake gourd

The qualitative characters of fifteen advanced mutant lines of snake gourd, along with control have been recorded and furnished in Table-1. Several macro-mutations were noted in the mutant lines which differed from the control e.g., stem pubescence (densely pubescent to pubescent), stem colour (dark green to green), lamina type (entire to serrated or multifid), fruit rind colour (dark green with white striped to light green) and fruit shape (short and club-shaped to elongated or medium shaped). Among these mutants the ones which have different lamina type, stem colour, fruit rind colour, tendril type etc. may be used as markers in the breeding programmes after confirming its stability through subsequent generations and multi-location trials.

Mean performances

The mean performances of mutant lines and their control for different traits are presented in Table 2. The minimum mean values for days to 50 % flowering were considered desirable for earliness. Among the mutant lines, only four mutant lines BCSG-14 (52.80 days), BCSG-12 (53.20 days), BCSG-13 (54.40 days) and BCSG-9 (54.60 days) took lesser days for 50 % flowering than the parental control (57.10 days). [5] and [9] also reported the earlier flowering time in the mutated lines than parental line of snake gourd. There is an increase in the grand mean value (15.55) of fruiting nodes on the main stem than the control (15.22). Among the mutant lines, the sex ratio ranged from 9.20 (BCSG-8) to 11.00 (BCSG-5). Almost all the mutant lines have produced a higher number of female flowers than the control (11.64) except BCSG-4 (10.95), BCSG-5 (10.98) and BCSG-6 (10.85). The mean value (9.88) indicates a very satisfactory sex ratio in the putative mutants. [13] and [17] too found an enhanced femaleness in cucumber with gamma irradiation.

Maximum length of fruit was recorded in BCSG-6 (36.72 cm) followed by BCSG-5 (33.69 cm) and BCSG-3 (29.50 cm). An increase in fruit girth was noticed in BCSG-6 (19.60 cm) followed by BCSG-5 (19.20 cm), BCSG-4 (18.80 cm), BCSG3 (17.80 cm) and BCSG-9 (17.60 cm) over the parental control (17.20 cm). [5] and [9] noted an increased fruit circumference in mutant lines over the parent in snake gourd. For average fruit weight, the reduction in grand mean value was observed in M_5 generation as compared to the control (218.60 g). [3] and [13] reported an increase in sponge gourd fruit weight.

Increased fruit yield was noticed in many of the lines, namely BCSG-2 (7.60 kg) followed byBCSG-12(7.41 kg), BCSG-3 (6.58 kg), BCSG-7 (6.51 kg), BCSG-9 (6.51 kg), BCSG-8 (6.09 kg), BCSG-6 (6.03 kg), and BCSG-4 (5.82 kg) over the control (4.96 kg). The lines BCSG-12, BCSG-13 and BCSG-14 recorded the maximum number of fruits per plant i.e., 33.80, 30.20 and 32.60, respectively. The grand mean value for fruits per plant (26.71) was higher than the parental line (24.85). Similar results have also been reported by [1] in tomato and [6] in okra with gamma treatment. The maximum total soluble solid content was recorded in BCSG-15 (4.80) followed by BCSG-13 (4.20) and BCSG-5 (4.10) while, lines BCSG-9, BCSG-8, BCSG-7 and BCSG-13 showed maximum content of ascorbic acid. [12] registered the same pattern of results in total soluble solid and ascorbic acid content in sponge gourd.

Principal component analysis

Principal Component Analysis (PCA) is employed to explain the variance-covariance structure of a set of variables through linear combinations and often to reduce the dimensionality of large data sets. The PCA was done to obtain a basic interpretation of the relationship between the characters that explained 100% contribution toward the divergence. The PCA reduced the fourteen quantitative traits into four Principal Components having eigenvalues >1 that explains 83.054% of the total variation (Table 3).

The first component (PC1) explained 39.491 % of total variation that was mainly defined by length of the main stem, days to 50 % flowering, length of fruit, average fruit weight, fruit yield per plant, number of seeds per fruit and ascorbic acid. The second component (PC2) accounted for 24.727 % of total variation and was related to the number of nodes on main stem and number of fruiting nodes on main stem. The third component (PC3) explained 11.016 % of total variation and was defined by sex ratio and number of fruits per plant. The fourth component (PC4) explained 7.82 % of total variation, which was defined by number of fruiting nodes on main stem, total soluble solid and ascorbic acid (Table 4). [8] also analysed the same to determine the genetic diversity and morphological relationship in round gourd genotypes and reported a similar trend as that of the present investigation. The principal component analysis of 16 snake gourd genotypes based on correlation matrix of yield and yield contributing traits yielded the 6 Eigen roots or Eigenvalues. Eigen roots along with percentage of variation explained by each Eigenvalues (Latent roots) have been presented in Table 3. Principal component analysis revealed that the highest Eigen value (5.134) was noted in the first principal axis. The Eigen root of the first principal component was accounted to be approximately 39.491% of the total variation, followed by second to fourth components, which accounted for 24.727, 11.016, and 7.820 % of the total variation existing among the genotypes, respectively. The first four PC axes explained 83.054% of the variations, indicating that the first four principal axes are adequate to explain the variation in reduced dimension.

The combined two dimensions (F1 and F2) of the bi-plot explain a substantial proportion (63.44%) of the variance and variables contributing heavily to F1 distinguish the two colour groups significantly (Figure-1). It also suggests that the higher-numbered groups (3 and 4) are more similar to each other. A clear group separation along the first principal component is evident and secondary variation is visible along F2 axis with distinguishable clustering patterns, which might through some

light on variable loadings. So, it can be inferred that the lines BCSG-1, BCSG-3, BCSG-4 and BCSG-5 have closer relationship with that of the control whereas, BCSG-10, BCSG-11, BCSG-13 and BCSG-14 were closely related to each other but different from the rest which could form separate clusters.

Genetic diversity of putative mutant lines of snake gourd

Based on divergence, all the sixteen lines are grouped into five clusters (Table 5). Cluster I, the largest one, comprises of five lines i.e., BCSG-1, BCSG-2, BCSG-3, BCSG-4, along with the parental control. Cluster III, IV and V have 3 lines in each. However, Cluster II, the smallest one had only two lines (BCSG-5 and BCSG-6). The intra- and inter-cluster distances of sixteen lines of snake gourd indicated that Cluster IV had the highest intra-cluster value representing that the lines present in the cluster (BCSG-10, BCSG-11 and BCSG-12) were extremely diverse (Table 7). Among the inter-cluster values, the maximum values were noted between cluster III and V, followed by the cluster IV and V indicating that the genotypes in those clusters were widely diverse. Choosing parents from widely separated clusters are most likely to give desirable recombinants [11].

The cluster mean values of sixteen lines of snake gourd genotypes explained that the mean values of the clusters varied in magnitude for all the characters studied (Table 6).The maximum cluster mean was observed in cluster III for fruit yield per plant along with average fruit weight, number of nodes on main stem, number of fruiting nodes on main stem, length of main stem, number of seeds per fruit and ascorbic acid; while cluster V for earliness (days to 50% flowering), short fruit length and average fruit weight. Hence, it can be suggested from the present study that an early flowering short fruited high yielding type with considerable total soluble solids and ascorbic acid could be bred by utilizing the genotypes from cluster III and V as the parents in future breeding programme.

The contribution of individual characters towards genetic divergence (in terms of the number of times it ranked first) has been given in Table 8 and Figure-4. Ascorbic acid contributed the maximum (41.67%) percentage towards genetic divergence followed by the number of seeds per fruit (18.33%) and fruit size (17.50%) showing the probability for selection of these characters. [12] also found the same trend for yield and its related component traits. Additionally, the dendrogram has been provided, plotted following Ward's method (Figure-2) by using squared Euclidean distance, to make it clearly evident that there was high diversity among these sixteen lines of snake gourd vis-a-vis somewhat strong relationships among the genotypes for certain characters.PC1 primarily reflects yield-attributing and quality traits, while PC2 points toward the morphological and reproductive trade-offs. The multivariate genotype evaluation with genetic diversity assessment in a non hierarchical clustering minimizes intra cluster distances and maximizes inter-cluster distances in an efficient way (Figure-3). Principal Component Analysis (PCA) in putative mutant lines of snake gourd (*Trichosanthes anguina* L.) revealed significant trait variability, aiding selection of superior genotypes. Evaluating a large population enhanced genetic diversity understanding, supporting breeding for higher yield and quality. This contributes to ensuring food and nutritional security through sustainable crop improvement as this crop is somewhat hardy and can grow with less inputs and care, particularly in the homestead gardens as well as in the undescript lands with somewhat below-average nutritional status, acts as a buffer crop if a main crop fails.

Table 1: Qualitative morphological characters of snake gourd mutant lines and the control

Characters	Growth habit	Stem pubescence	Stem colour	Stem shape	Petiole pubescence	Lamina type	Lamina colour
BCSG-1	Viny	Pubescent	Green	Angular	Pubescent	Serrated, entire, multifid	Light green
BCSG-2	Viny	Pubescent	Green	Angular	Pubescent	Serrated, entire, multifid	Light green
BCSG-3	Less viny	Pubescent	Dark green	Angular	Less pubescent	Less serrated, entire	Dark green
BCSG-4	Viny	Pubescent	Green	Angular	Pubescent	Serrated, multifid	Light green
BCSG-5	Viny	Pubescent	Green	Angular	Pubescent	Serrated	Light green
BCSG-6	Viny	Pubescent	Green	Angular	Pubescent	Serrated, multifid	Light green
BCSG-7	Viny	Densely pubescent	Dark green	Angular	Pubescent	Serrated, multifid	Dark green
BCSG-8	Viny	Pubescent	Green	Angular	Pubescent	Serrated, entire	Light green
BCSG-9	Viny	Pubescent	Light green	Angular	Pubescent	Less serrated, entire	Light green
BCSG-10	Viny	Pubescent	Green	Angular	Pubescent	Serrated, entire, multifid	Light green
BCSG-11	Viny	Pubescent	Green	Angular	Pubescent	Serrated	Light green
BCSG-12	Viny	Pubescent	Green	Angular	Pubescent	Serrated, entire	Light green
BCSG-13	Viny	Pubescent	Green	Angular	Pubescent	Serrated, entire	Light green
BCSG-14	Viny	Pubescent	Green	Angular	Pubescent	Serrated, entire, multifid	Light green
BCSG-15	Viny	Pubescent	Green	Angular	Pubescent	Serrated, entire, multifid	Light green
Parental Control	Viny	Densely pubescent	Dark green	Angular	Pubescent	Entire	Light green

Continued to next page

Characters	Tendril type	Flower colour	Fruit rind colour	Fruit surface	Rind thickness	Fruit shape	Seed colour
BCSG-1	Coiled & branched	White	Light green, white strips	Smooth & Striped	Thin	Elongated, medium	Off white
BCSG-2	Coiled & branched	White	Dark green, white strips	Smooth & Striped	Thick	Elongated, medium	Off white
BCSG-3	Coiled & branched	White	Dark green, white strips	Smooth & Striped	Thick	Elongated	Off white

BCSG-4	Coiled & branched	White	Light green, white strips	Smooth & Striped	Thin	Elongated, medium	Off white
BCSG-5	Coiled & branched	White	Light green, white strips	Smooth & Striped	Thin	Elongated	Off white
BCSG-6	Coiled & branched	White	Light green, white strips	Smooth & Striped	Thick	Elongated, medium	Off white
BCSG-7	Coiled & branched	White	Dark green, white strips	Smooth & Striped	Thick	Short, club shaped	Off white
BCSG-8	Coiled & branched	White	Light green, white strips	Smooth & Striped	Thin	Short, club shaped	Off white
BCSG-9	Coiled & branched	White	Light green, white strips	Smooth & Striped	Thin	Short, club shaped	Off white
BCSG-10	Coiled & branched	White	Light green, white strips	Smooth & Striped	Thin	Short, club shaped	Off white
BCSG-11	Coiled & branched	White	Light green, white strips	Smooth & Striped	Thick	Short, club shaped	Off white
BCSG-12	Coiled & branched	White	Light green, white strips	Smooth & Striped	Thin	Short, club shaped	Off white
BCSG-13	Coiled & branched	White	Light green, white strips	Smooth & Striped	Thin	Short, club shaped	Off white
BCSG-14	Coiled & branched	White	Dark green, white strips	Smooth & Striped	Thick	Short, club shaped	Off white
BCSG-15	Coiled & branched	White	Light green, white strips	Smooth & Striped	Thin	Short, club shaped	Off white
Parental Control	Coiled & branched	White	Dark green, white Stripe	Smooth & striped	Thin	Elongated, medium	Off white

Table 2: Mean performance for fourteen quantitative morphological characters of mutant lines and the parental control

Lines	Length of main stem	Number of primary branches per plant	Days to 50% flowering	No. of nodes on main stem	No. of fruiting nodes on main stem	Sex ratio (F:M)	Length of fruit (cm)	Girth of fruit (cm)	Average fruit weight (g)	Number of fruits per plant	Fruit yield per plant (kg)	Number of seeds per fruit	Total soluble solids (^{0}Brix)	Ascorbic acid (mg/100g)
BCSG-1	277.00	3.80	61.40	43.20	17.60	9.42	28.00	15.60	180.32	24.50	4.32	14.65	3.80	9.80
BCSG-2	343.00	3.35	58.80	34.40	18.20	9.30	22.40	13.20	264.70	29.70	7.60	15.54	3.60	10.40
BCSG-3	272.50	3.30	59.20	30.80	15.80	9.25	29.50	17.80	251.24	26.40	6.58	17.82	3.60	9.80
BCSG-4	286.20	2.60	65.60	33.60	18.60	10.95	27.39	18.80	227.50	25.60	5.82	18.46	4.00	12.00
BCSG-5	382.50	3.20	60.60	32.40	15.40	11.00	33.69	19.20	228.80	23.50	5.38	21.35	4.10	11.60
BCSG-6	323.00	3.40	61.40	39.20	11.20	10.85	36.72	19.60	260.10	21.20	6.03	22.28	3.80	11.80
BCSG-7	421.80	1.80	59.50	43.40	20.60	9.35	21.90	15.20	248.36	26.20	6.51	26.68	3.30	12.20
BCSG-8	385.80	2.15	58.40	36.60	17.40	9.20	19.00	13.20	239.89	25.40	6.09	28.16	3.30	12.53
BCSG-9	388.20	1.90	54.60	37.40	16.20	9.22	21.10	17.60	252.20	25.80	6.51	29.40	4.00	12.60
BCSG-10	316.40	1.70	61.80	35.80	12.40	9.60	11.70	10.80	110.00	28.30	3.11	21.88	3.20	9.10
BCSG-11	303.80	1.65	57.50	32.60	12.80	9.52	10.60	10.30	116.40	29.40	3.42	20.52	3.50	7.80
BCSG-12	343.40	1.50	53.20	37.40	15.60	9.48	10.40	9.79	218.60	33.80	7.41	23.62	3.20	8.45
BCSG-13	324.20	1.95	54.40	36.20	14.20	9.95	15.20	11.80	120.35	30.20	3.03	17.28	4.20	12.20
BCSG-14	312.20	1.80	52.80	35.80	15.80	9.80	17.00	15.00	175.40	32.60	5.60	19.45	4.00	11.70
BCSG-15	303.60	2.15	57.40	32.60	11.80	9.70	17.60	14.80	135.00	25.30	3.42	13.65	4.80	10.40
Parental Control	341.50	2.87	57.10	36.20	15.22	11.64	28.42	17.20	198.50	24.85	4.96	22.22	3.70	10.67
Mean	332.82	2.45	58.36	36.10	15.55	9.88	21.91	14.99	191.70	26.71	5.05	20.81	3.76	10.82
Range	272.50-421.80	1.50-3.80	52.80-65.60	30.80-43.40	11.20-20.60	9.20-11.64	10.40-36.72	9.79-19.60	98.50-264.70	21.20-33.80	3.03-7.60	13.65-29.40	3.20-4.80	7.80-12.60
C.V.%	6.331	11.859	5.840	9.099	12.392	6.967	8.087	11.819	8.035	13.303	15.502	9.084	4.296	3.915
S.Em.	12.165	0.167	1.967	1.896	1.113	0.395	1.023	1.023	8.893	2.058	0.449	1.091	0.093	0.244
C.D. 5%	35.136	0.483	5.682	5.477	3.213	1.141	2.955	2.955	25.686	5.944	1.296	3.152	0.269	0.706

Table 3: Principal component analysis (PCA) for quantitative characters contributing to divergence

Parameters	PC1	PC2	PC3	PC4
Eigenvalue (Root)	5.134	3.215	1.432	1.017
Explained Variation (%)	39.491	24.727	11.016	7.820
Cumulative explained variation (%)	39.491	64.218	75.234	83.054

Table 4: Correlation between original variables and the first four principal components (having Eigenvalues > 1)

Characters	PC1	PC2	PC3	PC4
Length of main stem	0.314	0.260	0.195	0.041
Number of primary branches	0.112	-0.460	-0.295	-0.113
Days to 50% flowering	0.377	0.046	-0.315	0.052
Number of nodes on main stem	0.202	0.239	0.201	-0.143
Number of fruiting nodes on main stem	0.060	0.310	0.444	0.272
Sex ratio	-0.188	-0.413	0.300	-0.022
Length of fruit	0.277	-0.426	-0.002	0.006
Average fruit weight	0.413	0.073	-0.104	-0.025
Number of fruits per plant	-0.158	-0.266	0.509	0.041
Fruit yield per plant	0.282	-0.347	0.150	0.249
Number of seeds per fruit	0.411	0.006	0.076	-0.189
Total soluble solids	-0.164	0.038	-0.315	0.789
Ascorbic acid	0.347	-0.126	0.226	0.407

Table 5: Clustering of sixteen snake gourd lines in classes, based on the morphological traits

Cluster	Lines
I	BCSG-1, BCSG-2, BCSG-3, BCSG-4, BCSG-16
II	BCSG-5, BCSG-6
III	BCSG-7, BCSG-8, BCSG-9
IV	BCSG-10, BCSG-11, BCSG-12
V	BCSG-13, BCSG-14, BCSG-15

Table 6: Cluster means for fourteen traits among sixteen snake gourd lines

Cluster	Length of main stem	Number of primary branches	Days of 50% flowering	No. of Nodes on main stem	No. of fruiting nodes on main stem	Sex ratio	Length of fruit	Girth of fruit	Average fruit weight	Number of fruits per plant	Fruit yield of the plant	No. of Seeds per fruit	Total Soluble Solid	Ascorbic acid
I	304.04	3.18	60.42	35.64	17.08	9.91	27.14	16.52	228.47	26.01	5.99	17.74	3.74	10.53
II	352.75	3.30	61.00	35.80	13.30	10.92	35.21	19.40	244.45	22.35	5.43	21.82	3.95	11.70
III	398.60	1.95	59.90	39.13	18.07	9.26	20.67	15.33	246.82	25.80	6.37	28.08	3.53	12.44
IV	321.20	1.62	55.10	35.27	13.60	9.53	10.90	10.30	108.30	30.50	3.28	22.01	3.30	8.45
V	313.33	1.97	54.87	34.87	13.93	9.82	16.60	13.87	123.53	28.37	3.49	16.79	4.33	11.43

Table 7: Intra - (diagonal) and inter-cluster D2 distances of sixteen snake gourd lines

Cluster	I	II	III	IV	V
I	**7.880**	67.146	200.489	129.355	55.873
II		**7.332**	114.874	66.835	31.392
III			**11.297**	69.572	187.754
IV				**31.413**	102.201
V					**0.000**

Table 8: Per cent contribution to divergence of different characters

Source	Contribution %	Times Ranked 1st
Length of main stem	1.67	2
Number of primary branches	2.50	3
Days of 50% flowering	0.01	0
Nodes of the main stem	1.67	2
Fruiting nodes on main stem	3.33	4
Sex Ratio	0.01	0
Fruit length and girth	17.46	21
Average fruit weight	5.83	7
Number of fruits per plant	0.01	0
Fruit yield per plant	0.01	0
Number of seeds per fruit	18.33	22
Total soluble solids	7.50	9
Ascorbic acid	41.67	50

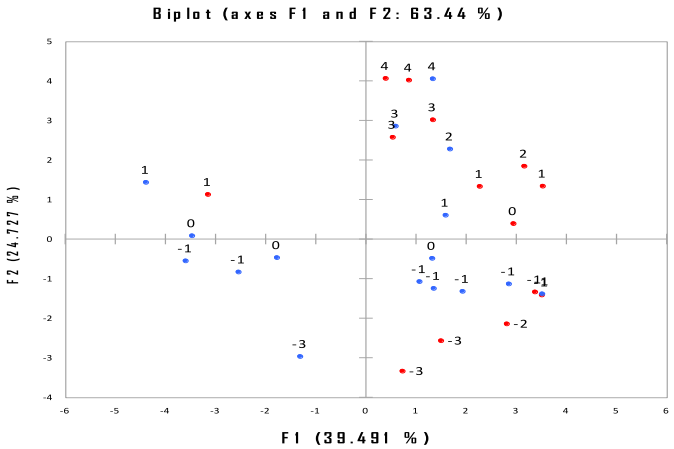

Figure 1: Bi-plot graph for different variances and mutant lines

Variances (Var): Length of main stem (Var 1), number of primary branches (Var 2), days to 50% flowering (Var 3), nodes of the main stem (Var 4), number of fruiting nodes on the main stem (Var 5), sex ratio (Var 6), fruit length (Var 7), fruit girth (Var 8), fruit weight (Var 9), fruits per plant (Var 10), yield of the plant (Var 11), number of seeds per plant (Var 12), TSS (Var 13) and Ascorbic acid (Var 14).

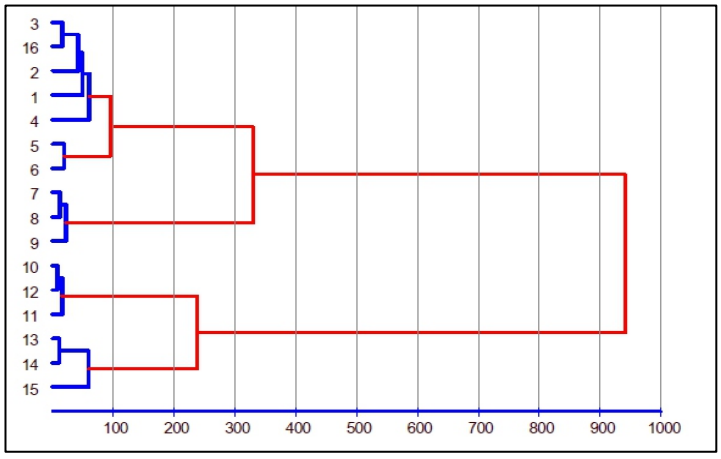

Figure 2: Dendrogram plotted following Ward's method by using squared Euclidean distance

Mutant Lines: 1: BCSG-1; 2: BCSG-2; 3: BCSG-3; 4: BCSG-4; 5: BCSG-5; 6: BCSG-6; 7: BCSG-7; 8: BCSG-8; 9: BCSG-9; 10: BCSG-10; 11: BCSG-11; 12: BCSG-12; 13: BCSG-13; 14: BCSG-14 and 15: BCSG-15

Parental control: 16

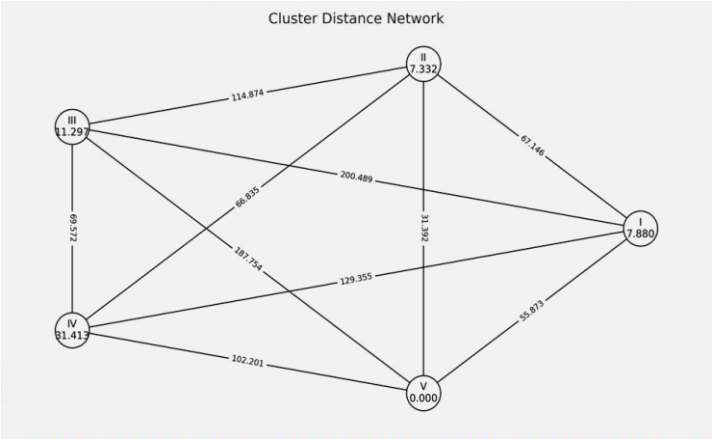

Figure 3: Mahalanobis Euclidean distance through Tocher's method

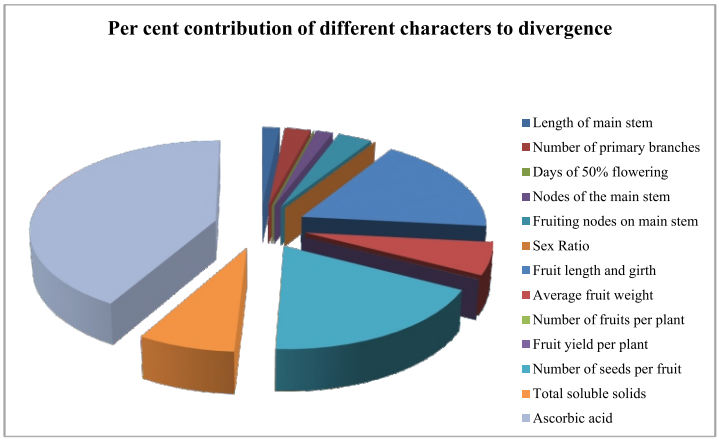

Figure 4: Pie chart of per cent contribution of different characters to divergence

Conclusion

Based on the above findings, it is concluded on the basis of the importance of some characters, namely shorter fruit shape, satisfactory yield, desirable sex ratio, fruits per plant, days to 50% flowering and ascorbic acid content, divergence trend and *per se* performances of the mutant lines, BCSG-2, BCSG-9, BCSG-12 and BCSG-14 have been found to be promising ones and may be rewarding when employed as parents in future breeding programmes.

Future scope

Primarily the putative mutant lines are carefully selected and validated over several generations to confirm genetic stability of the desired traits. Future studies on putative mutant lines of snake gourd (*Trichosanthes anguina* L.) should focus on integrating breeding strategies with value-added utilization of the lines as source parental lines of novel traits. Promising genotypes identified through PCA can further be improved for enhanced yield, nutritional quality, and pigment content. These lines may serve as raw materials for developing natural dyes and chutneys, promoting agri-based entrepreneurship. Additionally, the cone portion of the fruit, when cut in the middle, with suitable curing manipulations, can be explored as a biodegradable base or holder for fast-food industries. Combining genetic improvement with post-harvest and processing innovations will strengthen the crop's economic and industrial potential. Study on the putative mutants can identify a mutation in a specific gene and the connection to the resulting phenotypes, gene expression, marker-assisted selection, genome editing, which can be accelerated by high-efficiency techniques like TILLING (Targeting Induced Local Lesions in Genomes) and pooled CRISPR.

Declaration

All the authors have declared that there is no conflict of interest.

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