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Genetic diversity study of bacterial wilt-tolerant bell pepper (*Capsicum annuum*) genotypes using cluster and principal component analysis

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ABSTRACT

The present study was carried out during summer and rainy (*kharif*) seasons of 2019 and 2020 at the research farm of Himachal Pradesh Krishi Vishvavidyalaya, Palampur, Himachal Pradesh to study the genetic diversity among 43 bacterial wilt tolerant genotypes of bell pepper (*Capsicum annuum* L. var. *grossum* Sendt), focusing on 12 quantitative and 3 qualitative traits along with disease resistance. The experiment was laid out in a randomized complete block design (RCBD) with 3 replications. Following hierarchical cluster analysis, the 43 unique genotypes were categorized into 5 separate clusters. Cluster I consisted of maximum number of genotypes (39), followed by clusters II, III, IV and V, each having only one genotype. The greatest inter-cluster distance was observed between cluster II and V (3090.70), while the smallest distance was noted between cluster II and III (1299.21). Cluster I displayed the greatest intra-cluster distance (803.75), while clusters II, III, IV and V showed the lowest distances (0.00). The primary contributors to genetic divergence were found to be capsanthin content (23.4%), followed by ascorbic acid (21.4%), fruit length (12.5%), plant survival (11.1%) and marketable fruit yield/plant (10.9%). Principal component analysis indicated that the initial six principal components, each possessing an eigen value surpassing one, jointly accounted for 70.62% of the total observed variability. PCA biplot revealed that, the traits namely, marketable fruits/plant, average fruit weight, harvest duration and plant height were the major yield contributing traits. The results underscored the importance of genetic characterization and the selection of diverse genotypes for hybridization, facilitating the development of improved bell pepper cultivars with enhanced productivity and quality.

Keywords: Bell pepper, Cluster, PCA, Quality traits, Yield

Bell pepper (*Capsicum annuum* L. var. *grossum* Sendt.; 2n=2x=24), a prominent member of the Solanaceae family, holds significant agricultural importance, particularly in India. Its cultivation extends throughout the country, thriving in hilly regions during the summer and serving as an autumn crop in eastern states West Bengal, Bihar and in southern states such as Tamil Nadu and Karnataka (Devi *et al.* 2015). In India, bell peppers are cultivated across approximately 37000 ha yielding a production output of 563000 metric tonnes (Anonymous 2021). The distinctive characteristics of bell pepper consist of blocky, square-shaped fruits with thick flesh, typically three to four lobes, and a lack of pungency. In every 100 g of fruit, bell peppers have a great nutritional composition that includes 20 kcal of energy, 4.6 g of carbohydrates, 1.7 g of fibre, 0.9 g of protein, 370 IU of vitamin A, 80.4 mg of vitamin C, 7.4 mg of vitamin K, 0.2 mg of vitamin B6, 0.3 mg of iron, 10 mg of magnesium, 20 mg of phosphorus and 175 mg of potassium (Devi *et al.* 2021 and Dhillon *et al.* 2022).

Breeding efforts aimed at enhancing the potential of bell pepper cultivars are still in their infancy, primarily due to a lack of research endeavors. Evaluating the genetic diversity within the existing gene pool is crucial for the development of improved and superior bell pepper cultivars. Moreover, bell pepper varieties released by the public sector have become obsolete, exhibiting poor yields and susceptibility to insect-pests and diseases (Rana *et al.* 2015). Thus, there is an urgent need to characterize the available germplasm to identify specific traits for cultivar development. While molecular markers provide accurate estimates of genetic divergence among accessions, understanding phenotypic traits through morphological and agronomical descriptors remains important. Acknowledging the importance of germplasm characterization as a crucial bridge between the conservation and utilization of plant genetic resources, this study seeks to assess genetic diversity in bell pepper germplasm through the analysis of morphological traits.

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MATERIALS AND METHODS

The experiment was conducted during summer and rainy (*kharif*) seasons of 2019 and 2020 at the research farm of Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur, Himachal Pradesh. A total of 43 bacterial wilt tolerant genotypes [including susceptible check (California Wonder), moderately resistant check (Kandaghat Selection) and two resistant checks (EC-464107 and EC-464115)] collection was utilized to assess genetic divergence (Supplementary Table 1). The experiment was laid out in a randomized complete block design (RCBD) with 3 replications. Seedlings aged 45-days were transplanted under open field conditions, with a spacing of 60 cm × 45 cm between each plant. Each genotype had two rows accommodating seven plants in each row. The recommended cultural practices and necessary measures for plant protection were meticulously adhered for ensuring optimal crop development. Observations were recorded from 10 randomly selected plants in each replication for a total of 12 morphological traits, including days to 50% flowering (DFF), days to first picking (DFP), plant height (PH) (cm), primary branches/plant (PBPP), pericarp thickness (PT) (mm), lobes/fruit (L/F), harvest duration (HD) (days), fruit length (FL) (cm), fruit width (FW) (cm), average fruit weight (AFW) (g), marketable fruits/plant (MF/P) and marketable fruit yield/plant (MFY/P) (g). Additionally, three biochemical parameters-capsanthin content (CC) (ASTA units), ascorbic acid (AA) (mg/100 g) and total soluble solids (TSS) (°Brix) were also measured. Procedure outlined in AOAC (1980) was used to quantify capsanthin content in ripened fruits. Handheld digital refractometer was used for determination of TSS content while volumetric method as described by Ranganna (1977) was employed for estimation of ascorbic acid content in freshly harvested fruits.

The classification of all the genotypes into different clusters was conducted utilizing the procedure developed by Mahalanobis (1936), initially suggested by Rao (1952). We computed the relative contributions of different variables to overall genetic divergence using the Singh and Choudhary (1985) methodology. Principal component analysis (PCA) was also performed using factoextra and GG biplot packages, as recommended by Jeffers (1967). The entire analysis was carried out using R software.

RESULTS AND DISCUSSION

The results indicate that all genotypes were grouped into five clusters (Supplementary Table 2) based on Mahalanobis D^2 statistics. Among these clusters, cluster I comprised 39 genotypes, namely G1-G17, G19-G31, G33-G40 and G42, while each of the next clusters–cluster II (G18), cluster III (G32), cluster IV (G41), and cluster V (G43) only had one genotype. The arrangement of genotypes into different clusters indicates that there was no correlation between clustering patterns and geographic diversity. The impact of genotype genetic makeup on the clustering pattern is revealed when genotypes with the same geographical distribution fall into various clusters (Rahevar *et al.* 2021). As a result, when choosing genotypes for hybridization, prioritizing genetic diversity over geographic origins appears more pertinent. This notion aligns with the results of Hasan *et al.* (2015) and Razzaq *et al.* (2016), noticed analogous outcomes by categorizing 30 and 25 genotypes into five clusters, respectively.

The distances between and within the clusters are helpful in understanding how different or similar the genotypes are to each other. This information makes it easier to pick out diverse genotypes that would work well together for hybridization. The largest inter-cluster distance in this investigation, reaching 3090.70 units, was found between clusters II and V. Subsequently, the second, third, and fourth highest inter-cluster distances were observed between cluster III and cluster IV (2607.02), cluster II and cluster IV (2570.49), and cluster I and cluster II (2216.48), respectively. By incorporating genotypes from these diverse clusters into hybridization programme, it is possible to develop desirable segregates. Beneficial genes might get accumulate over the generations which eventually results into improvement of various traits. On the other hand, cluster II and cluster III had the least inter-cluster distance (1299.21), indicating that the genotypes within these clusters were more genetically similar and showed less variability (Table 1). There appears to be significant variation across the genotypes as the inter-cluster distances exceeded the intra-cluster distances (Kumar *et al.* 2014). Specifically, cluster I, consisting of 39 genotypes, displayed the highest intra-cluster distance, indicating that these genotypes share a high degree of genetic similarity (Supplementary Fig. 1). In contrast, clusters II, III, IV, and V exhibited null intra-cluster distances, as they each contained only one genotype, signifying unique genetic variations that are not shared with other genotypes. This pattern aligns with findings reported by Devi *et al.* (2018) regarding inter- and intra-cluster distances in capsicum.

A wide range of variation for both quantitative and qualitative attributes was shown by the cluster mean (Table 2). Notably, cluster IV demonstrated outstanding performance, displaying the maximum mean values for PH (57.33 cm), PBPP plant (3.43), HD (43.00 days), FL (7.77 cm), AA content (110.99 mg/100 g), and PS rate (98.75%). Therefore, the genotypes in cluster IV would be valuable for enhancing the structural efficiency, quality, and extending the harvesting duration of disease-resistant bell pepper varieties. Further, cluster III was observed to encompass a desirable performance for HD (43.0 days), MF/P (12.80), MFY/P (390.30 g) and CC (149.53 ASTA units). Therefore, genotypes from this cluster could be utilized as a donor to introduce traits that contribute to higher yield in desirable genotypes. Following the above, cluster II showed highest mean for harvest duration (43.00 days), fruit width (4.37 cm), average fruit weight (46.08 g) and TSS (4.47 °Brix). Consequently, these genotypes hold promise for enhancing both yield and fruit quality. Cluster I and cluster V showed highest mean pericarp thickness (3.87 mm) and lobes/fruit (3.67), respectively. Therefore, the genotypes within these clusters could be harnessed to improve the shelf life and shape of bell pepper fruits. Cluster III and cluster IV showed the lowest mean scores for DFF (35.00 days) and DFP (54.00 days), respectively, in contrast to the highest mean values seen for most characters. This implies that in the near future, genotypes from these clusters might aid in the creation of early maturing cultivars. Comparable results about landrace diversity in bell pepper were reported by Misra *et al.* (2011) and Rana *et al.* (2015).

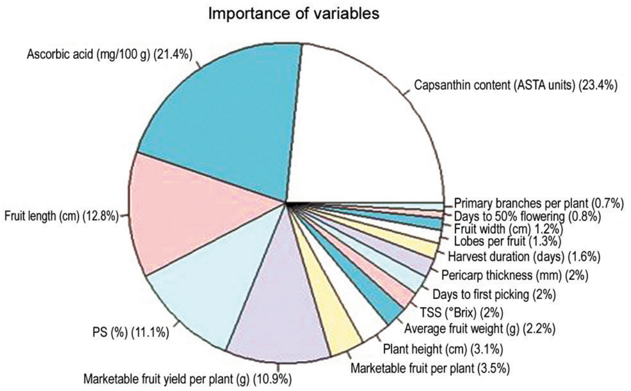

Fig. 1 Relative contribution (%) of individual traits to the genetic divergence among bell pepper genotypes.

PS, Percent survival percentage; TSS, Total soluble solids (ºBrix).

Table 2 and Fig. 1 illustrate the relative percentage contribution of each individual trait to the genetic divergence among the bell pepper genotypes studied. CC exhibited the highest contribution at 23.4%, followed by AA at 21.4%, FL at 12.5%, PS at 11.1%, MFY/P at 10.9%, MF/P at 3.5%, PH at 3.1%, AFW at 2.2%, DFP at 2%, PT at 2%, TSS at 2%, HD at 1.6%, L/F at 1.3%, and FW at 1.2%. For the purpose of heterosis breeding in bell peppers, choosing divergent parents based on these traits will be beneficial. The remaining traits, specifically DFF (0.8%) and PBPP (0.7%), contributed minimally to the total divergence among bell pepper genotypes. Earlier research by Farhad *et al.* (2010) also highlighted plant height's significant contribution towards genetic divergence. As a result, distinct genotypes showed divergence for different qualities based on cluster means, and these genotypes can be used as markers to choose different parents for particular traits in hybridization programmes.

Table 1 Average intra and inter cluster distance (D^2) among bell pepper genotypes

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
Cluster 1	803.75	2216.48	1463.97	1404.37	1453.90
Cluster 2		0	1299.21	2570.49	3090.70
Cluster 3			0	2607.02	1696.36
Cluster 4				0	1924.54
Cluster 5					0

When calculating the cluster mean, it is important to note that the presence of inferior or intermediate genotypes within the same cluster can diminish the perceived superiority of a genotype for a particular characteristic.

Table 2 Cluster means of different traits and relative contribution of individual traits to the genetic divergence among bell pepper genotypes

Character	I	II	III	IV	V	Mean	Maximum	Minimum	Contribution (%)
DFF	42.32	43.33	35.00	43.33	41.33	41.06	43.33	35.00	0.8 %
DFP	57.16	56.67	64.00	54.00	56.67	57.70	64.00	54.00	2.0 %
PH	53.28	45.01	49.25	57.33	44.87	49.95	57.33	44.87	3.1 %
PBPP	3.11	3.25	3.32	3.43	3.23	3.27	3.43	3.11	0.7 %
HD	41.68	43.00	43.00	43.00	17.67	37.67	43.00	17.67	1.6 %
FL	4.87	6.50	5.17	7.77	5.33	5.93	7.77	4.87	12.8 %
FW	4.30	4.37	4.22	3.55	3.96	4.08	4.37	3.55	1.2 %
PT	3.87	3.27	3.83	3.23	3.67	3.57	3.87	3.27	2.0 %
L/F	3.25	2.92	3.10	2.33	3.67	3.05	3.67	2.33	1.3 %
AFW	34.29	46.08	30.49	33.63	30.92	35.08	46.08	30.49	2.2 %
MF/P	9.94	7.60	12.80	8.70	2.90	8.39	12.80	2.90	3.5 %
MFY/P	338.46	350.33	390.3	292.72	90.32	292.43	390.30	90.32	10.9 %
CC	96.36	80.31	149.53	91.43	104.24	104.37	149.53	80.31	23.4 %
TSS	3.76	4.47	3.20	3.53	3.60	3.71	4.47	3.20	2.0 %
AA	96.63	84.65	109.18	110.99	109.18	102.13	110.99	84.65	21.4 %
PS	89.79	37.5	95.83	98.75	6.25	65.62	98.75	6.25	11.1 %

DFF, Days to 50% flowering; DFP, Days to first picking; PH, Plant height (cm); PBPP, primary branches/plant; HD, Harvest duration (days); FL, Fruit length (cm); FW, Fruit width (cm); PT, Pericarp thickness (mm); L/F, Lobes/fruit; AFW, Average fruit weight (g); MF/P, Marketable fruits per plant; MFY/P, Marketable fruit yield/plant (g); CC, Capsanthin content (ASTA units); TSS, Total soluble solids (ºBrix); AA, Ascorbic acid (mg/100 g); PS, Plant survival percentage.

Therefore, in addition to selecting genotypes from clusters with larger inter-cluster distances for hybridization, it is also advisable to choose parents based on the degree of divergence for specific traits. This approach ensures that the selected genotypes truly represent superior characteristics and contribute effectively for breeding programmes.

Principal component analysis (PCA) is a robust statistical technique used for dimensional reduction. It transforms a large set of correlated variables into a smaller set of uncorrelated variables called principal components, which capture the most significant variance in the data. By focusing on these principal components, PCA retains the essential patterns and structures within the dataset, thereby simplifying the analysis while preserving most of the original information. The eigen values and variations explained by each of the principal components are presented in screen plot (Fig. 2 and Table 3). The PCA's findings clarified that the initial six principal components (PCs) had eigen values greater than one and accounted for a total of 70.62% of the variance, playing a crucial role in depicting the patterns of genetic variations among breeding materials. The components with eigen value less than 1 are less informative and account for lesser variance and hence not included in the study. Janaki *et al.* (2015) also reported six major principal components with eigen values more than 1 in their respective studies. With an eigen value of 2.93, the first principal component (PC1) provided the explanation for 18.30% of the total variation which was mostly due to DFP (0.30), FL (0.30), TSS (0.21) and DFF (0.19). PC2 accounted for 15.25% variation and was attributed mainly due to L/F (0.70), PT (0.66), FW (0.53), AA (0.20) and CC (0.13). PC3 contributed 11.46% of the total variance maximum through AFW (0.66), FW (0.65), DFF (0.33), TSS (0.30), L/F (0.20) and PH (0.17). PC4 accounted 9.52% of the total variance chiefly through PBPP (0.60), PH (0.44), DFP (0.33) and MF/P (0.30). PC5 contributed 8.58% of variation primarily through DFF (0.63), DFP (0.51), PH (0.32), HD (0.26), TSS (0.25), AA (0.17), PT (0.16) and CC (0.15). PC6 accounted for 7.31% variation that was attributed mainly by DFP (0.40), PS (0.18) and PT (0.16). Following PT, AFW, FW, DFF, PBPP, DFP, and PH, the observation that L/F produced the highest positive value across all principal components suggests their significant contribution to the variability within the dataset. These traits can indeed serve as effective criteria for selecting parental lines aimed at yield improvement and other breeding objectives.

The biplot drawn between PC1 and PC2, which collectively explain 33.50% of the variance, emphasized the importance of different genotypes and morphological features in explaining the variation between accessions and comprehending species relationships (Fig. 3). The scatter diagram revealed that the majority of genotypes were distinct, as they were positioned in different corners of the biplot. This dispersion underscores the diversity present within the dataset and emphasizes the unique characteristics exhibited by individual genotypes, thereby providing valuable insights for further analysis and selection

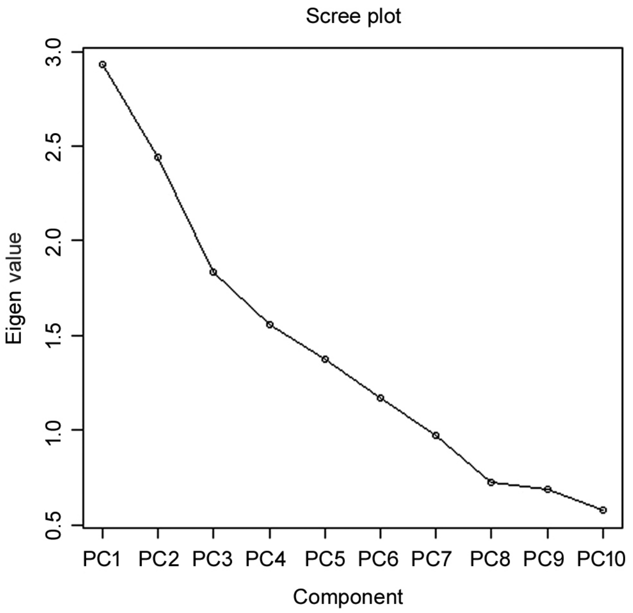

Fig. 2 Scree plot showing eigen values variation.

Table 3 Principal component analysis for variables

	PC1	PC2	PC3	PC4	PC5	PC6
Eigen value	2.93	2.44	1.83	1.56	1.37	1.17
Variability (%)	18.30	15.25	11.46	9.72	8.58	7.31
Cumulative variability (%)	18.30	33.55	45.00	54.73	63.30	70.62
Variable			Eigen vector			
DFF	0.19	-0.01	0.33	-0.13	0.63	-0.25
DFP	0.30	0.04	-0.05	0.33	0.51	0.40
PH	-0.17	-0.37	0.17	0.44	0.32	-0.47
PBPP	0.04	0.02	-0.13	0.60	-0.38	-0.45
HD	-0.77	-0.23	0.07	-0.17	0.26	0.01
FL	0.30	-0.78	-0.26	-0.23	-0.10	0.04
FW	-0.27	0.53	0.65	0.10	-0.05	0.02
PT	-0.08	0.66	-0.41	-0.15	0.16	0.16
L/F	0.19	0.70	0.20	0.22	0.07	-0.13
AFW	-0.13	-0.11	0.66	-0.54	-0.23	0.01
MF/P	-0.84	-0.09	-0.19	0.30	-0.05	0.03
MFY/P	-0.90	-0.12	0.11	0.03	-0.18	0.04
CC	-0.14	0.13	-0.61	-0.26	0.15	-0.28
TSS	0.21	-0.57	0.30	0.17	0.25	0.02
AA	-0.06	0.20	-0.09	-0.48	0.17	-0.61
PS	-0.60	0.01	-0.15	0.01	0.40	0.18

DFF, Days to 50% flowering; DFP, Days to first picking; PH, Plant height (cm); PBPP, primary branches/plant; HD, Harvest duration (days); FL, Fruit length (cm); FW, Fruit width (cm); PT, Pericarp thickness (mm); L/F, Lobes/fruit; AFW, Average fruit weight (g); MF/P, Marketable fruits per plant; MFY/P, Marketable fruit yield/plant (g); CC, Capsanthin content (ASTA units); TSS, Total soluble solids (ºBrix); AA, Ascorbic acid (mg/100 g); PS, Plant survival percentage

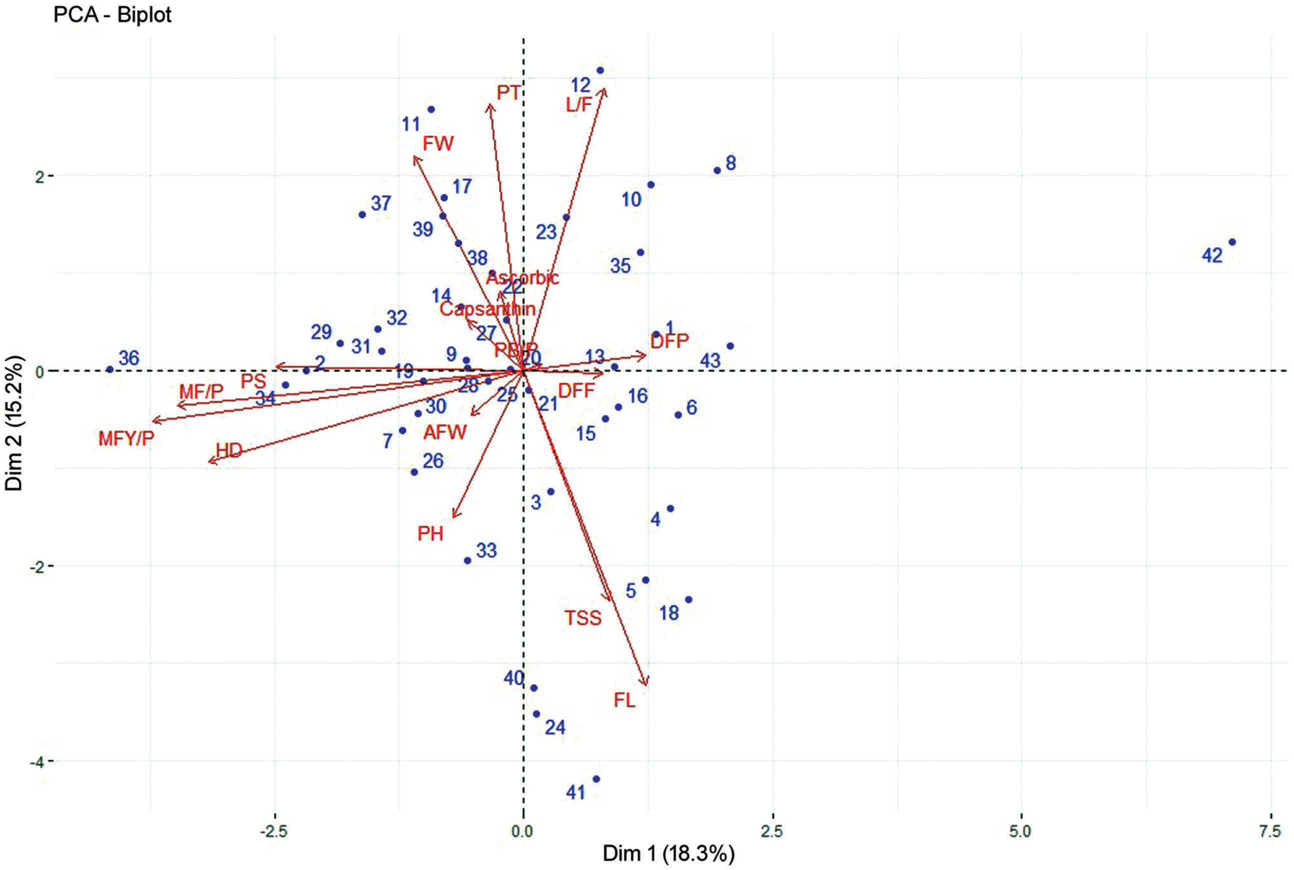

Fig. 3 PCA biplot for PC1 and PC2.

DFF, Days to 50% flowering; DFP, Days to first picking; PH, Plant height (cm); PBPP, primary branches/plant; HD, Harvest duration (days); FL, Fruit length (cm); FW, Fruit width (cm); PT, Pericarp thickness (mm); L/F, Lobes/fruit; AFW, Average fruit weight (g); MF/P, Marketable fruits per plant; MFY/P, Marketable fruit yield/plant (g); CC, Capsanthin content (ASTA units); TSS, Total soluble solids (ºBrix); AA, Ascorbic acid (mg/100 g); PS, Plant survival percentage

in breeding programmes. Genotypes with yields higher than the mean were G23, G12, G10, G8, G35, G13, G1, G43, G21, G15, G16, G6, G3, G4, G5, G18, G24, G40, G41, and G42, whereas genotypes G22, G27, G38, G17, G11, G37, G39, G9, G32, G31, G29, G19, G2, G36, G34, G28, G30, G7, G26, and G33 performed poorer than the average. Among all genotypes, G42 and G36 were poles apart in their performance and these were highest and lowest yielding genotypes, respectively. The angle between the vectors indicates the correlation of the characteristics with each other. When the two vectors are near to one another tends to forms an acute angle (less than 90°), the variables are found to be correlated in positive manner. When the angle between the vectors is at 90°, the variables are not correlated. Similarly, when the vectors are much diverged at a larger angle (close to 180°), the variables are negatively correlated. FW exhibited a near-perfect negative correlation with FL. Similarly, PH and FW were negatively correlated, and AFW showed no correlation with either FW or FL. FL displayed an almost perfect positive correlation with TSS. The biplot indicated that PT had a negative correlation with FL but a positive correlation with FW. AA and CC were found to be positively correlated with FW and negatively correlated with both FL and TSS. The MFY/P was positively correlated with MF/P, AFW, HD, and PH, whereas it was negatively correlated with PBPP, DFF, and DFP.

This study highlights the genetic diversity and distinct clustering patterns among 43 bacterial wilt-tolerant bell pepper genotypes, demonstrating the minimal impact of geographic origin on genetic diversity. The significant heterogeneity observed across various morphological and biochemical traits underscores the necessity of thorough genotype characterization. Key traits identified through PCA, such as marketable fruits/plant, average fruit weight, harvest duration, and plant height, serve as vital markers for selecting desirable genotypes in future breeding programmes. Incorporating diverse genotypes from different clusters into hybridization programmes can foster the development of superior cultivars with enhanced yield and quality. This comprehensive understanding of genetic diversity and yield-contributing traits provides a foundation for targeted breeding strategies, aiming to improve bell pepper varieties for better agricultural productivity and resilience.

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