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Characterization of pepper (*Capsicum* spp.) germplasms based on morphological and phytochemical characters in Bangladesh

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Abstract

Pepper (*Capsicum* spp.) is a major spice crop around the globe. The major goal of the experiment was to evaluate the genetic diversity amongst 30 pepper germplasms for twelve morphological and phytochemical parameters. The investigation was conducted between November 2019 and April 2020 using a randomized complete block design with three replications in the experimental field of Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh. The results revealed a notable disparity across the genotypes for all studied traits. The genotype C80 displayed the highest fresh fruit weight (20.60 g) and dry fruit weight (1.20 g). Once again, the genotype YF1 had the highest chlorophyll and vitamin-C contents. The most significant correlations were revealed between fresh fruit weight and dry fruit weight (r = 0.83***) followed by between fruit diameter and dry fruit weight (r = 0.80***), and between fruit diameter and fresh fruit weight (r = 0.79***). The Wards-D method was used to cluster thirty genotypes into four clusters based on Euclidean distances. Cluster IV consisted of a maximum of 13 pepper genotypes. Cluster I yielded the greatest fresh fruit weight, measuring 11.75 g, whereas cluster III contained the highest Vitamin-C content, measuring 23.61 mg/ 100 g. The clusters I and III had the highest inter-cluster distances (6.45), while cluster I had the highest intra-cluster distance (2.36). PC1 and PC2 explained 32.8% and 18.3% of the total variances, respectively. In the biplot, the genotypes C54 and C80 favored fresh and dry fruit weights as well as fruit diameters, while the genotypes C29 and YF1 positively favored chlorophyll and vitamin-C contents. Therefore, the diverse pepper genotypes C54, C80 from cluster I and C29 and YF1 from cluster III could be included in future hybridization of pepper breeding.

Keywords Pepper, Genetic diversity, Vitamin-C, Clustering, Biplot

Introduction

A group of Solanaceous fruiting plants whereas the heatless varieties are commonly known as capsicum, sweet pepper, bell pepper, paprika or just pepper, and hot ones are known as chilli (Madala and Nutakki 2020). The diverse range of edible peppers, along with other spices and vegetables, that the genus *Capsicum* produces has led to its widespread cultivation worldwide (Herath et al. 2021). The Latin name 'Capsa', meaning chest or box, is the origin of the English word '*Capsicum*', which accurately characterizes the shape of the fruit and its function

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in safeguarding the seeds. The prevailing belief is that the Portuguese introduced it to India 1600 years ago (Bhalabhai et al. 2021). It is native to the Americas' warm regions and the Caribbean (EPPO 2023).

Peppers can be found all over the world in various sizes, colors, and, shapes with tastes spanning from sweet to spicy. The astounding range of colors enhances the visual appeal of any dish. It is traditionally associated with the red and green varieties, almost all begin life as green and eventually change to other colors. It is cultivated for its non-toxic and edible fruits. Uses for peppers are as varied as there are huge germplasms under the *Capsicum* genus. Among these, *Capsicum annuum* L. (2n = 2x = 24), is an important pepper species grown around in Bangladesh. In the year 2021–22, the area and production under pepper cultivation was 243 thousand acres and 625 thousand metric tons, respectively in Bangladesh (BBS 2023). The agro-ecological conditions are favorable for year-round pepper production. Various local pepper varieties are cultivated in different regions of Bangladesh, particularly in Chuadanga (Chuadanga local), Nilphamari (Bindu, Panisaka, and Zira), Chattogram (Halda), Hathajari (Halda), Jashore (Tangrakhali Morich), Kumilla (Kumilla local), Magura (Tanghrakhali morich and Magura local), Meherpur (Meherpur local), Manikgonj (Bindu), Pabna (Bindu and Upda Morich), Panchagarh (Bindu), Thakurgaon (Bindu), and so on. All cultivars are used in both fresh and preserved conditions and have a strong taste. Availability of sweet pepper is scarce in the country and demand of it increasing day by day. Although the average fresh pepper yield in Bangladesh is lower at 1.54 t/ha, this does not necessarily indicate limited crop potential (Uddin 2022). Hence, it is imperative to prioritize breeding efforts towards the development of new pepper types with both a sweet taste and a high yield potential.

Pepper cultivars exhibit substantial differences in their flowering and fruiting times, as well as their yields and other qualitative traits (Maurya et al. 2017). Plant breeders have employed a diverse array of breeding techniques in order to improve the economically advantageous characteristics of peppers. Peppers contain proteins, lipids, carbohydrates, calcium, iron, phosphorus, fibers, vitamins A, B2, B12, C, D, E, K as well as minerals including calcium, phosphorus and iron (El-Ghoraba et al. 2013). Furthermore, pepper contains substantial amounts of essential elements such as magnesium and potassium. Additionally, it has wide applications in the pharmaceutical industry (Herath et al. 2021).

The rapid increase in pepper's yield, along with its associated traits, is significantly facilitated by heterosis breeding. This strategy, among several others in plant breeding, has the potential to enhance yield and other important economic characteristics (Herath et al. 2021). Again, fruit yield and other important plant characteristics viz. plant height, number of main branches, number of fruits per plant, stem girth, fruit length, fruit breadth, and average fruit weight are linked together (Bundela et al. 2018).

The current availability of germplasm screening facilitates the evaluation of germplasms and diversity, as well as the classification and identification of superior parental candidates for future hybridization (Bertan et al. 2007). The genetic divergence studies of pepper genotypes, including Bell, Poblano, Jalapeño, Serrano, Cayenne, and Pepperoncini, have unveiled variations that may be useful in this regard. Following that, the breeding effort may make improvement of the nation's larger pepper germplasm stock. Thus, the purpose of this study was to (i) conduct an analysis of the average performances of twelve morphological and phytochemical characteristics, (ii) determine correlations between characters, and (iii) evaluate genetic diversity of the pepper genotypes.

Materials and methods

Plant materials

The seeds of 30 unique pepper (*Capsicum* spp.) genotypes were used as plant materials in this investigation. They have diverse origins and were collected from Phytotech Laboratory Pty Ltd., Sydney, Australia. The list of experimental genotypes with types, origins and reasons for selection are presented in Table 1.

Context of the research

The experiment took place from November 2019 to April 2020 in the rabi season at the experimental field of Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh. The experimental field was situated at a latitude of 24.00^0 N and a longitude of 90.25^0 E, with an elevation of 34 m above the sea level. The field is situated in the Agro Ecological Zone (AEZ) known as the Old Himalayan Piedmont Plain of Bangladesh. The soil utilized for this experiment exhibited a silty loam texture, included organic matter, and possessed a slightly acidic pH. The soil characteristics are reported in supplementary Table 1.

The experimental site is located in a subtropical environment characterized by considerable rainfall during the kharif season (March-August) and scarce rainfall during the rabi season (October-February). During the growth phase of this crop, the ambient temperature reduced due to the rabi season, which is winter in Bangladesh. The Weather Yard, a division of the Bangladesh Meteorological Department, collected data on the monthly average temperature, humidity, and rainfall specifically during the crop growing time. The research period recorded a maximum temperature of 30.5 °C in April 2020 and a minimum temperature of 10.6 °C in January 2020. In

Table 1 List of pepper genotypes with their source, type and origins

Sl. No	Genotype	Type	Origin	Reasons for selection
1	C1	Poblano	Puebla, Mexico	Mild heat and rich, smoky flavor
2	C10	Long Green chilli	Mexico	Vibrant flavor and crisp spicy texture
3	C190	Bell	Mexico, Central America and South America	Sweet, juicy flesh, and a burst of color
4	C28	Bell	Mexico, Central America and South America	Sweet, juicy flesh, and a burst of color
5	C29	Jalapeno	Jalapa, Mexico	Spicy and tangy flavor
6	C31	Poblano	Puebla, Mexico	Mild heat and rich, smoky flavor
7	C37	Bell	Mexico, Central America and South America	Sweet, juicy flesh, and a burst of color
8	C43	Serrano	Puebla and Hidalgo Mexico	Crisp texture and a fiery heat
9	C44	Pablano	Puebla, Mexico	Mild heat and rich, smoky flavor
10	C46Best	Pepperoncini	Italy and Greece	Mild heat and slightly sweet, tangy flavor
11	C47	Jalapeno	Jalapa, Mexico	Spicy and tangy flavor
12	C51	Jalapeno	Jalapa, Mexico	Spicy and tangy flavor
13	C51 Hot	Jalapeno	Jalapa, Mexico	Spicy and tangy flavor
14	C51hot2	Jalapeno	Jalapa, Mexico	Spicy and tangy flavor
15	C54	Bell	Mexico, Central America and South America	Sweet, juicy flesh, and a burst of color
16	C55KNI	Jalapeno	Jalapa, Mexico	Spicy and tangy flavor
17	C55 Thickblant	Serrano	Puebla and Hidalgo Mexico	Crisp texture and a fiery heat
18	C55 Thickpointed	Serrano	Puebla and Hidalgo Mexico	Crisp texture and a fiery heat
19	C71	Jalapeno	Jalapa, Mexico	Spicy and tangy flavor
20	C72	Pablano	Puebla, Mexico	Mild heat and rich, smoky flavor
21	C78	Bell	Mexico, Central America and South America	Sweet, juicy flesh, and a burst of color
22	C80	Bell	Mexico, Central America and South America	Sweet, juicy flesh, and a burst of color
23	C82	Pablano	Puebla, Mexico	Mild heat and rich, smoky flavor
24	C83	Pablano	Puebla, Mexico	Mild heat and rich, smoky flavor
25	C85	Pepperoncini	Italy and Greece	Mild heat and slightly sweet, tangy flavor
26	C88	Cayenne	Cayenne, French Guiana	Intense heat and bold, earthy flavor
27	GS1	Serrano	Puebla and Hidalgo Mexico	Crisp texture and a fiery heat
28	GS3	Serrano	Puebla and Hidalgo Mexico	Crisp texture and a fiery heat
29	YF1	Pablano	Puebla, Mexico	Mild heat and rich, smoky flavor
30	Z4	Pablano	Puebla, Mexico	Mild heat and rich, smoky flavor

February 2020, the maximum relative humidity of 68.2% was recorded, while the lowest relative humidity of 51.6% was noted in November 2019. The maximum precipitation of 71.5 mm was documented in April 2020.

Experimental set-up

The seeds were subjected to surface sterilization by immersing them in a 1% (w/v) solution of mercuric chloride for 5 min, and then rinsing them with 70% (v/v) ethanol. Afterwards, the seeds were rinsed an additional five times and then placed on a bed of coco peat to begin germination. The experimental containers were outfitted with both irrigation and drainage systems. The necessary techniques for managing the nursery, such as shade, watering, thinning, forking, and hardening off, were implemented to ensure the successful development of robust seedlings. After a period of 30 days from the time of sowing, the seedlings were transferred to pots. Seedling transplantation was conducted in the afternoon, when the temperature was lower, to minimize the stress associated with transplanting. We created the potting soil by combining well-decomposed bovine manure and top soil in a 2:1 ratio. The genotypes were allocated randomly to one of three replications using a randomized complete block design (RCBD). After transplanting the seedlings, intercultural activities such as watering, weeding, and top dressing were carried out to promote the healthy growth and development of the pepper plants. Following the application of a layer of fertilizer, we promptly commenced the process of earthing up. Fungal infections greatly impeded agricultural productivity. After a light rainfall, the plot was affected by anthracnose. A double dose of Tilt 250 EC (0.5 ml per liter of water) was administered. To prevent viral diseases such as leaf curl, the

plant was treated with a solution of 1 ml of Malathion 50 EC per liter of water.

Data collection

In total twelve morphological and phytochemical traits were measured for 30 pepper genotypes. The detailed methods of measuring data of these traits are discussed here below.

Morphological parameter estimation

Thirty pepper genotypes were subjected to three replications, from which ten morphological parameters were collected. Among these, the days to flowering was measured as the interval between seed planting and the onset of blooming. The plant height was measured in centimeters (cm) from the ground soil to the apex of their tallest stems. The primary, secondary, and tertiary branches per plant were quantified on each plant in every replicate. The length of each individual fruit was calculated by measuring its diameter using a meter scale, and the measurement was expressed in centimeters (cm). The average diameter of each fruit was estimated using slide calipers by taking measurements at different distances. An electronic balance was used to measure the weight of fresh fruit in gram (g). Afterwards, the fruits were let to dry and the weight of the dried fruit was then measured and stated in gram (g). One hundred fresh fruits were thoroughly cleansed and dehydrated, and the mass of their seeds was quantified using an electronic digital scale. Consequently, this value was converted into the weight of 1000 seeds (g).

Estimation of phytochemical parameters

The approach involved estimating two phytochemical parameters, namely chlorophyll content and Vitamin-C content. The chlorophyll content was computed using a SPAD502 plus chlorophyll meter (Konica Minolta, Japan) by determining the SPAD value of the three most mature leaves. Here, vitamin-C content in pepper genotypes, three distinct working solutions were prepared, namely meta-phosphoric acid, dye, and standard vitamin C solutions. A solution of meta-phosphoric acid was created by dissolving 30 g of meta phosphoric acid in 80 ml of glacial acetic acid. This solution was then transferred to a volumetric flask with a capacity of 1000 ml and further diluted with distilled water until the desired volume was reached. A dye solution was prepared by dissolving 260 mg of 2,6-dichlorophenol indophenol and 210 mg of sodium bicarbonate in 1000 ml of distilled water. Lastly, 100 mg of Vitamin-C, or L-ascorbic acid, completely dissolved in a 1000 ml solution of Meta phosphoric acid, commonly referred to as the standard vitamin C solution. Once more, a 5 ml aliquot of a standard vitamin C solution was transferred into a 100 ml conical flask and subsequently titrated with a dye solution dispensed from a burette. Subsequently, a quantity of 10 mg of fruit was combined with 50 ml of a solution containing metaphosphoric acid. Then, the mixtures were strained using a white cotton fabric and subsequently poured into a volumetric vial having a 100 ml capacity. A conical flask was utilized to contain a 10 ml portion of fruit sample, which was subsequently titrated with dye solution dispensed from a burette. Afterwards, the vitamin C content was calculated using the following equation.

$$\begin{aligned}&\text{Vitamin C content}\left(\text{mg}/100\text{g}\right)\\&=\left(\text{Titrate value}\times\text{dye factor}\right.\\&\quad\left.\times\text{volume of sample made up}\times 100\right)/\\&\quad\left(\text{volume of sample used}\times\text{weight of sample}\right)\end{aligned}$$

Quantitative analyses

The data for each character was entered into a Microsoft Excel spreadsheet for each entry. The data were examined using the randomized complete block design model developed by Cochran and Cox (1950). Following this, the analysis was performed by R statistical software of version 4.0.3 (R Core Team 2019) using the following model:

$$Yij = gi + rj + \varepsilon ij$$

Here,

Yij represents observed values of the i^{th} genotype in j^{th} replication, *gi* represents effects of the i^{th} genotype, *rj* represents effects of the j^{th} replication, εij represents the residual error of the i^{th} genotype in the j^{th} replication.

The replications were considered as random variable and genotype were fixed variable.

The correlation coefficient (r) was calculated for chosen traits in 30 genotypes using the formula by Singh and Chaudhary 1985. In this analysis, p-values were calculated using the 'corr.test' function from the 'R psych package' (R Core Team 2019).

The use of biometrical methods has facilitated the measurement and choice of genetically varied parents for a hybridization program (Rao 1952). Multivariate analytic approaches, such as principal component analysis (PCA) and cluster analysis, are useful tools for evaluating genetic diversity by measuring variations across various quantitative variables. In this study, we used aggregated mean data to perform clustering analysis using Python software (Pilgrim and Willison 2009). Here, we followed a hierarchical agglomerative method, specifically Ward's method, which calculates Euclidean distances. Ward's minimum variance approach (Ward 1963) is a clustering

process that is designed to reduce the variance within each group. The ideal number of clusters was obtained by identifying the point at which the overall variance within each cluster showed a substantial decrease.

Results and discussion

The objective of the study was to examine the average performances, character association, and genetic diversity of 30 pepper genotypes. The pepper genotypes were analyzed to investigate 12 specific morphological and phytochemical characteristics. The results of the experiment are presented and discussed under the following heads.

Analysis of variance

An analysis of variance on 12 morphological and phytochemical traits of 30 pepper genotypes are presented in Table 2. The traits plant height (cm), primary branches per plant, secondary branches per plant, tertiary branches per plant, chlorophyll content (SPAD), fresh fruit weight (g), fruit diameter (cm), fruit length (cm), dry fruit weight (g), 1000- seed weight (g) and vitamin C-content varied significantly across the genotypes. A substantial amount of genetic diversity exhibited among the genotypes due to large variances revealed among them. These variances, which can be traced back, indicate how populations have evolved over time and allow natural selection to establish a gene pool that is more likely to be successful. Thilak et al. (2019) found notable differences in the genotypes of pepper regarding average fruit weight, length, girth, number of seeds per plant, and yield per plot. The coefficient of variation (CV %) is founded on the principle that as the average magnitude increases, the level of variance also increases. The CV (%) values can indicate the variability within a population, the stability of phenotypic traits, the variation in plot sizes in uniformity trials, and other situations where individual variability is assessed. Belay et al. (2019) state that the coefficient of variation can be employed to assess the extent to which the statistical model accounts for the observed variation. Our investigation revealed significant variances, ranging from 1.75% in vitamin C content to 32.01% in fresh fruit weight (g).

Mean performances

The 30 pepper genotypes appeared different size, shape and colors those are presented in Fig. 1. The average mean performances of 30 pepper genotypes for 12 morphological and phytochemical traits were presented in Table 3. According to Table 3, the days to flowering was variable in 30 pepper genotypes. Here, late flowering was exhibited for the genotype C71 (89 days) and early flowering was for C31 genotype (76.67 days). Negi and Sharma (2019) observed the highest days to flowering 42.67, while the lowest 33.33 days in red ripe chilli genotypes. The longest fruit length 8.08 cm was exhibited in GS3 genotype, whereas the YF1 genotype measured lowest 3.24 cm. Bekele et al. (2023) reported maximum (11 cm) fruit length in pepper genotypes V.AVPP.0411 whereas the minimum (4 cm) in V-Unknown-2, 9086, 9099, and 9082. Again, Nankar et al. (2020) recorded the maximum fruit length 24.33 cm while the minimum was recorded in 1.27 cm and the maximum fruit weight 195.57 g while the minimum was recorded in 1.07 g. Fruit diameter ranged

Table 2 Analysis of variance of twelve morpho-physiological characters of 30 pepper genotypes

Character	Sources of variation with mean sum of square			
	Replication	Genotype	Error	Coefficient of variation (%)
Days to flowering	4.47^{NS}	29.33***	10.70	3.88
Plant height (cm)	155.33^{NS}	252.72*	140.94	24.21
Primary branches per plant	3.07*	4.99***	0.72	21.18
Secondary branches per plant	2.53^{NS}	10.72***	2.67	23.46
Tertiary branches per plant	26.17^{NS}	87.68***	12.37	22.00
Chlorophyll content (SPAD)	2.96^{NS}	73.91***	27.09	12.66
Fruit length (cm)	2.25^{NS}	4.52***	1.38	21.90
Fruit diameter (mm)	0.74^{NS}	9.05***	1.08	16.60
Fresh fruit weight (g)	14.87^{NS}	50.61***	6.34	32.01
Dry fruit weight (g)	0.03^{NS}	0.15***	0.03	28.62
1000- seed weight (g)	0.10^{NS}	7.03 ***	0.19	16.12
Vitamin C-content (mg/ 100 g)	0.01^{NS}	83.54***	0.02	1.75

NS indicates not-significant

'*', '**' and '***' indicates significant at 5%, 1% and 0.1% levels of probability, respectively

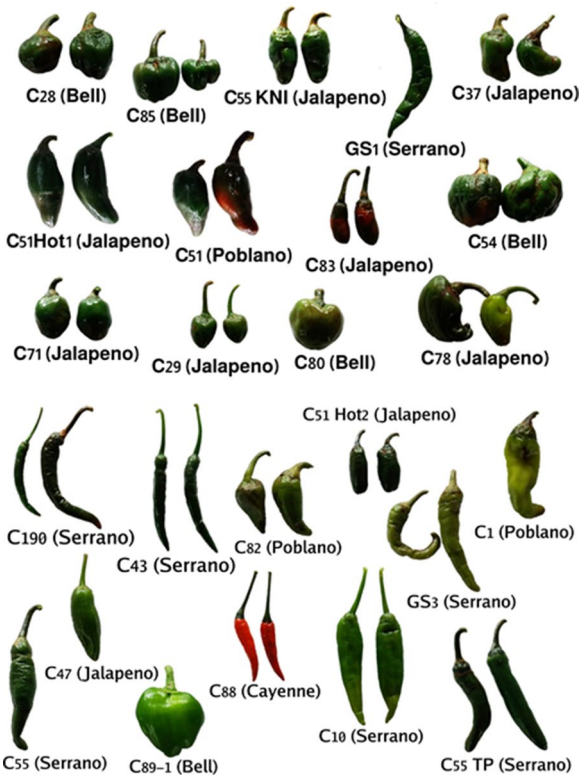

Fig. 1 Thirty pepper fruits exhibited phenotypic diversity through their size, shape and colours

from 3.65 mm for the C88 genotype to 11.30 mm for the C54 genotype. Negi and Sharma (2019) found maximum fruit diameter as 11.9 mm, while minimum as 7.6 mm in red chilli genotypes. The C72 genotype produced the tallest plant at 66 cm, while the C78 genotype produced the shortest at 31.43 cm with a mean of 49.05 cm. Almost similar findings were revealed by Bekele et al. (2023) exhibiting 49.90 cm mean plant height in pepper genotypes with a range of 41.00–62.16 cm. Primary branches per plant ranged from 7.67 for the C43 genotype to 2.33 for the C28 genotype. The C55Thickblant genotype produced the highest average secondary branches per plant at 10.67 whereas the C28 genotype gave the lowest value 4.0. The maximum tertiary branches per plant (29.33) was generated by the genotype C85, whereas the minimum was for the genotype C28. Overall, the mean primary, secondary and tertiary branches per plant in the study were 4.01, 6.96 and 15.98, respectively. Misra et al. (2011) revealed 7, 8 and 5 primary, secondary and tertiary branches per plant among 38 *Capsicum* accessions. Different genotypes have varying chlorophyll contents (SPAD value) that ranged from 55.4 in YF1 to 34.27 in C31. Srideepthi, et al. (2017) recorded highest chlorophyll content 59.35 and lowest 5.60. The fresh fruit weight from the C80 genotype averaged was 20.60 g, but those from the C88 genotype only weighed 1.94 g on an average. The dry fruit weight ranged from 0.22 g for C88 to 1.20 g for C80. Negi and Sharma (2019) observed maximum dry fruit weight 0.81 g, while minimum 0.35 g in chilli genotypes. The 1000-seed weight (g) was found in C10 (6.31 g), with the lowest 1000-seed weight was found in C29 and C55KNI. Vitamin-C content varied greatly across the genotypes, with the highest being found in YF1 (28.42 mg/ 100 g) and the lowest being found in C54 (2.81 mg/ 100 g). Nankar et al. (2020) observed that the maximum Vitamin-C content 273.47 mg/100 g FW while the minimum was recorded in 4.77 mg/100 g FW.

Correlation analysis

Comprehending the interaction between fruit yield and its components is crucial since yield is determined by the interplay of various distinct yield components and their interaction with the developing plant. Simple correlation co-efficient values between 12 morphological and phytochemical parameters were discovered in the study and presented Fig. 2. Here, the number of secondary branches per plant exhibited a strong and positive correlations with tertiary branches per plant (0.72***) followed by primary branches per plant (0.66***) and plant height (0.51**). This correlation is logical and increasing branch number per plant with plant height might produce higher pepper yield. Shumbulo et al. (2017) observed significant and positive correlations between branch number per plant and plant height (cm) in hot pepper genotypes. Among the fruit traits, the fresh fruit weight (g) exhibited a positive and strongest correlations with dry fruit weight (g) ($r = 0.83^{***}$). Again, fruit diameter (mm) showed stronger correlations with dry fruit weight (g) ($r = 0.80^{***}$) and fresh fruit weight (g) ($r = 0.79^{***}$). Negi and Sharma (2019) found a strong and positive correlation of average dry fruit weight with fruit length and fruit diameter. In another study, fruit weight per fruit revealed positive and strong correlations with fruit width, fruit length and fruit yield per plant (Luitel et al. 2013). Pardeshi et al. (2021) observed negative and insignificant correlations with fruit parameters viz. fruit length and fruit weight. In our study, the fruit diameter (mm) revealed significant and negative correlations with primary branches per plant, secondary branches per plant and tertiary branches per plant. Negi and Sharma (2019) also revealed negative correlations between fruit width and primary branches per plant ($r_p = -0.201$), but positive correlations with secondary branches per plant ($r_p = -0.115$). In the study,

Table 3 Mean performance of 30 genotypes of pepper for 12 morphological and phytochemical characters

Genotype	Mean ± SE						
	Days to flowering	Plant height (cm)	Primary branches per plant	Secondary branches per plant	Tertiary Branches per plant	Chlorophyll content (SPAD)	Fruit length (cm)
C1	82.67 ± 3.48	46.33 ± 9.21	3.00 ± 0.58	5.00 ± 1.15	12.67 ± 2.19	50.15 ± 1.20	6.01 ± 0.29
C10	85.33 ± 1.45	54.33 ± 4.18	4.33 ± 0.33	8.67 ± 0.67	19.00 ± 1.73	37.23 ± 2.27	6.51 ± 1.17
C190	89.00 ± 0.58	58.67 ± 4.10	3.67 ± 0.33	7.00 ± 0.58	15.00 ± 1.53	37.87 ± 0.91	7.61 ± 0.31
C28	84.00 ± 2.00	35.67 ± 1.86	2.33 ± 0.33	4.00 ± 0.00	8.00 ± 0.58	40.26 ± 2.92	4.00 ± 0.51
C29	82.33 ± 2.33	36.83 ± 5.40	3.33 ± 0.33	6.33 ± 0.88	14.67 ± 2.40	46.43 ± 6.23	3.86 ± 0.59
C31	76.67 ± 0.88	55.00 ± 0.58	6.00 ± 0.58	7.33 ± 0.33	9.33 ± 0.33	34.27 ± 0.72	3.33 ± 0.29
C37	85.33 ± 1.45	53.00 ± 2.65	3.67 ± 0.33	6.33 ± 0.33	15.00 ± 0.58	40.90 ± 5.78	5.10 ± 0.12
C43	88.00 ± 0.58	57.67 ± 9.40	7.67 ± 1.20	8.00 ± 0.58	19.00 ± 4.16	40.80 ± 3.93	6.27 ± 0.07
C44	85.00 ± 4.16	38.33 ± 3.48	3.33 ± 0.33	5.33 ± 0.33	10.00 ± 1.15	44.19 ± 1.33	5.15 ± 0.45
C46Best	82.67 ± 2.73	49.00 ± 6.43	4.67 ± 0.33	7.00 ± 0.58	15.00 ± 1.53	42.63 ± 5.14	6.50 ± 0.88
C47	80.00 ± 1.53	51.33 ± 6.96	2.67 ± 0.33	5.33 ± 0.67	17.67 ± 1.20	36.33 ± 2.99	6.50 ± 0.84
C51	88.33 ± 0.33	45.33 ± 9.17	3.00 ± 0.58	5.00 ± 0.58	13.33 ± 2.03	44.43 ± 0.55	4.72 ± 0.36
C51 Hot	87.00 ± 1.53	62.67 ± 2.91	3.67 ± 0.33	5.67 ± 1.45	10.00 ± 1.15	37.87 ± 3.52	5.41 ± 0.59
C51hot2	86.67 ± 0.88	51.33 ± 5.17	2.67 ± 0.33	5.33 ± 0.33	12.67 ± 0.67	39.53 ± 3.67	5.67 ± 0.67
C54	85.00 ± 1.15	45.00 ± 1.15	3.33 ± 0.33	5.67 ± 0.33	11.67 ± 0.88	35.17 ± 0.44	6.25 ± 1.01
C55KNI	86.33 ± 1.45	42.00 ± 9.61	4.33 ± 0.88	7.33 ± 1.45	19.33 ± 2.96	37.64 ± 4.15	4.08 ± 0.33
C55Thickblant	80.00 ± 1.15	65.33 ± 9.87	5.33 ± 0.33	10.67 ± 0.67	28.33 ± 3.76	46.00 ± 1.39	6.22 ± 1.03
C55Thickpointed	82.00 ± 1.73	49.33 ± 7.84	6.00 ± 0.58	10.33 ± 1.20	24.00 ± 3.06	37.70 ± 4.84	5.63 ± 1.59
C71	89.00 ± 1.15	43.33 ± 8.84	5.00 ± 1.00	10.33 ± 1.76	21.33 ± 3.28	37.23 ± 2.14	4.38 ± 0.37
C72	86.33 ± 0.33	66.00 ± 10.02	3.67 ± 0.33	8.00 ± 0.58	22.67 ± 2.33	40.57 ± 2.57	5.31 ± 0.22
C78	85.67 ± 1.20	31.43 ± 1.44	2.67 ± 0.33	5.00 ± 0.58	21.00 ± 2.08	35.97 ± 0.84	5.40 ± 0.15
C80	80.00 ± 1.53	43.00 ± 3.46	3.67 ± 0.33	6.67 ± 0.33	11.67 ± 1.67	43.70 ± 1.18	6.25 ± 0.80
C82	81.00 ± 3.51	33.50 ± 2.02	4.33 ± 0.33	5.00 ± 1.00	12.00 ± 1.15	36.05 ± 2.40	5.17 ± 0.29
C83	87.33 ± 1.20	44.33 ± 7.84	3.33 ± 0.33	7.67 ± 0.33	19.67 ± 2.73	34.53 ± 2.28	3.53 ± 0.16
C85	86.33 ± 0.88	60.33 ± 12.25	3.33 ± 0.33	10.33 ± 1.33	29.33 ± 2.40	41.23 ± 2.07	5.33 ± 1.27
C88	82.67 ± 1.45	56.67 ± 1.86	7.00 ± 0.58	10.33 ± 2.60	13.67 ± 1.45	44.13 ± 3.96	4.22 ± 0.23
GS1	87.67 ± 0.88	45.00 ± 5.69	4.00 ± 0.58	6.00 ± 1.00	14.33 ± 1.20	44.37 ± 1.20	6.93 ± 0.54
GS3	81.33 ± 2.96	47.00 ± 6.81	4.00 ± 0.58	7.00 ± 0.00	14.67 ± 1.33	43.60 ± 2.33	8.08 ± 0.51
YF1	85.33 ± 2.03	46.67 ± 12.44	3.33 ± 0.33	6.33 ± 0.33	10.67 ± 2.19	55.40 ± 1.00	3.24 ± 0.33
Z4	81.67 ± 2.03	57.00 ± 6.93	3.00 ± 0.58	6.00 ± 1.00	14.00 ± 1.53	47.60 ± 0.81	4.57 ± 1.12
Range	76.67–89	31.43–66	2.33–7.67	4–10.67	8–29.33	34.27–55.4	3.24–8.08
Mean	84.36	49.05	4.01	6.96	15.98	41.13	5.37
SE (±)	0.57	1.68	0.24	0.35	0.99	0.91	0.22
Std. dev (±)	3.13	9.18	1.29	1.89	5.41	4.96	1.23

Genotype	Mean ± SE				
	Fruit diameter (mm)	Fresh fruit weight (g)	Dry fruit weight (g)	1000 Seed Weight (g)	Vitamin C-content (mg/100 g)
C1	7.00 ± 0.58	8.06 ± 1.07	0.63 ± 0.13	5.05 ± 0.08	9.14 ± 0.09
C10	4.57 ± 0.38	4.68 ± 1.30	0.49 ± 0.06	6.31 ± 0.05	6.85 ± 0.13
C190	4.69 ± 0.43	7.77 ± 1.25	0.75 ± 0.05	3.90 ± 0.07	10.81 ± 0.06
C28	9.43 ± 0.61	6.35 ± 0.78	0.79 ± 0.16	2.36 ± 0.07	4.62 ± 0.06
C29	5.26 ± 0.10	3.46 ± 0.32	0.26 ± 0.01	0.01 ± 0.01	18.80 ± 0.06
C31	6.46 ± 0.74	6.31 ± 1.15	0.53 ± 0.08	1.46 ± 0.03	7.90 ± 0.06
C37	6.24 ± 0.95	13.66 ± 1.35	0.83 ± 0.11	2.60 ± 0.06	4.24 ± 0.03
C43	3.84 ± 0.25	3.92 ± 0.61	0.40 ± 0.09	1.49 ± 0.01	17.41 ± 0.06

Table 3 (continued)

Genotype	Mean ± SE				
	Fruit diameter (mm)	Fresh fruit weight (g)	Dry fruit weight (g)	1000 Seed Weight (g)	Vitamin C-content (mg/100 g)
C44	7.22 ± 0.44	7.49 ± 0.32	0.94 ± 0.02	1.52 ± 0.06	7.84 ± 0.03
C46Best	5.70 ± 0.23	7.98 ± 0.99	0.45 ± 0.04	2.30 ± 0.09	6.95 ± 0.02
C47	6.23 ± 0.54	7.19 ± 1.90	0.48 ± 0.18	2.04 ± 0.08	11.92 ± 0.03
C51	6.22 ± 0.40	5.96 ± 0.90	0.70 ± 0.09	1.48 ± 0.12	5.72 ± 0.06
C51 Hot	6.72 ± 0.36	8.97 ± 1.64	0.53 ± 0.09	4.45 ± 0.16	3.76 ± 0.13
C51hot2	6.56 ± 0.57	8.25 ± 1.65	0.65 ± 0.19	2.63 ± 0.14	8.19 ± 0.01
C54	11.30 ± 0.65	19.31 ± 0.83	1.04 ± 0.03	0.90 ± 0.17	2.81 ± 0.06
C55KNI	5.72 ± 0.43	7.03 ± 0.66	0.61 ± 0.08	0.01 ± 0.01	6.05 ± 0.03
C55Thickblant	6.05 ± 0.03	10.28 ± 0.44	0.64 ± 0.02	3.84 ± 0.36	8.13 ± 0.06
C55Thickpointed	5.58 ± 0.56	7.47 ± 1.77	0.57 ± 0.11	1.40 ± 0.06	8.33 ± 0.00
C71	6.84 ± 0.57	9.96 ± 0.91	0.60 ± 0.05	1.59 ± 0.05	5.51 ± 0.03
C72	6.63 ± 0.36	7.46 ± 0.65	0.50 ± 0.04	2.99 ± 0.10	6.39 ± 0.03
C78	6.34 ± 0.48	7.61 ± 0.70	0.79 ± 0.06	4.09 ± 0.31	5.90 ± 0.06
C80	10.20 ± 1.66	20.60 ± 4.89	1.20 ± 0.17	2.03 ± 0.09	3.39 ± 0.09
C82	7.67 ± 0.77	10.80 ± 1.81	0.86 ± 0.25	1.88 ± 0.01	5.10 ± 0.06
C83	5.12 ± 0.28	3.57 ± 0.30	0.41 ± 0.05	5.41 ± 0.15	7.21 ± 0.06
C85	4.62 ± 0.12	4.76 ± 0.83	0.29 ± 0.05	4.33 ± 1.22	5.33 ± 0.24
C88	3.65 ± 0.51	1.94 ± 0.43	0.22 ± 0.03	4.28 ± 0.04	9.43 ± 0.04
GS1	3.90 ± 0.38	5.38 ± 0.14	0.44 ± 0.03	2.90 ± 0.23	13.81 ± 0.06
GS3	5.58 ± 0.34	7.80 ± 1.51	0.63 ± 0.08	3.18 ± 0.06	7.83 ± 0.03
YF1	5.13 ± 0.19	3.69 ± 0.31	0.44 ± 0.02	3.28 ± 0.04	28.42 ± 0.24
Z4	7.22 ± 1.16	8.30 ± 3.28	0.65 ± 0.12	3.14 ± 0.09	7.58 ± 0.01
Range	3.65–11.3	1.94–20.6	0.22–1.2	0.01–6.31	2.81–28.42
Mean	6.26	7.86	0.61	2.76	8.51
SE (±)	0.32	0.75	0.04	0.50	0.91
Std. dev (±)	1.74	4.11	0.22	1.53	3.74

phytochemical parameters viz. vitamin-C content and chlorophyll content (SPAD) revealed positive and strong correlations (0.56). But, vitamin-C content exhibited significant and negative correlations with fresh fruit weight (g) ($r = -0.52^{**}$), fruit diameter (mm) ($r = -0.50^{**}$) and dry fruit weight ($r = -0.49^{**}$). Srinivas et al. (2020) observed insignificant correlations of vitamin-c content with fruit diameter and dry fruit weight, and significant but weak correlations with fresh fruit weight.

Genetic diversity analysis

Estimates of genetic diversity were generated using a wide range of statistical methods. One technique for calculating genetic diversity and dissimilarity is cluster analysis. In this case, members of the same cluster group is grouped together based on similar attributes. The two main categories of clustering techniques are hierarchical and non-hierarchical techniques. Because of its robustness, a hierarchical cluster analysis method was used in the study.

Hierarchical cluster analysis

Hierarchical clustering technique is a common place for examining genetic diversity. This method unites the individuals that are closest to each other first, and then these initial groupings are further combined based on their common features. Applying Ward's method, the 30 pepper genotypes were grouped into a total four distinct clusters viz. Cluster I, II, III and IV (Fig. 3). Cluster IV contained the highest quantity of pepper genotypes, totaling 13 (Table 4). The second highest number (9) included in cluster II which is followed by cluster I (6) and cluster III (2). Yatung et al. (2014) grouped 30 chilli genotypes into 6 clusters where the cluster III contained maximum 14 genotypes. Belay et al. (2019) observed 7 clusters for 64 hot pepper genotypes where maximum 20 genotypes were included in cluster I.

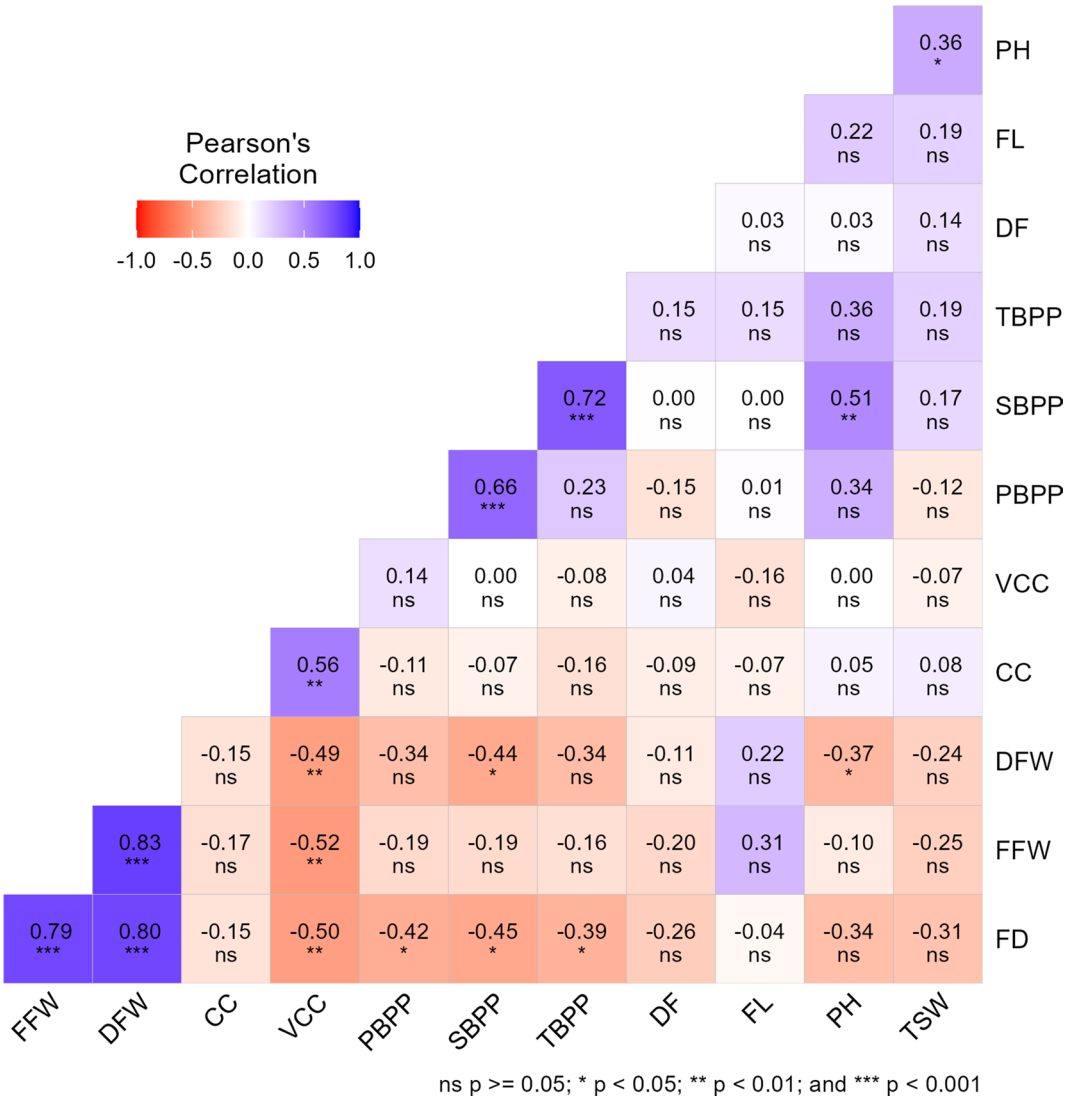

Fig. 2 Simple correlation coefficient between yield and yield contributing traits in thirty pepper genotypes. Here, *DF* Days to Flowering, *PH* Plant height (cm), *PBPP* Primary branches per plant, *SBPP* Secondary branches per plant, *TBPP* Tertiary branches per plant, *CC* Chlorophyll content (SPAD), *FL* Fruit length (cm), *FD* Fruit diameter (mm), *FFW* Fresh fruit weight (g), *DFW* Dry fruit weight (g), *TSW* 1000- seed weight (g), and *VCC* Vitamin-C content (mg/ 100 g)

Cluster I consisted of six different jalapeno genotypes. This cluster had the highest average fruit diameter (8.67 mm), fresh fruit weight (11.75 g), and dry fruit weight (0.92 g) based on the clustering mean data provided in Table 5. Additionally, cluster I exhibited typical characteristics such as a flowering time of 84.25 days after sowing (DAS), chlorophyll content of 40.63 SPAD, fruit length of 5.26 cm, and 1000-seed weight of 1.7 g. In addition, it exhibited the lowest number of primary branches per plant, secondary branches per plant and tertiary branches per plant (3.33, 5.28, and 11.11, respectively), as well as the shortest plant height (40.14 cm) and the lowest vitamin C content (4.91 mg/ 100 g). Cluster II comprised of nine distinct genotypes, as illustrated in Table 4. These genotypes exhibited longest days to flowering at 84.67 days. Additionally, they exhibited the greatest value for 1000-seed weight at 2.37 g, the tallest plant height at 55.07 cm, and the most primary, secondary, and tertiary branches per plant at 5.37, 9.19, and 20.78, respectively (Table 5). This cluster exhibited the lowest chlorophyll content, measuring 39.95 SPAD. Table 4 demonstrates that Cluster III included merely two distinct genotypes viz. C29 and YF1. Based on the clustering analysis of the mean data (Table 5), these genotypes

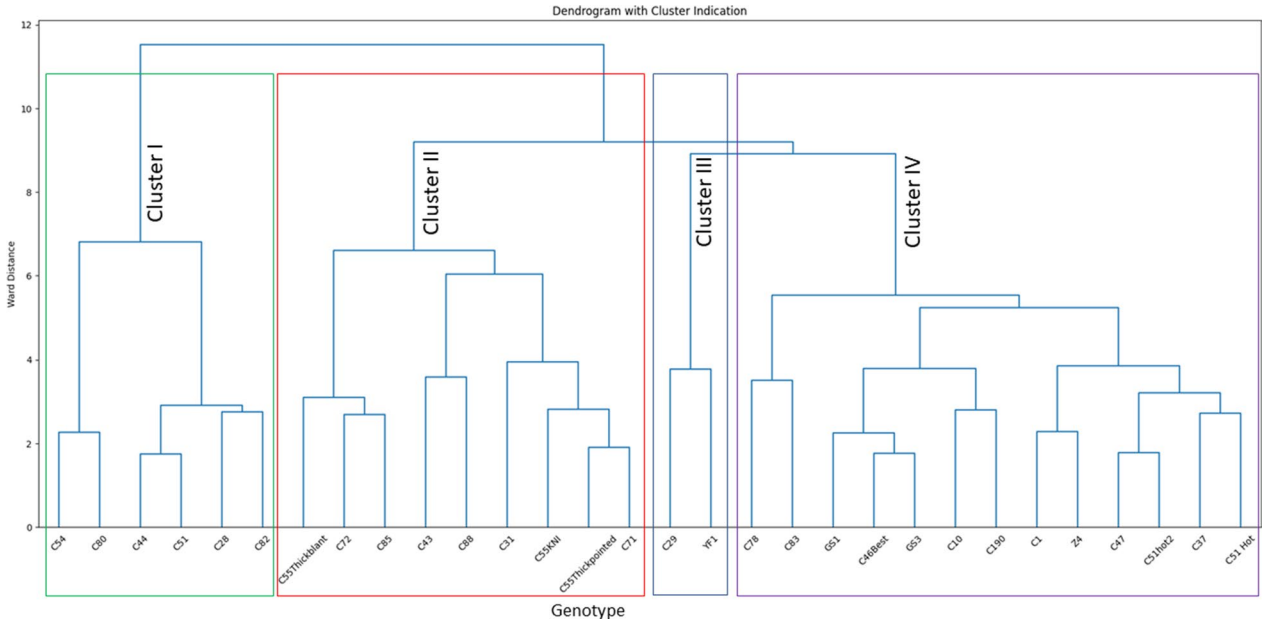

Fig. 3 Dendrogram based on 12 quantitative traits of 30 pepper genotypes

Table 4 Distribution of 30 pepper genotypes into four Wards-D clusters based on Euclidean distances

Cluster group	Number of genotypes	Genotype
Cluster I	6	C28, C44, C51, C54, C80, C82
Cluster II	9	C31, C43, C55KNI, C55Thickblant, C55Thickpointed, C71, C72, C85, C88
Cluster III	2	C29, YF1
Cluster IV	13	C1, C10, C190, C37, C46Best, C47, C51Hot, C51hot2, C78, C83, GS1, GS3, Z4

exhibited superior performance compared to the highest value in terms of flowering time (86.38 DAS), chlorophyll content (50.92 SPAD), and vitamin C content (23.61 mg/100 g). The mean plant height in this cluster was 41.75 cm. The measurements of this cluster's fruit length, fruit diameter, fresh fruit weight, dry fruit weight, and 1000-seed weight exhibited comparatively lesser values than other clusters. Table 4 displays thirteen distinct pepper genotypes that were categorized in Cluster IV. During the clustering analysis, the genotypes exhibited the highest average value (5.99 cm) for fruit length, as shown in Table 5. This group had the most minimal average days to flowering, with a value of 83.00 DAS. In a study, Belay et al. (2019) categorized 64 different types of hot pepper into seven distinct groups. They observed that genotypes with early flowering, fruiting, and maturity periods were primarily found in Cluster I, whereas

Table 5 Cluster mean values for twelve morpho-phytochemical characters of 30 pepper genotypes

Trait	Cluster			
	I	II	III	IV
Days to flowering	84.25 (I)	84.67 (I)	86.38 (H)	83.00 (L)
Plant height (cm)	40.14 (L)	55.07 (H)	41.75 (I)	50.11 (I)
Primary branches per plant	3.33 (L)	5.37 (H)	3.33 (L)	3.49 (I)
Secondary branches per plant	5.28 (L)	9.19 (H)	6.33 (I)	6.31 (I)
Tertiary branches per plant	11.11 (L)	20.78 (H)	12.67 (I)	15.44 (I)
Chlorophyll content (SPAD)	40.63 (I)	39.95 (L)	50.92 (H)	40.66 (I)
Fruit length (cm)	5.26 (I)	4.97 (I)	3.55 (L)	5.99 (H)
Fruit diameter (mm)	8.67 (H)	5.49 (I)	5.2 (L)	5.84 (I)
Fresh fruit weight (g)	11.75 (H)	6.57 (I)	3.57 (L)	7.63 (I)
Dry fruit weight (g)	0.92 (H)	0.49 (I)	0.35 (L)	0.59 (I)
1000- seed weight (g)	1.7 (I)	2.37 (H)	1.64 (L)	3.69 (I)
Vitamin-C content (mg/ 100 g)	4.91 (L)	8.28 (I)	23.61 (H)	8.02 (I)

Here, H, I and L represents high, intermediate and low values, respectively

Table 6 Intra (diagonal) and inter-cluster cluster distances of 30 pepper genotypes

Cluster	I	II	III	IV
I	2.36	5.85	6.45	4.51
II		2.7	6.01	4.49
III			1.89	5.58
IV				2.28

high-yield genotypes were predominantly present in Cluster III.

Diverse methodologies have been effectively employed to analyze genetic diversity and generate varied genotypes. The most prevalent and effective technique among them is the morphological and phytochemical characterization, which is widely utilized to evaluate genetic diversity in the majority of breeding programs (Phougat et al. 2017). The genotypes were grouped into several clusters and subclusters mostly based on physical distinctions rather than geographical proximity (Tanwar et al. 2023). Table 6 presented measurements of both intra-cluster and inter-cluster distances. The values that are highlighted in bold along the diagonal indicate the distances within each cluster, which demonstrate the level of variety within each cluster. From the intra-cluster distances, it is evident that Cluster III (1.89) has the lowest level of divergence, whereas Cluster I (2.36) exhibits the highest level of divergence. The remaining results illustrate the inter-cluster distances, which indicate the genetic divergence between pairs of clusters. The greatest inter-cluster distance is recorded between clusters I and III, measuring 6.45. Next in line is the proximity between clusters II and III, which measures 6.01. This is succeeded by the distance between clusters I and II, which is documented as 5.85. The clusters II and IV have the shortest inter-cluster distance of 4.49, indicating a close genetic relationship between them. Belay et al. (2019) discovered that the largest gap was observed between clusters III and VII, measuring 189.09 units. Conversely, the smallest gap was identified between clusters I and V, measuring 29.24 units. In their study, Sharma et al. (2017) examined nine different genotypes and found that the inter-cluster distance varied between 10.42 and 22.43. The study found that the clusters with the largest inter-cluster genetic difference were IV and VII, followed by I and III, as well as VII and VIII. Crossing genotypes from these clusters may produce a diverse range of segregating populations, as they show significant genetic diversity between them Belay et al. (2019). When considering hybridization, genotypes from clusters with larger inter-cluster distances, such as clusters I and III as well as II and III, might be beneficial. This suggested that there may be a chance to enhance genotypes by hybridizing using any two identified diverse clusters.

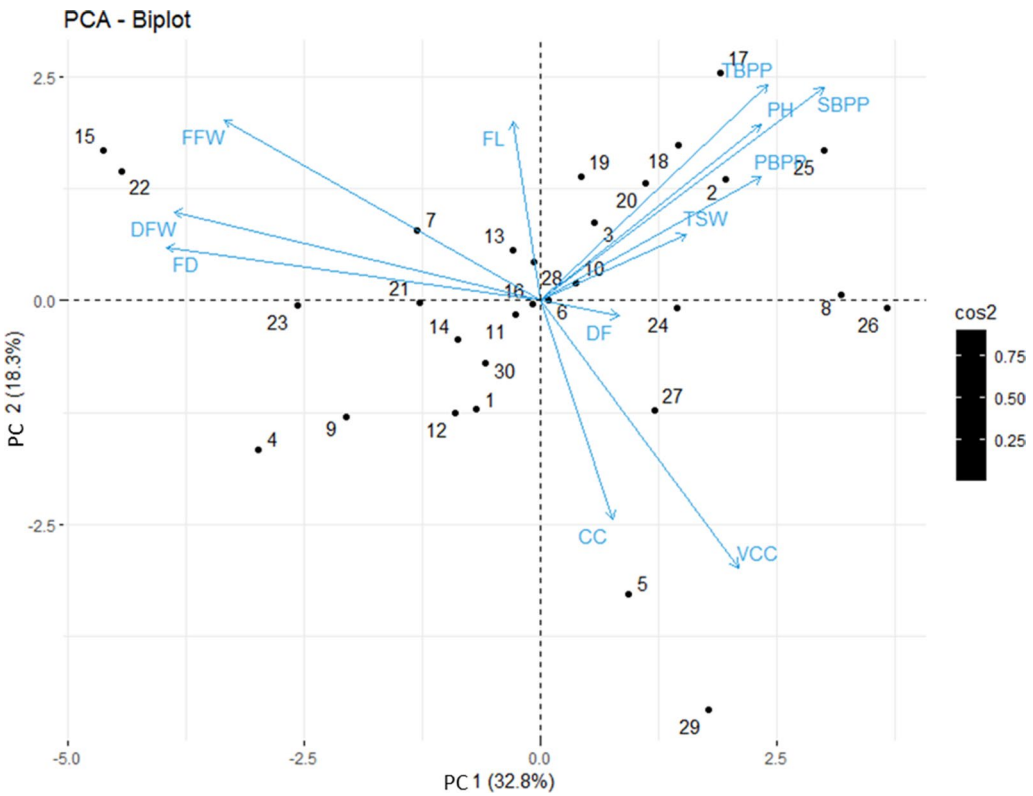

Fig. 4 PCA-biplot showing genotype × trait interactions. Here, *DF* Days to Flowering, *PH* Plant height (cm), *PBPP* Primary branches per plant, *SBPP* Secondary branches per plant, *TBPP* Tertiary branches per plant, *CC* Chlorophyll content (SPAD), *FL* Fruit length (cm), *FD* Fruit diameter (mm), *FFW* Fresh fruit weight (g), *DFW* Dry fruit weight (g), *TSW* 1000- seed weight (g), and *VCC* Vitamin-C content (mg/ 100 g). The serial number of genotypes are shown in Table 1

Biplot analysis

According to the biplot (Fig. 4), smaller angles between two adjacent vectors pointing in the same direction indicate a significant relationship between a trait and genotype identification, whereas an angle of 90^0 indicates no correlation. The first two axes (PC1 and PC2) of a principal component analysis (PCA) biplot depicting the relationships between factors and genotypes explained 32.8 and 18.3 percent, respectively, of the total variance. Moon et al. (2023) observed that the first (PC1) and second (PC2) principal component explained 32.8% and 18.3% of the total variance, respectively for 513 pepper accessions. Genotypes and traits that are spread out throughout the graph have a higher reproductive value compared to those that are located in the center. In this biplot, the superior genotypes were those with relatively high expression levels of favorable trait combinations. Figure 4 depicts the distribution of genotypes across all dimensions.

The traits primary, secondary, and tertiary branches per plant, plant height and 1000- seed weight showed positive loadings for both PC1 and PC2 axes. These traits were highly correlated with each other and the genotypes C10, C55Thickblant, C72 and C85 commonly favored these trait performances. The phytochemical traits chlorophyll content and vitamin-C content had positive PC scores for PC1 and negative for PC2 were highly correlated. So, the genotypes C29 and YF1 positively favored the phytochemical traits. Here, a small arrow observed for days to flowering that indicates lower correlations with genotypes. The genotypes C80 and C54 located near the arrowhead of the traits fruit diameter, dry fruit weight and fresh fruit weight. These traits are correlated and the genotypes were linked with the traits. According to the biplot analysis conducted by Akand et al. (2016), the optimal genotype is located near the arrowhead. According to their findings, G25 is located in cluster V and positively favored the traits fruit weight (g), fruit length (cm), and yield per plant (g). In our study, a clear co-segregation was revealed for major trait groups fruit, shoot and phytochemical parameters. Therefore, the outcome is logical and genotypes linked to different phenotypic traits should be taken consideration for further pepper breeding.

Conclusion

The 30 pepper genotypes exhibited noteworthy variability in days to flowering, plant height, fruit characteristics, and phytochemical parameters, with significant differences observed. The most significant correlations identified in between fresh fruit weight and dry fruit weight. Based on Ward's D-statistics, the 30 pepper genotypes were classified into four clusters. The largest inter-cluster distance was identified between clusters I and III. Crossing genotypes from these clusters would be beneficial for obtaining superior fruit architectures with greater chlorophyll and vitamin-C contents. Biplot analysis reveals that genotypes C54 and C80 from cluster I exhibit a preference for greater values in fresh fruit weight, dry fruit weight, and fruit diameter. The genotypes C29, YF1, C54, C80, C55Thickblant, C72, C85, and C31 exhibit higher performances and divergence in specific traits. These genotypes can be effectively utilized in future hybridization programs to improve the quality of peppers.

Supplementary Information

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Supplementary material 1.

Supplementary material 2.

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Author contributions

Conceptualization, M.A. and E.U.A.; Data collection and analysis, N.P., M.S.M. and S.J.; Original draft, N.P.; Review and editing, M.A., M.T.S.T., MAA, M.A.K. and E.U.A.; Funding acquisition, N.P. and R.I.A. All authors provided intellectual inputs, read the manuscript, approved for submission and agreed to the published version of the manuscript.

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Availability of data and materials

The availability of data and materials in this research is subject to the policy set by the corresponding author upon reasonable request.

Declarations

Competing interests

The authors declare that they have no competing interests exists.

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