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Assessment of genetic divergence in snake gourd (*Trichosanthes anguina* L.) through multivariate techniques

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Abstract
Maintaining, assessing, and characterizing traditional crop diversity is crucial for ensuring sustainable farming and food security. This study focuses on snake gourd (*Trichosanthes anguina* L.), a relatively underutilized, monoecious cucurbit predominantly cultivated with minimal inputs on marginal lands in West Bengal. Significant genetic diversity exists among the snake gourd genotypes in this region, encompassing both landraces and local cultivars. In the present study, thirty-two morphologically diverse snake gourd genotypes were collected from various agro-climatic zones of West Bengal and evaluated during the spring-summer season of 2022–2023 based on morphological and yield traits; genetic divergence among genotypes was estimated using multivariate analysis employing Mahalanobis' D^2 statistic, clustering them by Tocher's method. Among the six distinct clusters, Cluster-I included the highest number of genotypes (13), followed by Cluster-II (11 genotypes), Cluster-III (5 genotypes), while Clusters-IV, V, and VI, each contained a single genotype, suggesting substantial genetic variability among the genotypes. Low intra-cluster distances indicated relative homogeneity within individual clusters, whereas the maximum inter-cluster divergence was observed between Cluster-I and Cluster-V (D^2 = 18.003), reflecting high genetic dissimilarity. Based on key agronomic traits, like days to first female flower, days to first fruit set, days to first harvest, fruit weight, number of fruits per plant, and fruit yield, fiver genotypes namely, BCSG-9, BCSG-6, BCSG-11, BCSG-36, and BCSG-38 were identified as promising candidates for future hybridization programs aimed at developing superior segregating populations.

Keywords: Snake gourd; multivariate analysis; mahalanobis' D^2; genetic divergence

Introduction

Snake gourd (*Trichosanthes anguina* L.) is indigenous to India, exhibiting significant diversity particularly in the eastern states, the north-eastern region, and peninsular India (Mehra & Arora, 1982; Revathy & Kanthaswamy, 2015) [8]. This vegetable, prized for its nutritional value, is an important summer crop in West Bengal and across India, thriving optimally within a temperature range of 30–35 °C. Growth and fruit development are adversely affected when temperatures drop below 20 °C (Gildemacher *et al*., 1994; Ashwini *et al*., 2014) [4, 1]. Due to its adaptability, snake gourd can be cultivated year-round in regions with mild winters.

West Bengal in particular harbors a wide spectrum of variability in snake gourd populations, which is crucial for any breeding or hybridization program focused on improving desirable traits (Chowdhury *et al*., 1975; Deepa Devi *et al*., 2013) [3]. Estimating genetic diversity plays a vital role in identifying valuable gene sources within germplasm collections (Tomooka, 1991; Revathy & Kanthaswamy, 2015) [14]. Despite its agronomic potential and capacity to grow on marginal lands with minimal inputs, snake gourd remains an underutilized and neglected crop within India, with limited advancement in its genetic improvement (Deepa Devi *et al*., 2013) [3]. The crop also holds cultural and economic importance within rural homestead gardens. Therefore, assessing the genetic variability among geographically isolated genotypes is essential for developing elite breeding lines (Revathy &Kanthaswamy, 2015; Ashwini *et al.,* 2014) [1]. This study aims to evaluate the extent of genetic divergence among collected snake gourd genotypes to find out suitable genotypes for utilization in breeding programmes.

Materials and methods

Thirty-one genotypes, collected from different parts of the state were employed for morphological diversity study during spring-summer seasons of 2022-23, in a randomized block design with three replications. Spacing given was 2.5 × 2 m. Plants were mended on trellis. Standard crop husbandry recommendations were followed to raise the crop. Observation on twenty three morphological and yield characters namely, days to seedling emergence, number of primary branches per plant, number of secondary branches per plant, number of tertiary branches per plant, length of vine (m), petiole length (cm), number of nodes/plant, peduncle length (cm), node number of first male flower opening, node number of first female flower, days to first female flower opening, days to 50% female flowering, days to 50% male flowering, days to first fruit set, days to 50% fruit set, node number of first fruit set, length of fruits (cm), weight of fruits (g), diameter of fruits (cm), total number of fruits/plant, days to first harvest, yield (kg/plant) were recorded from average of five plants per plot. Genetic diversity was worked out following Mahalanobis generalize distance, i.e. D^2 statistic (Mahalanobis, 1936) [7] extended by Rao (1952) to clustering in Tocher's method. The mean performance of 32 genotypes of snake gourd has been furnished in Table-4.

Results and discussions

Clustering of genotypes

The multivariate analysis considering all quantitative characters together as a constellation serves as a powerful tool in quantifying the degree of divergence among the genotypes. The genotypes have been grouped into six clusters based on the quantitative characters (Table-1).

Table 1: Distribution of 32 genotypes of snake gourd in different clusters

Cluster	Number of genotypes	Genotypes	Places of collection from different parts of West Bengal, India
Cluster I	13	BCSG-31	Chakdah, Nadia
		BCSG-3	Madanpur, Nadia
		BCSG-32	Bongaon, 24 Parganas(N)
		BCSG-12	Malda Local, Malda
		BCSG-33	Bongaon, 24 Parganas(N)
		BCSG-34	Chunakhali, Murshidabad
		BCSG-9	Awalsiddhi, 24 Parganas(N)
		BCSG-35	Sindrani, 24 Parganas(N)
		BCSG-36	Chakdah, Nadia
		BCSG-37	Habra, 24 parganas (N)
		BCSG-6	Chandmari, Nadia
		BCSG-38	Basirhat, 24 Parganas
		BCSG-39	Krishnanagar, Nadia
Cluster II	11	BCSG-15	Nimpith, 24 Parganas(S)
		BCSG-16	Joynagar, 24 Parganas(S)
		BCSG-17	Amta, Howrah
		BCSG-20	Udainarayanpur, Howrah
		BCSG-28	Rahimpur, Hoogly
		BCSG-21	Joynagar, 24 Parganas(S)
		BCSG-23	Basantapur, Nadia
		BCSG-24	Kharagpur, Midnapur
		BCSG-26	Sonamukhi, Bankura
		BCSG-29	Contai Local, Purba Midnapur
		BCSG-30	Barasat,24 parganas(N)
Cluster III	5	BCSG-1	Joynagar, South 24 parganas
		BCSG-4	Haldia, Midnapur
		BCSG-5	Balagarh, Hoogly
		BCSG-10	Kaliyaganj, Uttar Dinajpur
		BCSG-11	Fatehpur, Nadia
Cluster IV	1	BCSG-2	Ramnagar, Midnapur
Cluster V	1	BCSG-25	Kaliyaganj, UttarDinajpur
Cluster VI	1	BCSG-14	Paddapukuria, UttarDinajpur

However, revelation of a relatively lesser number of clusters indicates either a common character constellation manifested simultaneously in the genotypes or a mutual balancing of characters operative in the genotypes.

Comparing the clustering pattern and grouping of the genotypes, it has been found that many of the genotypes from the same or nearby areas have fallen in the same clusters, whereas some genotypes collected from the same region did not fall into a single cluster. This indicates that geographical proximity does not always corroborate with genetic similarity. Genetic drift and natural selection in different environments result in huge diversity in genotypes, and there is no fixed relationship between geographic and genetic diversity (Upadhya & Murty, 1970; Revathy & Kanthaswamy, 2015; Deepa Devi *et al*., 2013) [15, 3]. Thus, factors other than geographical diversity are responsible for genetic diversity, and it is possible for genotypes of the same geographic location to have different genetic constitution.

Morphological diversity becomes evident through the clustering patterns, and the results of the present study are in tune with Khatun *et al.* (2010) [6] as well as recent findings in cucurbits (Rai *et al.,* 2016; Singh *et al*., 2018) [10]. It can further be said that the diversity present in the genotypes in any particular area may not be distinct, but there exists wide genetic diversity among the genotypes collected from different areas and hence these can be effectively used for crop improvement. Kalloo *et al*. (1980) [5] suggested that crosses between selected genotypes from widely separated clusters are most likely to give rise to desirable recombinants, which is also supported by more recent diversity studies (Ashwini *et al.,* 2014; Revathy &Kanthaswamy, 2015) [1].

The important and economic characters from a horticultural point of view must be taken into account before designing breeding strategies for efficient and effective harnessing of potential yield attributes. Traits such as early female flowering, early fruit set, more fruits per plant, and high yield are good indicators of the superiority of a genotype to be utilized in crop improvement (Deepa Devi *et al*., 2013; Singh *et al*., 2018) [3]

Intra and inter-cluster distances

The intra-cluster distance was the highest in cluster III (containing 5 genotypes) followed by cluster II (11 genotypes) and I (13 genotypes) (Table-2).

Table-2. Average intra and inter cluster distances for 31 snake gourd genotypes.

Clusters	I	II	III	IV	V	VI
I	**7.221**					
II	15.791	**7.758**				
III	12.772	10.878	**8.201**			
IV	15.512	9.600	12.699	**0.000**		
V	18.003	11.059	14.696	12.729	**0.000**	
VI	16.785	11.454	12.990	13.474	11.540	**0.000**

The lowest intra-cluster distance was recorded in the clusters IV, V and VI. The inter-cluster D^2 values ranged from 9.60 to 18.003 indicating considerable diversity among the genotypes. The highest inter-cluster distance was noted between I and V, followed by VI and I. The lowest distance was recorded in IV and II. The clustering pattern revealed a wide range of genetic variability among the genotypes studied. The highest inter-cluster distance was observed between Cluster I and Cluster V ($D^2 = 18.003$), suggesting these genotypes possess the greatest genetic divergence and may be utilized as parental lines to maximize heterosis. The low intra-cluster distances indicated genetic homogeneity within clusters, supporting the reliability of the clustering approach.

The genotypes grouped in I and V, with maximum inter-cluster distance, are expected to have greater variability and a high heterotic expression in hybridization programme is not unlikely. Genotypes with moderate inter-cluster distances, when crossed, may also result in significant positive heterosis (Mian and Bhal, 1989) [9]. In the present investigation, inter-cluster distances were higher in all the cases than the intra-cluster distances indicating wide genetic diversity among the different group of genotypes.

Cluster means

Cluster-wise mean values for six economically important characters of snake gourd have been presented in Table-3.

Table-3. Cluster mean values for six economically important characters of snake gourd.

Characters	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Per cent contribution Towards divergence
Days to first female flower	56.03	75.57	63.20	82.67	75.67	71.00	4.23
Days to 50% female flower	65.28	84.07	69.40	93.00	84.00	82.00	15.73
Days to first fruiting	59.15	78.06	66.22	86.00	78.67	79.00	4.03
Length of vine (m)	4.81	4.74	4.36	4.16	4.74	5.80	6.25
Length of fruits (cm)	35.73	35.32	41.99	34.33	79.28	62.63	14.92
eld of fruit/plant (kg.)	1.37	1.48	1.45	1.29	1.40	1.38	5.65

Table-4: Mean performance of 32 genotypes of Snake gourd

Parameters	BCSG-1	BCSG-2	BCSG-3	BCSG-4	BCSG-5	BCSG-6	BCSG-9	BCSG-10	BCSG-11	BCSG-12
Days to seedling emergence	12.33	10.33	11.00	12.33	12.00	9.33	10.33	12.00	14.00	9.00
Days to first male flower opening	40.00	73.00	51.67	50.00	66.33	52.67	52.00	50.67	52.00	48.67
Node no. of first male flower opening	11.67	11.33	14.67	12.33	14.33	12.00	12.67	11.67	10.67	13.33
Days to first female flower opening	56.67	82.67	60.33	66.00	73.00	58.00	56.33	57.67	62.67	57.33
Node no. of first female flower opening	20.00	25.33	25.00	21.33	24.67	23.33	26.00	22.67	22.33	26.33
Sex ratio	11.94	24.61	19.80	21.14	17.14	19.68	20.05	14.91	20.12	24.05
Total number of primary branches/ plants	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Total number of secondary branches/plants	2.33	2.33	2.67	2.33	2.67	2.00	3.00	2.00	2.00	3.00
Total number of tertiary branches/plants	7.00	5.00	6.00	5.67	5.67	6.33	5.67	5.67	7.67	8.33
Total number of nodes/plants	38.33	39.33	46.67	40.67	40.00	45.00	40.67	39.67	42.67	47.67
Days to 50% male flower opening	42.67	75.67	55.00	53.33	70.33	55.00	55.33	55.00	56.00	52.67
Days to 50% female flower opening	64.67	93.00	66.00	71.67	78.00	63.67	63.67	65.00	67.67	64.33
Days to first fruit setting	59.67	86.00	63.00	69.00	75.67	62.33	60.33	61.00	65.67	60.33
Days to 50% fruit setting	65.33	94.33	69.67	71.67	79.33	68.33	66.67	66.67	70.00	66.67
Node number of first fruit set	20.00	25.33	25.00	23.00	24.67	23.33	25.67	22.67	22.33	26.33
Petiole length (cm)	7.60	7.13	7.17	6.03	7.13	6.57	6.57	5.97	7.10	6.57
Peduncle length (cm)	2.16	2.07	2.16	2.87	1.48	1.82	2.22	2.87	4.15	1.72
Average length of fruits (cm)	38.71	35.73	31.39	29.98	31.27	48.37	33.07	48.53	61.43	26.03
Average diameter of fruits (cm)	3.85	3.81	4.60	3.57	3.81	4.18	4.15	4.30	3.35	3.99
Total number of fruits per plant	4.00	3.67	4.00	4.67	4.00	5.00	5.33	3.67	4.67	3.67
Average yield (kg/plant)	1.35	1.29	1.25	1.41	1.25	1.63	1.88	1.58	1.69	1.16
Length of vine (in m)	4.17	4.16	4.97	4.19	4.38	4.74	3.73	4.20	4.87	4.90
Days to first harvest	75.33	111.33	76.00	77.67	83.00	73.33	72.67	74.00	74.00	76.00

Parameters	BCSG-14	BCSG-15	BCSG-16	BCSG-17	BCSG-20	BCSG-21	BCSG-23	BCSG-24	BCSG-25	BCSG-26	BCSG-28
Days to seedling emergence	13.33	13.67	12.33	12.67	12.00	13.00	13.00	14.00	13.00	13.00	13.67
Days to first male flower opening	58.67	55.00	54.00	58.00	62.00	63.67	81.00	59.67	63.33	58.00	61.33

Node no. of first male flower opening	15.67	8.00	10.00	7.00	14.00	15.33	28.00	12.33	13.67	8.33	13.67
Days to first female flower opening	71.00	79.67	76.67	75.33	75.67	75.33	77.67	74.33	75.67	76.67	75.67
Node no. of first female flower opening	24.67	27.33	18.67	20.33	27.00	24.00	30.33	26.00	25.33	25.00	26.33
Sex ratio	22.35	25.78	26.59	28.38	36.75	21.07	9.20	17.05	24.56	32.86	28.00
Total no. of primary branches/ plant	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Total no. of secondary branches/plant	2.33	3.00	2.00	2.00	2.00	2.67	2.00	2.00	2.00	2.33	2.00
Total number of tertiary branches/plants	7.67	8.00	5.00	6.00	6.67	7.33	7.67	6.67	3.33	6.67	8.67
Total number of nodes/plants	56.33	47.67	43.67	43.67	52.33	42.33	49.67	48.00	55.00	46.67	52.33
Days to 50% male flower opening	63.00	59.67	58.67	62.67	66.00	67.00	86.00	64.33	68.00	63.00	65.33
Days to 50% female flower opening	82.00	89.00	84.00	82.00	84.00	82.67	87.33	83.33	84.00	86.00	84.67
Days to first fruit setting	79.00	83.00	79.00	78.33	78.67	78.67	81.00	78.00	78.67	80.00	78.67
Days to 50% fruit setting	84.67	90.00	85.33	83.33	85.67	86.00	88.00	84.67	87.00	90.33	86.00
Node number of first fruit set	24.67	28.33	18.67	20.33	27.00	24.00	30.33	26.00	26.33	25.00	26.33
Petiole length (cm)	7.77	5.23	6.85	3.97	8.84	7.53	10.55	6.11	7.20	7.48	7.87
Peduncle length (cm)	2.87	1.48	2.63	2.06	1.81	1.92	2.20	2.57	3.75	2.80	2.32
Average length of fruits (cm)	62.63	34.85	32.20	29.71	25.61	31.21	49.33	45.00	79.28	21.92	36.75
Average diameter of fruits (cm)	3.81	5.41	4.43	3.80	5.85	6.00	5.02	5.35	3.69	5.48	5.75
Total number of fruits per plant	3.67	3.67	3.33	3.33	4.33	4.67	2.67	4.67	3.33	4.00	5.00
Average yield (kg/plant)	1.38	1.30	1.41	1.17	1.51	1.69	1.08	1.52	1.40	0.65	1.73
Length of vine (in m)	5.80	5.47	4.17	4.45	5.68	4.00	4.17	3.86	4.74	4.01	4.97
Days to first harvest	95.33	98.00	97.00	95.33	98.00	103.67	106.33	98.00	103.67	121.00	115.00
Parameters	**BCSG-29**	**BCSG-30**	**BCSG-31**	**BCSG-32**	**BCSG-33**	**BCSG-34**	**BCSG-35**	**BCSG-36**	**BCSG-37**	**BCSG-38**	**BCSG-39**
Days to seedling emergence	12.67	13.00		7.33	6.33	7.00	7.00	7.00	7.00	7.00	7.00
Days to first male flower opening	66.67	64.67		54.67	53.00	49.67	52.00	51.00	48.33	51.00	48.67
Node no. of first male flower opening	15.67	17.00		14.67	13.33	14.33	10.67	18.00	14.33	17.00	18.67
Days to first female flower opening	75.67	70.67		57.67	56.67	57.67	55.00	52.67	53.67	55.33	52.00	55.67
Node no. of first female flower opening	26.33	31.33		23.00	23.33	19.67	21.33	24.00	23.33	23.00	21.00	26.67
Sex ratio	20.79	29.47		18.33	12.89	18.94	20.89	33.16	38.94	29.77	21.77	36.39
Total number of primary branches/ plants	1.00	1.00		1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Total number of secondary branches/plants	2.00	2.00		2.00	2.00	2.00	2.00	2.33	2.00	2.33	2.00	2.00
Total number of tertiary branches/plants	8.00	6.33		7.00	6.67	7.67	5.67	7.33	8.00	5.67	6.67	7.33
Total number of nodes/plants	50.67	54.33		44.33	47.00	46.67	40.67	51.33	52.67	49.00	47.67	52.67
Days to 50% male flower opening	70.67	69.00		59.00	57.33	53.67	55.67	55.00	52.00	56.00	53.33	53.33
Days to 50% female flower opening	85.33	79.67		64.67	64.33	68.33	67.00	68.67	64.33	65.67	63.00	65.00
Days to first fruit setting	78.00	73.67		60.67	59.33	60.33	58.67	55.67	56.67	58.67	54.33	58.67
Days to 50% fruit setting	84.67	80.67		68.00	68.00	68.67	67.33	64.67	63.67	67.00	62.67	65.67
Node number of first fruit set	26.33	31.00		23.00	23.33	19.67	21.33	24.00	23.33	23.00	21.00	26.67
Petiole length (cm)	8.43	9.59		8.19	9.88	8.39	7.25	8.86	10.01	7.93	10.19	8.76
Peduncle length (cm)	2.31	2.22		2.03	2.23	2.28	2.38	2.67	2.58	2.25	1.97	1.93
Average length of fruits (cm)	43.63	52.32		34.67	36.87	29.47	33.61	31.82	38.61	39.43	37.18	30.98
Average diameter of fruits (cm)	5.32	4.07		5.50	6.16	6.11	5.88	5.23	4.61	5.31	5.32	5.26
Total number of fruits per plant	5.33	5.00		4.33	2.67	3.00	3.00	4.33	6.67	4.33	5.33	4.33
Average yield (kg/plant)	2.20	1.66		1.46	1.09	0.88	1.10	1.35	1.81	1.40	1.52	1.32
Length of vine (in m)	5.46	5.32		4.49	4.31	4.85	4.38	5.60	5.82	5.10	4.51	5.15
Days to first harvest	109.33	103.67		78.33	82.67	85.00	80.67	80.67	76.00	80.67	76.00	76.00

The characters which have contributed maximum towards D^2 matrix includes days to 50% female flowering (15.73%), length of fruits (14.92%), length of vine (6.25%), yield of fruits (5.65%), days to first female flowering (4.23%) and

days to first fruit set (4.03%) (Figure-1). The earliest female flower was produced in the genotypes of cluster I and the latest by those in cluster IV. The same trend was recorded in case of days to 50% female flower. Quite expectedly, the earliest and latest fruiting were noted in cluster I and IV, respectively. Cluster IV also recorded the lower mean values for length of vine, length of fruits and yield which might be due to the lowest performing genotypes.

With respect to yield, cluster II performed the best closely followed by cluster III and cluster V. The maximum cluster mean was observed in cluster II for yield of fruits per plant and cluster V for fruit length (Table-3 and Figure 1).

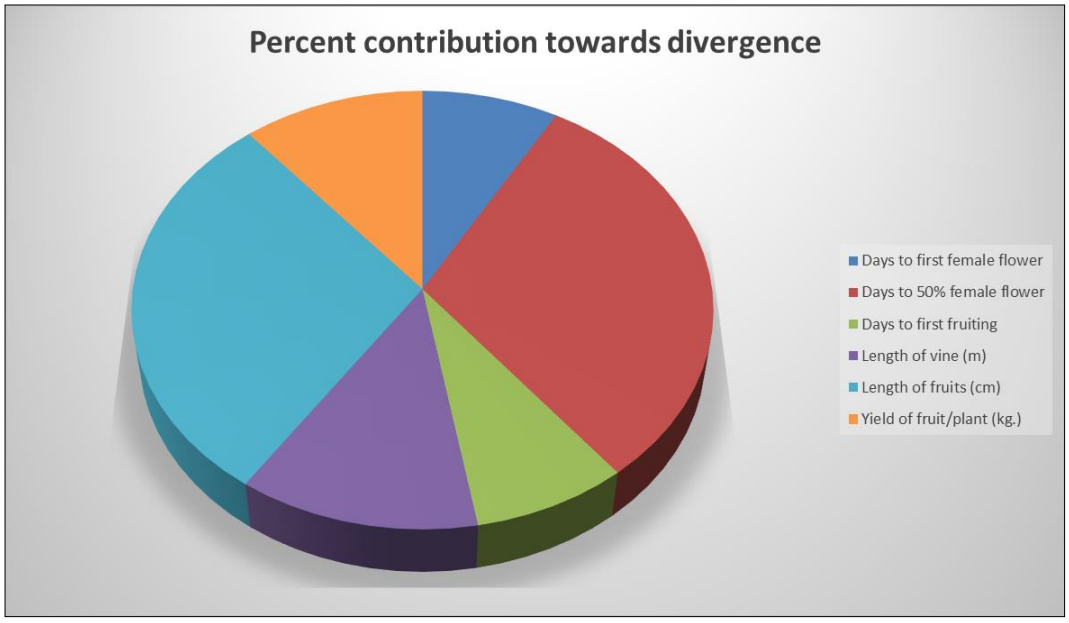

Fig 1: Percent Contribution towards divergence

These clusters could be regarded as useful sources of gene for yield component traits. However, cluster I genotypes required the least number of days for first female flowering, 50% female flowering and first fruit setting with an almost average vine length among the six clusters. This can be helpful for breeding an early type. Hence, it can be suggested from the present study that a high yielding early plant can be bred by utilizing the genotypes from cluster I and cluster II. A high yielding type with longer fruits could be bred by utilizing the genotypes from cluster II and cluster V. Intergroup crossing of these genotypes is expected to produce heterotic hybrids.

Considering the genetic divergence and manifestation of important yield components, the genotypes BCSG-6, BCSG-9, BCSG-11, BCSG-36 and BCSG-38 emerged as the best, which can be used as the base materials for direct selection as well as in hybridization programme. It is expected that the derivatives of cross combinations among these genotypes will throw wide range of segregates for effecting successful selection of the best ones.

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