Genetic Diversity Analysis among *Capsicum annuum* Mutants Based on Morpho-Physiological and Yield Traits

K. M. Rezaul Karim [1], Mohd Y. Rafii [1,*], Azizah Misran [2], Mohd Firdaus Ismail [2], Abdul Rahim Harun [3], Raihana Ridzuan [4], Mst. Farhana Nazneen Chowdhury [2], Monir Hosen [1], Oladosu Yusuff [1] and Md Azadul Haque [1]

1   Laboratory of Climate-Smart Food Crop Production, Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia (UPM), Serdang 4300, Malaysia
2   Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia (UPM), Serdang 43400, Malaysia
3   Agrotechnology and Bioscience Divisions, Malaysian Nuclear Agency, Bangi, Kajang 43600, Malaysia
4   Department of Biosciences, Faculty of Science, Universiti Teknologi Malaysia (UTM), Johor Bahru 81310, Johor, Malaysia
*   Correspondence: mrafii@upm.edu.my; Tel.: +60-193096876

Abstract: It is crucial to assess genetically superior parents when developing novel hybrids. This experiment was conducted to find out the diversity of 27 *Capsicum annuum* mutant lines derived from two varieties.To achieve the objective, 23 morpho-physiological and yield traits were recorded through two planting seasons. Highly significant differences ($p < 0.01$) were recorded among the studied traits. There was a strong to moderately positive phenotypic association between yield and all other morphological traits except first bifurcation length, stem diameter, pedicle length, flowering date, and maturity date. A higher Genotypic Coefficient of Variation (GCV) and Phenotypic Coefficient of Variation (PCV), combined with moderate to high heritability and high hereditary progress, have been found in the number of fruits per plant, fruit yield per plant, and number of seeds per fruit. High heritability was found in yield characteristics, vis-à-visnumber of seeds per fruit, number of fruits per plant, and indicated high genetic advance. The studied genotypes were divided into six groups after the cluster analysis. Based on the correlation matrix of 23 quantitative characteristics, principal component analysis revealed that the percentage of variation for PC1 and PC2 is 28%and 19%, respectively, andPC1 represents the largest percentage of the overall total variation. The calculated genetic distance also explains the potential of heterosis breeding. The revealed findings might be helpful for breeders to target quantitative characters and the parental lines of *C. annuum* during the execution of their future breeding programmes for developing high-yielding and climate-resilient chilli varieties.

Keywords: chilli; mutant; genetic diversity; cluster analysis

1. Introduction

Chilli (*Capsicum annuum* L.) of the genus Capsicum includes about 25 regularly used species with four cultivar groups: Chinense (West Indies chilli), Frutescens (bird chilli), Annuum (hot chilli), and the sweet pepper group [1]. It is said to have originated in South and Central America and belongs to the Solanaceae family. It is a spice crop that can also be used as a vegetable and is widely produced across the world [2,3]. The nutritional and functional benefits of chilli pepper fruits, such as capsaicinoids, carotenoids, antioxidant vitamins, and phenolic components, make them an important vegetable. In addition, hot pepper fruits are utilized as food coloring and flavoring [4]. *Capsicum annuum* genotypes have a variety of different and intriguing growth and yield characteristics, including fruit size, weight of fruit, fruit colour, pungency, flowering, plant height, and maturity, which may be somehow beneficial for breeding purposes. Many experiments have shown that

critical yield traits in C. annuum genotypes have high genetic changeability, heritability, and hereditary advancement [5–8].

It was reported that there is a wide range of variance in the capacity of chilli genotypes for flowering, setting fruit, yield, and other qualitative characteristics [9,10]. The systematic breeding process includes numerous phases, such as collecting germplasm, analysing genetic diversity, producing genetic variability, implementing selection, and preparing chosen genotypes for commercial distribution [11]. Investigation and improved knowledge of the variability available in a crop population is necessary for efficient and successful breeding research, so that plant breeders may use it for crop development. Furthermore, the effectiveness of any crop development effort is determined not just by the quantity of genetic diversity contained in a crop, but also by the degree of variation heritable from parent to progeny [12]. A large range of diversity in genotypes gives enough opportunity for boosting fruit production and other desired features through systematic breeding. Estimating the genetic diversity inherent in a crop's germplasm is a prerequisite for having a successful breeding programme [13]. The long-term sustainability of plant populations and their capacity to adapt to changing climatic and environmental circumstances may both be significantly influenced by genetic variety, which is another reason why it is crucial to ensure this. Development of new varietiesis a continuous process to mitigate various demands of growers, so the information aboutparental lines can help the breeders to use the germplasm more confidently.

Several chilli researchers concluded that PCV was higher than GCV for the different characteristics tested [14–16]. Fruit yield per plant, seed yield per plant, fruit length, green chilli fruit weight, and fruit production per hectare each had extremely high heritability [17]. Knowledge of genetic distance between parents is vital forbenefiting from transgressive segregation [18,19]. The breeder must have information on the degree of genetic divergence in order to select the proper type of parent for targeted hybridization in heterosis breeding [19,20]. Furthermore, choosing varied parents within a suitable range increases the odds of improving various features in the progeny. One of the fundamental prerequisites for establishing efficient breeding procedures is a careful assessment of the type and extent of variability in the germplasm resource [21]. The genetic variability, correlations, and associations between qualitative and quantitative features, as well as heritability estimates, all play a role in determining the best breeding approach for improving yield and its components in each crop. High heritability together with high hereditary advancement indicated the role of the additive gene for selected characters [22]. Information on genetic distance between parents is also important in order to benefit transgressive segregation [18]. Hence, clustering analysis and genetic distance determination are also essential [23]. The breeder can select the best type of parents for deliberate hybridization in heterosis breeding by having complete knowledge of the nature and degree of genetic difference [24]. The following objectives were pursued in this experiment: (a) to calculate the genetic diversity of 27 chilli genotypes based on their morphological, physiological, and yield traits; and (b) to estimate the genetic variance components, heritability, and genetic advance as a selection criterion for further chilli breeding initiatives.

2. Materials and Methods

2.1. Experiment Location

The experiment was conducted under a rain shelter in the nethouse at the Institute of Tropical Agriculture, Faculty of Agriculture, Universiti Putra Malaysia (UPM), located at 03°00′′12.6′N, 101°47′′22.4′E. Over the period of two seasons, the evaluation was repeated in a humid tropical climate. The first season was conducted in 2019 and the second was in 2020. The average daily temperature ranged from 19 to 36 °C and relative humidity was recorded between 80–90% during the experimental tenure.

2.2. Plant Materials

A total of 27 selected advanced chilli mutant lines in the M4 generation were used in this study, which were derived from two varieties, viz. Chilli Bangi 3 and Chilli Bangi 5 (Table 1). The advanced mutants were selected based on their excellent agronomic performance.

Table 1. Selected 27 Capsicum annuum genotypes from mutant lines with gamma source.

Code	Variety	Gamma Source Type
G1	Chilli Bangi 3	acute
G2	Chilli Bangi 3	acute
G3	Chilli Bangi 3	acute
G4	Chilli Bangi 3	acute
G5	Chilli Bangi 3	chronic
G6	Chilli Bangi 3	chronic
G7	Chilli Bangi 3	chronic
G8	Chilli Bangi 3	chronic
G9	Chilli Bangi 3	chronic
G10	Chilli Bangi 3	chronic
G11	Chilli Bangi 3	chronic
G12	Chilli Bangi 3	chronic
G13	Chilli Bangi 3	chronic
G14	Chilli Bangi 5	acute
G15	Chilli Bangi 5	acute
G16	Chilli Bangi 5	acute
G17	Chilli Bangi 5	acute
G18	Chilli Bangi 5	chronic
G19	Chilli Bangi 5	chronic
G20	Chilli Bangi 5	chronic
G21	Chilli Bangi 5	chronic
G22	Chilli Bangi 5	chronic
G23	Chilli Bangi 5	chronic
G24	Chilli Bangi 5	chronic
G25	Chilli Bangi 5	chronic
G26	Chilli Bangi 5	chronic
G27	Chilli Bangi 5	chronic

2.3. Experimental Design and Layout

The seeds were first sown in seed trays containing peat moss with one or two seeds per cell and were later transplanted after four weeks to prepared polythene pots (17 × 30 cm) filled with cocoa dust, having small holes to drain excess water. The experiment was laid in a randomized complete block design with three replications. Two pots were assigned for each genotype in each replication (54 pots per replication) and were oriented east to west (spaced 75 cm × 150 cm). Seedlings emerged within 3–10 days after sowing and were transplanted 4 weeks after sowing. Fertigation system of cropping was adopted for both irrigation and fertilization. Drip system of irrigation was applied. Throughout the cropping season, intercultural activities including supplemental irrigations and plant protection approaches were carried out as required. Agronomic recommendations were followed.

2.4. Data Collection

2.4.1. Measuring the Morphological, Physiological, and Yield Components

Table 2 contains data on morphological, physiological, and yield parameters that were assessed and reported 90 days after transplantation. The quantitative morphological features obtained in this study include plant height, stem diameter, fruit number, and fruit weight, which could be counted or quantified using particular measuring instruments.

Table 2. The quantitative characteristics of chosen chilli genotypes with detail description.

Sl. No.	Parameter	Denotation	Description
1	Germination%	GP	Germination was counted at tenth day after sowing.
2	First bifurcation length (cm)	FBL	The length between soil base and first bifurcation is measured.
3	Number of primary branches (nos.)	PB	Number of branches produced from the main stem wascounted.
4	Number of secondary branches (nos.)	SB	Number of branches produced from the primary branch wascounted
5	Plant height (cm)	PH	Each plant's height was measured from the soil surface up to the tip of the plant with a measuring tape.
6	Stem diameter (mm)	SD	The stem diameter was taken using an Absolute Digimatic calliper 5 cm from the base of the plant (Mitutoyo, Japan).
7	Number of leaf/plant (nos.)	NLP	Total number of leaves were counted for each plant.
8	Days to first flowering (nos.)	DF	The days from transplanting to the first fully open flower was observed.
9	Days to first fruit maturity (nos.)	DM	The days tofirst fruit ripening on the plant were counted.
10	Number of fruits/plant (nos.)	NFP	Total numberof fruits collected from the first harvest to 90 days after transplanting.
11	Fruit length (mm)	FL	The matured fruit length from calyx to the tip of fruit.
12	Fruit breadth (mm)	FB	The girth of one mature fruit (0.3 cm below the calyx).
13	Pedicle length (mm)	PL	From the base of calyx to the attachment point of branch.
14	Single fruit weight (gm)	FW	Weight of one mature fruit per plant.
15	Single fruit dry weight (gm)	FDW	Per plant, the weight of one dried ripe fruit.
16	Seed number/fruit (nos.)	NSF	Total number of seeds for each fruit were counted.
17	100 seeds weight (gm)	HSW	Counted hundred seeds' weight was taken by using electronic weighing balance.
18	Fruit wall thickness (mm)	FWT	The wall thickness of fully matured fruit was recorded at harvest using slide calliper.
19	TYP (kg)	YLD	All fruits'weight from the first harvest to 90 days after transplanting.
20	Relative chlorophyll content (SPAD value)	RCC	The relative amount of chlorophyll content present in the leaf, measured on the third or fourth leaf from the tips using SPAD-502 Plus (Konica Minolta, Japan).
21	Photosynthesis rate (μmol CO_2 m^{-2} s^{-1})	PR	Photosynthetic rate, stomata conductance, and transpiration rate measured on third or fourth leaf from the tips using
22	Stomata conductance ($mol H_2 O m^{-2} s^{-1}$)	SC	a photosynthesis portable system (LI-6400xt, LI-COR, Lincoln, NE, USA).
23	Transpiration rate ($mmol H_2 O m^{-2} s^{-1}$)	TR	

2.4.2. Genetic Variance, Heritability, and Advance

An analysis of variance was performed to detect genotype differences and to assess genetic and environmental impacts on several attributes.

(a) Calculation of genotypic variance using following formulae:

$$\sigma^2 g = \frac{MSG - MSE}{r}$$

(b) Calculation of phenotypic variance using following formula:

$$\sigma^2 p = \sigma^2{}_g + MSE$$

where $\sigma^2 g$ is the genotypic variance, $\sigma^2 p$ is the phenotypic variance, MSG is the meansquare of genotypes, MSE is mean square of error, and r is number of replications.

(c) Phenotypic and Genotypic Coefficient of Variation (PCV and GCV). Estimates of phenotypic and genotypic coefficient of variation were calculated according to Singh and Choudhary [25] as follows:

$$PCV = \frac{\sqrt{\sigma^2 p}}{\bar{x}} \times 100$$

$$GCV = \frac{\sqrt{\sigma^2 g}}{\bar{x}} \times 100$$

$$RD = \frac{PCV - GCV}{PCV} \times 100$$

$\sigma^2{}_p$ is the phenotypic variance, $\sigma^2{}_g$ is the genotypic variance, and $\bar{x}$ is the mean of the trait. GCV and PCV values were categorized as low (0–10%), moderate (10–20%), and high (20% and above) following Sivasubramanian and Madhavamenon [26].

(d) Broad sense heritability h^2B ratio of genetic variance ($\sigma^2{}_g$) to phenotypic variance ($\sigma^2{}g$).

The formula used for broad sense heritability is as follows:

$$h^2B \ (\%) = \frac{\sigma^2 g}{\sigma^2 p} \times 100$$

where $\sigma^2{}_g$ is the genotypic variance and $\sigma^2{}_p$ is the phenotypic variance. The heritability percentage was categorized as low (0–30%), moderate (30–60%), and high ($\geq$60%) in accordance with Johnson et al. [27].

(e) Estimated and Expected Genetic Advance. Expected genetic advance (GA) (as percentage of the mean) was calculated using the method of Assefa et al. [28] and selection intensity (K) was assumed to be 5%. Genetic advance was marked as low (0–10%), moderate (10–20%), and high (>20%) by following Johnson et al. [29].

$$GA \ (\%) = K \times \frac{\sqrt{\sigma^2 p}}{\bar{x}} \times 100$$

K is a constant which represents the selection intensity. When K is 5%, the value is 2.06. $\sigma^2{}_P$ is phenotypic standard deviation, $h^2{}_B$ is the heritability, and $\bar{x}$ is the mean of traits.

2.4.2.1. Data Analysis

The 27 accessions were characterised morphologically and agronomically using a randomised complete block design, with four replicates consisting of two pot plants from each accession as the source of variance. One-way ANOVA was used to examine all of the data sets using SAS 9.4 statistical analysis software (North Carolina State University, Raleigh, NC, USA). The significance level was set at >0.05, and the LSD test was used to see if there were any significant differences between the means. A correlation coefficient was also determined. The dendrogram was mapped using SAHN clustering of the UPGMA method through the application of NTSYS 2.1 (Numerical Taxonomy Multivariate Analysis System, Exeter Software, Setauket, NY, USA) software. In addition, principal component analysis (PCA) was employed to generate 2Dvisualisations.

3. Results and Discussion

3.1. Morpho-Physiological and Yield Component

Among the tested genotypes, the results showed that there are highly significant differences ($p < 0.01$) for all the parameters measured (Table 3).

Table 3. Analysis of variance (mean squares) of all studied characteristics of 27 chilli genotypes over two seasons.

SOV	GP	FBL	PB	SB	PH	SD	NLP	DF
Blocks(season)	11.72 ns	1.05 ns	0.67 ns	0.56 ns	5.22 ns	3.67 ns	1259.45 ns	37.00**
Seasons (S)	29.38 ns	10.88 *	0.09 ns	0.15 ns	42.91 ns	10.31 *	74.69 ns	9.97 *
Genotypes (G)	190.11 **	7.44 **	0.93 **	1.65 **	177.68 **	30.52 **	23,229.31 **	31.96 **
G × S	8.65 ns	1.20 ns	0.23 ns	0.33 ns	52.77*	1.41 ns	4032.98 *	2.41 *
Error	15.55	1.74	0.31	0.54 ns	29.70 ns	1.53 ns	2097.35 ns	1.46
SOV	DM	NFP	FL	FB	CL	FW	FDW	NSF
Blocks(season)	57.85 *	91.98 ns	3.08 ns	0.10 ns	10.24 ns	0.02 ns	0.02 ns	3.82 ns
Seasons (S)	90.37 *	71.02 ns	1.77 ns	0.37 ns	26.53 ns	0.38 ns	0.002 ns	1.04 ns
Genotypes (G)	131.49 **	2689.08 **	399.71 **	33.25 **	118.58 **	9.64 **	0.11 **	935.50 **
G × S	10.24 ns	15.14 ns	6.48 ns	0.54 ns	2.57 ns	0.12 ns	0.02 ns	1.97 ns
Error	8.01	81.20	31.54	1.42	6.86	0.19	0.006	32.46
SOV	HSW	FWT	RCC	PR	SC	TR	YLD	
Blocks(season)	0.001 ns	0.004 ns	3.72 ns	0.12 ns	0.0005 ns	0.001 ns	0.0006 ns	
Seasons (S)	0.0009 ns	0.0006 ns	12.21 ns	0.22 ns	0.002 ns	0.02 ns	0.003 ns	
Genotypes (G)	0.007 **	0.64 **	109.15 **	31.26 **	0.11 **	2.99 **	0.29 **	
G × S	0.001 ns	0.001 ns	6.23 ns	0.25 ns	0.0004 ns	0.01 ns	0.006 ns	
Error	0.001	0.03	10.41	0.57	0.002	0.05	0.03	

* Significant at 0.05 probability level. ** Highly significant at 0.01 probability level. ns not significant. GP, germination%; FBL, first bifurcation length; PB, primary branch; SB, secondary branch; PH, plant height; SD, plant diameter; NLP, number of leaf per plant; DF, days to first flowering, DM, days to maturity; NFP, number of fruit per plant; FL, fruit length; FB, fruit breadth; CL, calyx length; FW, fruit fresh weight; FWD, fruit dry weight; NSF, number of seed per fruit; HSW, hundred seed weight; FWT, fruit wall thickness; RCC, relative chlorophyll content; PR, photosynthesis rate; SR, stomata conductance; TR, transpiration rate; YLD, yield per plant.

3.1.1. Growth and Physiological Components

The highest germination percentage was observed for Genotype 5 (94.2%) and the lowest was recorded for Genotype 11 (75.3%). Genotype 9 (84.70 cm) was recorded as the tallest plant while Genotype 24 (66.96 cm) was the shortest one in respect of taken plant height (Table 3). In the case of stem diameter, Genotype 10 and Genotype 26 werethe lowest and the highest, respectively, where the value ranged between 10.58 and 17.84 cm. The highest leaf number was found in Genotype 27 (743.67), whereas for days to first flowering and maturity the Genotype 15 (18.6) andGenotype 27 (64.5 days) was the earliest, respectively.

Genotype 2 (39.63 mm) was found with the longest pedicle; on the contrary, Genotype 5 was the smallest (22.10 mm). Among studied physiological traits, it was found that in the case of relative chlorophyll content Genotype 15 and Genotype 13 werethe highest and lowest, respectively, as shown in Table 4. Within all the observed chilli genotypes, Genotype 16 was found with the highest photosynthesis rate (22.10 µmol CO_2 m^{-2}s^{-1}) followed by Genotype 22 and Genotype 7; however, the lowest photosynthesis rate (15.65 µmol CO_2 m^{-2}s^{-1}) was found for the Genotype 4. On the other hand, Genotype 15 (0.76 mol H_2O m^{-2}s^{-1}) was recorded for the highest stomata conductance, followed by Genotype 13 and Genotype 6, respectively, but the lowest value for this trait was recorded for the Genotype 20 (0.38 mol H_2O m^{-2}s^{-1}). For transpiration rate, the highest value was indicated by the Genotype 27 (6.86 mmol H_2O m^{-2}s^{-1}) and lowest was found for the Genotype 1 (4.72 mmolH_2O m^{-2}s^{-1}).

Table 4. Mean for morphological, physiological, and yield characteristics of the 27 studied chilli genotypes planted over two seasons.

Genotypes	GP	FBL	PB	SB	PH	SD	NLP	DF	DM	NFP	FL	FB	CL	FW	FDW	NSF	HSW	FWT	RCC	PR	SC	TR	YLD
Gen 1	93.5	9.83	3.3	5.5	79.53	16.48	574.7	24.4	75.7	133.2	83.68	20.13	28.27	13.49	1.13	67.7	0.496	2.33	50.20	17.13	0.43	4.72	1.43
Gen 2	94.0	7.83	3.5	7.5	82.20	14.57	710.5	20.1	66.7	144.2	89.68	18.40	39.63	11.00	1.03	79.3	0.518	2.70	55.01	21.52	0.68	6.47	1.74
Gen 3	93.7	7.33	3.5	6.8	83.70	14.41	731.5	20.3	68.2	110.7	88.31	21.22	35.45	11.97	1.13	77.8	0.584	2.46	55.91	20.86	0.61	6.72	1.57
Gen 4	83.7	9.50	2.7	6.5	84.20	11.57	631.7	25.4	76.8	131.8	93.06	22.71	35.03	9.67	0.91	66.3	0.487	1.71	47.15	15.65	0.41	5.09	1.54
Gen 5	94.2	7.33	3.2	7.5	82.70	13.10	685.2	20.9	66.8	139.0	72.25	22.70	22.10	14.05	1.21	84.5	0.527	2.66	56.07	20.41	0.55	6.68	1.62
Gen 6	86.3	8.00	3.2	6.8	71.53	14.80	739.2	20.4	69.5	140.0	95.50	18.79	28.82	13.22	1.26	69.0	0.509	2.73	55.61	21.06	0.73	6.76	1.60
Gen 7	87.5	9.83	3.0	6.8	77.03	14.56	662.5	19.6	69.8	124.0	78.37	21.54	33.14	13.59	1.26	68.8	0.487	2.71	55.71	22.02	0.62	6.53	1.29
Gen 8	80.3	9.67	2.7	5.8	77.87	17.48	633.3	24.1	77.5	97.5	83.67	17.54	29.89	11.09	0.89	68.7	0.497	2.03	46.78	18.09	0.42	4.83	1.29
Gen 9	87.0	8.50	3.2	7.5	84.70	14.22	706.0	19.6	68.5	154.3	82.09	17.17	23.75	13.23	1.16	71.5	0.516	2.56	55.99	21.85	0.61	6.60	1.63
Gen 10	83.0	10.33	2.8	6.8	81.20	10.58	555.0	24.5	77.3	107.5	79.23	18.62	29.30	12.07	0.96	65.5	0.480	2.45	48.49	17.62	0.43	5.13	1.39
Gen 11	75.3	10.33	2.5	7.2	79.03	17.35	671.3	24.5	77.3	107.7	86.68	17.02	30.48	14.09	1.31	63.5	0.497	2.17	47.92	16.95	0.49	5.18	1.33
Gen 12	81.2	9.67	2.8	6.2	83.72	12.72	614.3	24.4	75.7	109.8	95.01	18.85	36.32	12.26	1.03	63.8	0.493	2.58	48.12	17.81	0.51	5.36	1.26
Gen 13	84.2	9.67	2.5	6.7	83.05	11.19	593.3	23.8	76.0	128.2	95.55	19.11	32.38	14.96	1.19	60.5	0.500	2.90	46.32	18.00	0.39	5.45	1.25
Gen 14	91.8	10.50	2.3	7.2	80.05	11.17	618.3	24.8	76.3	95.7	87.68	16.93	35.23	12.52	1.25	45.2	0.447	2.65	47.83	16.45	0.43	5.37	1.15
Gen 15	93.3	8.17	2.2	7.2	69.59	14.56	707.0	18.6	66.7	125.0	102.78	16.77	39.44	11.69	1.23	53.2	0.461	1.60	57.20	22.02	0.76	6.81	1.28
Gen 16	90.3	8.67	2.5	7.5	74.93	14.13	706.5	21.1	65.0	120.2	80.24	17.36	39.09	11.67	1.08	51.5	0.461	1.82	56.23	22.10	0.59	6.67	1.25
Gen 17	77.0	9.67	2.2	6.8	83.59	16.87	669.8	24.3	75.2	157.2	81.71	13.53	29.37	9.97	0.93	54.7	0.443	2.44	46.63	17.52	0.39	5.42	1.18
Gen 18	81.0	10.50	2.5	6.2	74.96	11.27	609.0	23.9	73.3	104.8	70.36	12.40	31.36	10.91	1.01	39.5	0.435	2.33	46.61	17.22	0.43	5.32	0.98
Gen 19	91.5	8.83	2.5	6.8	75.63	17.43	552.2	23.5	76.0	122.3	75.14	20.59	35.44	12.34	1.03	55.2	0.452	2.19	46.37	16.94	0.42	5.41	1.35
Gen 20	85.0	8.83	2.2	7.3	78.46	16.95	734.5	23.4	73.8	107.7	77.89	19.61	37.96	12.77	1.20	52.0	0.462	2.18	47.47	17.20	0.38	5.62	1.25
Gen 21	80.0	8.50	2.8	7.2	80.29	11.60	655.0	24.2	76.3	104.3	73.58	20.62	30.94	12.25	1.22	44.5	0.463	2.35	47.84	17.29	0.42	5.92	1.09
Gen 22	86.2	10.00	3.2	6.5	67.46	15.13	739.5	19.0	67.2	121.7	89.03	19.65	36.58	13.24	1.33	48.0	0.449	1.99	54.24	22.04	0.68	6.62	1.42
Gen 23	89.2	10.17	2.8	7.5	72.96	14.72	652.3	20.4	65.5	114.3	79.11	17.13	32.34	11.35	1.32	49.3	0.450	2.13	55.06	21.73	0.74	6.44	1.24
Gen 24	88.5	10.33	3.2	6.8	66.96	16.82	725.7	19.7	66.2	146.7	77.53	19.35	34.21	12.48	1.22	51.8	0.480	2.28	55.66	21.79	0.72	6.46	1.47
Gen 25	77.5	11.33	2.8	6.5	76.63	16.46	551.3	24.4	74.3	78.2	73.24	18.87	31.33	10.98	0.94	40.7	0.440	2.13	46.96	17.13	0.41	5.40	0.89
Gen 26	84.3	11.17	2.5	6.5	69.96	17.84	615.0	25.7	76.0	104.0	86.60	17.47	33.67	12.88	1.02	39.7	0.438	2.10	49.12	17.17	0.42	5.58	0.91
Gen 27	90.3	8.17	2.5	6.5	71.29	14.52	743.7	20.0	64.5	71.0	82.84	19.37	37.74	13.37	1.27	52.3	0.459	2.52	56.86	21.51	0.71	6.86	1.04
LSD ($p < 0.05$)	4.52	1.51	0.63	0.85	6.24	1.42	52.43	1.38	3.24	10.32	6.43	1.37	2.99	0.5	0.09	6.52	0.04	0.20	3.69	0.86	0.05	0.24	0.18
Season 1	8586	9.09	2.75	6.79	77.01	14.28	658.1	22.2	71.2	117.9	83.63	18.60	32.53	12.29	1.12	58.60	0.47	2.31	50.96	19.11	0.53	5.89	1.30
Season 2	86.72	9.62	2.80	6.85	78.05	14.79	659.5	22.7	72.7	119.2	83.83	18.69	33.34	12.39	1.13	58.77	0.48	2.32	51.51	19.18	0.52	5.91	1.32
LSD ($p < 0.05$)	1.22	0.41	0.17	0.23	1.69	0.39	14.27	0.38	0.88	2.81	1.75	0.37	0.82	0.14	0.02	1.78	0.01	0.05	1.01	0.23	0.01	0.07	0.05

GP, germination %; FBL, first bifurcation length; PB, primary branch; SB, secondary branch; PH, plant height; SD, stem diameter; NLP, number of leaf per plant; DF, days to first flowering, DM, days to maturity; NFP, number of fruit per plant; FL, fruit length; FB, fruit breadth; CL, calyx length; FW, fruit fresh weight; FWD, fruit dry weight; NSF, number of seed per fruit; HSW, hundred seed weight; FWT, fruit wall thickness; RCC, relative chlorophyll content; PR, photosynthesis rate; SR, stomata conductance; TR, transpiration rate; YLD, yield per plant.

The differences at the gene level might be the cause behind the variation of the studied chilli genotypes. Ridzuan et al. [30] and Usman et al. [31] also concluded with similar findings after havingconducted experiments with different chilli genotypes. Overtwo different growing seasons most of the values were not significantly different except first bifurcation length, stem diameter, first flowering date, and maturity date (Table 4). Plant growth and development are dependenton physiological processes (e.g., photosynthesis) which in turn follow various factors in the environment in order to proceed optimally [32]. Chlorophyll content, photosynthesis rate, stomata conductance, and transpiration rate were the physiological characters measured in other experiments. Higher chlorophyll content values signify the more prominent dependability of a plant's chloroplast membranes prompting higher rates of photosynthesis, more dry matter accumulation, and higher productivity [31].

3.1.2. Yield and Yield Contributing Traits

The Genotype 17 produced the highest fruit number per plant with the number 157.2, which was followed by Genotype 9 (154.3) and Genotype 24 (146.7), respectively (Table 4). The lowest fruit number per plant was produced by Genotype 27 (71.0). For fruit length and breadth, Genotype 15 (102.8 mm) and Genotype 4 (22.7 mm) werefound the longest and thickest, respectively. On the other hand, Genotype 18 was recorded as the lowest for both of thesetraits, having the value 70.4mm for length and 12.4mm for breadth. The highest (14.96 g) average green fruit weight was found for Genotype 13, while Genotype 4 was the lowest (9.67 g). In respect of dry fruit weight, Genotype 22 and Genotype 8 werethe highest (1.33 g) and the lowest (0.89 g), respectively. Genotype 5 produced the highest (84.50) seed number in a single fruit, whileGenotype 18 produced the lowest number of seed with the value of 39.50 in a fruit. Genotype 3 and Genotype 13 wereobservedas the highest for hundred seed weight and fruit wall thickness, respectively. On the contrary, Genotype 18 and Genotype 15 were the lowest for these traits, respectively. The highestyield per plant was recorded for the Genotype 2 with the value 1.74 kg. This was followed by Genotype 9 and Genotype 5 with the values 1.63 and 1.62 kg, respectively. However, Genotype 25 was the lowest yielder (0.89 kg) among all the studied genotypes.

No significant difference was found for yield and yield contributing traits whenboth of the seasons were considered (Table 3). A slightly higher yield was found in the second season, which might havehappened due to environmental effect. Ridzuan et al. [33] found similar results when an experiment was done to find out the variability among different chilli genotypes.

3.2. *Correlation between Different Traits*

Pearson correlation coefficients were calculated to determine the relationships among the traits, with a significant level at $p \leq 0.05$ and high significant level at $p \leq 0.01$ (Table 5). Yield showed significantly positive correlation with the physiological traits. However, transpiration rate and relative chlorophyll content had low contribution, whereas photosynthesis rate and stomatal conductance had moderate contribution to yield. Among the morphological and growth traits, first bifurcation length, stem diameter, pedicle length flowering and maturity date showed negative correlation with yield. However, apositive correlation was seen in the cases of germination percentage, stem diameter, number of leaves per plant, and primary and secondary branches. Germination percentage, first bifurcation length and days to maturity moderately contributed to total yield per plant, which was also highly significant statistically. Moreover, in respect of yield related traits, fruit number, fruit breadth, number of seed per fruit, and hundred seed weight showedmoderately positive correlation with yield, which was also highly significant. The majority of characteristics do notexist in isolation; rather, they are linked to one another in intricate ways that have an influence on the yield. This relationship could be favourable or unfavourable. Raihana et al. also corroborate this finding [30].

Table 5. Combine analysis for correlation coefficient among 23 traits.

	FBL	PB	SB	PH	SD	NLP	DF	DM	NFP	FL	FB	CL	FW	FDW	NSF	HSW	FWT	RCC	PR	SC	TR	YLD
GP	−0.363 **	0.199 *	0.206 **	−0.073	−0.068	0.195 *	−0.386 **	−0.429 **	0.152	0.130	0.251 *	0.150	0.134	0.249 *	0.268 **	0.197 *	0.085	0.484 **	0.429 **	0.406 **	0.469 **	0.296 **
FBL		−0.086	−0.165 *	−0.129	0.123	−0.261 **	0.297 **	0.293 **	−0.189 *	−0.110	−0.204 *	−0.031	−0.042	−0.146	−0.351 **	−0.291 **	−0.121	−0.282 **	−0.265 **	−0.282 **	−0.381 **	−0.347 **
PB			0.078	−0.099	−0.072	0.057	−0.229 *	−0.127	0.206 *	−0.058	0.297 **	−0.164 *	0.101	0.039	0.297 **	0.343 **	0.169 *	0.225 *	0.252 *	0.252 *	0.212 *	0.350 **
SB				0.005	−0.068	0.182 *	−0.220 *	−0.301 **	0.121	−0.073	0.039	−0.032	0.057	0.286 **	0.048	0.124	0.027	0.164 *	0.245 *	0.244 *	0.334 **	0.089
PH					−0.216 *	−0.155 *	0.240 *	0.242 *	0.080	0.041	0.103	−0.242 *	−0.110	−0.269 *	0.359 **	0.274 *	0.278 *	−0.218 *	−0.258 *	−0.335 **	−0.269*	0.131
SD						0.114	−0.055	−0.077	0.046	−0.046	−0.029	0.047	0.013	−0.003	−0.046	−0.097	−0.244 *	0.051	0.079	0.048	−0.020	−0.009
NLP							−0.453 **	−0.503 **	0.193 *	0.152	0.031	0.156 *	0.096	0.366 **	0.154 *	0.120	0.001	0.502 **	0.546 **	0.531 **	0.604 **	0.259 *
DF								0.646 **	−0.248 *	−0.073	−0.096	−0.181 *	−0.170 *	−0.405 **	−0.217	−0.238 *	−0.077	−0.668 **	−0.788 **	−0.739 **	−0.740 **	−0.307 **
DM									−0.188 *	−0.045	−0.059	−0.147	−0.079	−0.375 **	−0.129	−0.104	−0.008	−0.624 **	−0.735 **	−0.699 **	−0.757 **	−0.202*
NFP										0.173 *	0.046	0.012	−0.233 *	0.028	0.421 **	0.231 *	0.134	0.207 *	0.263 *	0.214 **	0.214 **	0.537 **
FL											0.074	0.277 **	0.069	0.102 *	0.181 *	0.096	−0.053	0.120	0.125	0.228 *	0.072	0.183 *
FB												0.018	0.308 **	0.142	0.379 **	0.296 **	0.069	0.140	0.085	0.079	0.146	0.332 **
CL													−0.207 **	0.034	−0.256 **	−0.175	−0.293 **	0.099	0.151	0.198 *	0.140	−0.145
FW														0.565 **	0.146	0.183 *	0.405 **	0.173 *	0.169 *	0.120	0.176 *	0.098
FDW															−0.044	0.031	0.193 *	0.380 **	0.428 **	0.490 **	0.487 **	0.128
NSF																0.592 **	0.310 **	0.263 *	0.254 **	0.145	0.168 *	0.608 **
HSW																	0.312 **	0.185 *	0.152	0.149	0.173 *	0.422 **
FWT																		0.008	0.033	−0.013	0.095	0.178 *
RCC																			0.746 **	0.702 **	0.735 **	0.262 **
PR																				0.847 **	0.837 **	0.318 **
SC																					0.784 **	0.322 **
TR																						0.257 **

* Significant at 0.05 probability level. ** Highly significant at 0.01 probability level. GP, germination %; FBL, first bifurcation length; PB, primary branch; SB, secondary branch; PH, plant height; SD, stem diameter; NLP, number of leaf per plant; DF, days to first flowering, DM, days to maturity; NFP, number of fruit per plant; FL, fruit length; FB, fruit breadth; CL, calyx length; FW, fruit fresh weight; FWD, fruit dry weight; NSF, number of seed per fruit; HSW, hundred seed weight; FWT, fruit wall thickness; RCC, relative chlorophyll content; PR, photosynthesis rate; SR, stomata conductance; TR, transpiration rate; YLD, yield per plant.

Considering the plant height, it was observed that plant diameter, leaf number, and other physiological traits were negatively correlated. However, the flowering and maturity date, fruit length, and breadth showed low positive correlation with plant height. On the other hand, days to flowering was highly correlated with maturity date, relative chlorophyll content, photo synthesis rate, stomata conductance, and transpiration rate, and the magnitude of correlation was positive and statistically highly significant. Moreover, in case of days to maturity, highly positive and statistically significant correlation was also found with the studied physiological traits. Fruit number per plant was positively correlated with all other studied traits excluding first bifurcation length, pedicle length, flowering, and maturity date. Moreover, number of leaves showed moderate to high correlation with the physiological traits, which was statistically highly significant also.

3.3. Genetic Analysis, Broad-Sense Heritability, and Genetic Advance

The variance components, environmental variance, genotypic variance (GV) and phenotypic variance (PV), genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV), heritability (h^2_B), and genetic advance (GA) havebeen presented in Table 6. For all the studied traits, genotypic variance was higher than the phenotypic variance. The phenotypic coefficient of variation (PCV) was greater than the genotypic coefficient of variation (GCV) for all traits, showing that environment had minimal impact on trait expression and that a significant amount of variance was controlled by genotypic composition.

Table 6. Genetic variance, broad-sense heritability, and genetic advance for 23 traits in 27 Capsicum annuum genotypes from the combined analysis.

Traits	Mean	(σ^2_e)	(σ^2_g)	(σ^2_p)	PCV (%)	GCV (%)	RD (%)	(h^2_B) %	GA (%)
GP	86.3	14.3	29.3	43.6	7.7	6.3	18.0	67.3	10.6
FBL	9.4	1.7	1.0	2.7	17.4	10.5	39.9	36.1	13.0
PB	2.8	0.3	0.1	0.4	22.8	11.8	48.3	26.8	12.6
SB	6.8	0.5	0.2	0.7	12.2	6.4	47.0	28.1	7.0
PH	77.5	33.6	24.0	57.6	9.8	6.3	35.4	41.7	8.4
SD	14.5	1.6	4.8	6.4	17.4	15.1	13.4	75.0	26.9
NLP	658.8	2415.5	3469.0	5884.5	11.6	8.9	23.2	59.0	14.1
DF	22.4	2.3	5.0	7.2	12.0	9.9	17.1	68.8	17.0
DM	71.9	9.8	20.3	30.1	7.6	6.3	17.9	67.4	10.6
NFP	118.5	67.9	436.9	504.7	19.0	17.6	7.0	86.6	33.8
FL	83.7	26.0	62.3	88.3	11.2	9.4	16.0	70.6	16.3
FB	18.7	1.2	5.3	6.6	13.7	12.4	9.8	81.4	23.0
CL	32.9	6.1	18.7	24.9	15.2	13.1	13.2	75.3	23.5
FW	12.3	0.2	1.6	1.8	10.7	10.2	5.2	90.0	19.9
FDW	1.1	0.0	0.0	0.0	13.4	11.8	11.6	78.2	21.5
NSF	58.7	25.8	151.6	177.5	22.7	21.0	7.6	85.4	40.0
HSW	0.5	0.0	0.0	0.0	9.5	6.6	31.2	47.4	9.3
FWT	2.3	0.0	0.1	0.1	15.3	13.8	10.2	80.6	25.4
RCC	51.2	9.5	16.6	26.1	10.0	8.0	20.3	63.6	13.1
PR	19.2	0.5	5.1	5.6	12.4	11.8	4.5	91.2	23.3
SC	0.5	0.0	0.0	0.0	25.9	24.8	4.2	91.8	49.0
TR	5.9	0.0	0.5	0.5	12.3	11.9	3.6	92.9	23.6
YLD	1.3	0.0	0.1	0.1	19.7	16.3	17.3	68.5	27.8

GP, germination %; FBL, first bifurcation length; PB, primary branch; SB, secondary branch; PH, plant height; PD, stem diameter; NLP, number of leaf per plant; DF, days to first flowering; DM, days to maturity; NFP, number of fruit per plant; FL, fruit length; FB, fruit breadth; CL, calyx length; FW, fruit fresh weight; FWD, fruit dry weight; NSF, number of seed per fruit; HSW, hundred seed weight; FWT, fruit wall thickness; RCC, relative chlorophyll content; PR, photosynthesis rate; SR, stomata conductance; TR, transpiration rate; YLD, yield per plant.

High PCVwas recorded for primary branches, number of seeds per fruit, and stomata conductance. Moreover, most of the remaining traits were found with moderate PCV, excluding germination percentage, plant height, maturity date, and hundred seed weight, which were estimated as having a lower PCV value. The GCV value ranged from 6.3 to

24.8, indicating high variability among the traits. The highest GCV value was found for stomata conductance, followed by number of seeds per fruit and number of fruits per plant, signifying the potential to select these traits. Among the yield contributing traits, fruit length and hundred seed weight were found to have a lower GCV value. According to Falconer [34], heritability percentage is considered low when values range from 0 to 30%, moderate when values range from 30 to 60%, and high when values exceed 60%. Broad sense heritability of studied traits was high for most of the traits, except the primary and secondary branch numbers, which showed lower heritability. First bifurcation length, plant height, and hundred seed weight all had moderate heritability. All the yield contributing traits, including total yield per plant, revealed high heritability.

Among the studied 23 traits, the highest genetic advance (49%) was recorded for stomata conductance, followed by the number of seeds per fruit (40%), while the lowest value was recorded for secondary branches (7%), followed by plant height (8.4%). Raihana et al. [31] proposed that heritability estimates combined with genetic advancement are usually superior to heritability alone when it comes to selecting superior individuals. For number of fruits per plant, number of seeds per fruit, and stomata conductance, high heritability was combined with very high genetic advance as a percentage of mean, indicating that these traits were controlled by additive gene action and that standard selection procedures could be effective for the isolation of superior genotypes for these traits. These results are in accordance with results of earlier research by Chattopadhyay et al. [7], Kumar et al. [35], and Agasimani et al. [36] for fruit yield per plant, and Sreelathakumary and Rajamony [37] for number of fruits per plant. High heritability coupled with moderate genetic advance as percent of mean was observed for stem diameter, fruit breadth, fruit dry and fresh weight, transpiration rate, and total yield per plant, indicating the preponderance of additive and non-additive gene action. Further improvement of these traits would be possible through mass selection, progeny selection, and hybridization procedures intending to exploit the additive gene action that was reported by Tembhurne et al. [38] (2010) and Suryakumari et al. [39]. Low heritability was associated with low genetic advance as % of mean and which was observed for primary and secondary branches, indicating the presence of nonadditive gene action for these traits and that their improvement could be achieved through heterosis breeding.

3.4. Clustering and Principal Components Analysis

For selecting the desired parents, estimation of existing diversity among the genotypes through genetic diversity analysis plays avery important role. The summarized data on the degree and nature of genetic variability is essential for choosing the right parent for targeted crosses [22,40].

Based on different recorded traits, the studied 27 genotypes were successfully clustered into four major groups. Geleta et al. [41] also conducted an experiment with twenty-nine diversified genotypes and clustered them based on morphological character. The Euclidian distance was calculated by using the data of different traits, and the unweighted pair group method with arithmetic means (UPGMA) dendrogram was constructed using those values. The dendrogram explains that the genotypes with common trends remain in the same cluster. The genotypes were grouped into four clusters at a 0.27 dissimilarity coefficient. Groups I, II, and III consist of six, seven, and thirteen genotypes, respectively. There was only one genotype in Group IV. Group I had the highest yielding characteristics; Group II had the most fruits per plant; Group III had early flowering and high yielding characteristics; and Group IV had early maturity and larger fruit diameter. Similar observations were reported by Assefa et al. [26] while experimenting with different chilli populations. Figure 1 describes the result of PCA. Gen 15 was the genotype farthest from the centroid. Gen 23 and Gen 21 were more or less close to the centroid. Based on the combined data of the two seasons, PCA further explains cluster analysis, yielding the two-dimensional graphical illustration (Figure 2), showing that most of the genotypes were dispersed at close distances at PC1, while few were dispersed at great distances as revealed by the Eigenvector. The

variation percentages of PC1 and PC2 are 28% and 19%, respectively, with PC1 showing the highest of the total variation.
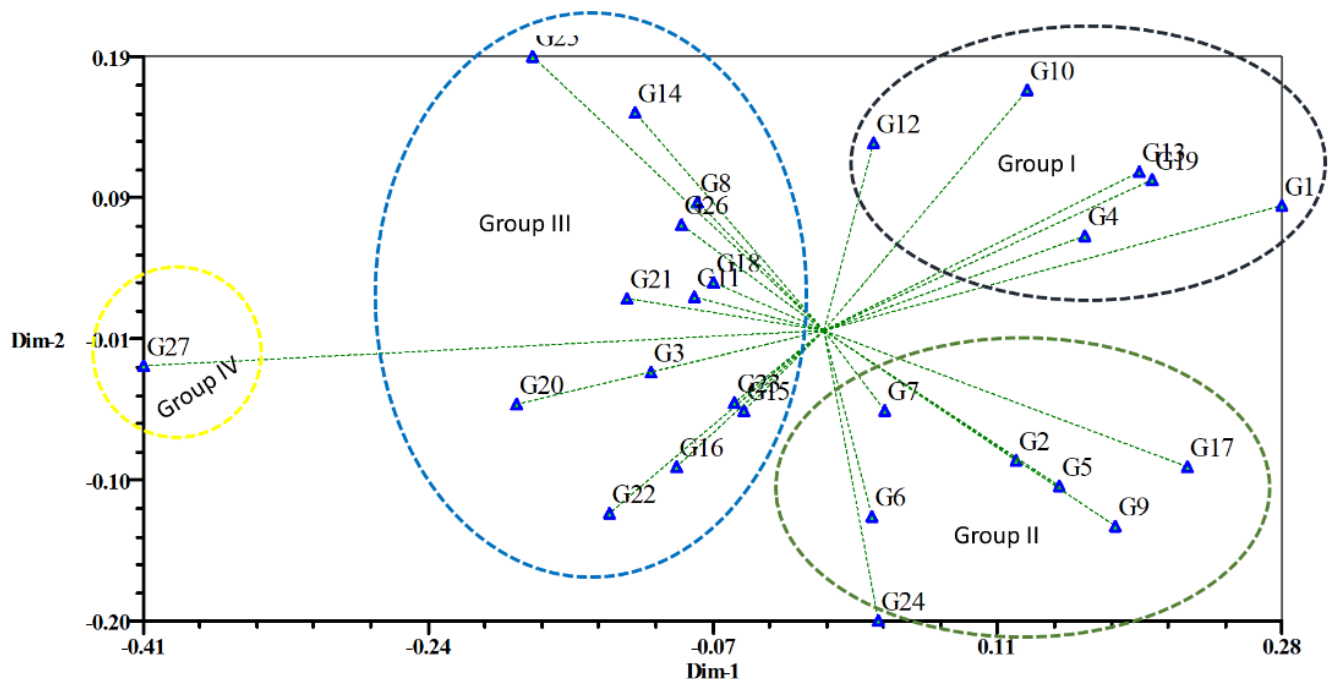
Figure 1. Principal component analysis showing the relationship among 27 chilli genotypes in a two-dimensional graph.
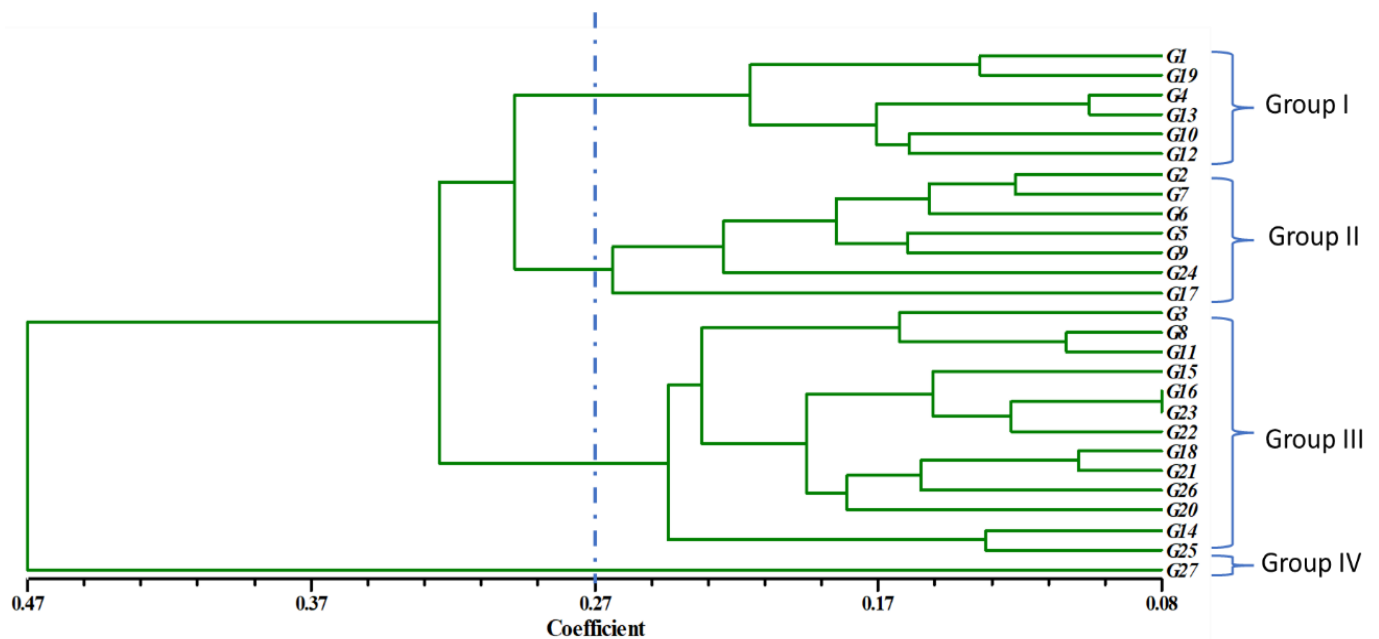
Figure 2. Relationship among the 27chilli genotypes based on 23 traits using SAHN clustering of UPGMA method.

4. Conclusions

Variability among the base generation of parental lines creates more scope for selecting the targeted genotypes to develop the recombinant type and for heterosis breeding. In the present study, information was gathered about 27 chilli genotypes regarding 23 morphological and yield-related traits. Obviously, this information will pave the way forusing

the better ones in various breeding programmes for the improvement of this crop. Hence, estimation of correlation, GCV, PCV, heritability in the broad sense, and genetic advance help to select the genotypes and the selection indices for their exact exploitation. The results of this experiment present an insight into the genetic diversity of the studied chilli population. Considering all the diversity patterns and analysis, the studied genotypes were allocated into four different groups. Group III had the highest (13) number of genotypes, whereas Group IV had only one. The calculated genetic distance also reveals the potential forheterosis breeding. Considering all the information and practical crop conditions, nine genotypes were selected for further hybridization. However, for developing yield and other quality traits, Gen 2, Gen 3, Gen 5, Gen 9, Gen 15, Gen 16, Gen 22, Gen 23, and Gen 27 were selected as better parents, having early flowering, high yielding, and highpungency level characteristics, to design an effective future breeding programme.

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