





Assessment of genetic variability parameters and trait association in chilli (*Capsicum annuum* L.)

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Abstract

A study conducted during the 2022–2023 season evaluated 15 chilli genotypes, including one check variety, to identify those with superior yield and quality traits. The genotypes exhibited significant genetic variability, indicating broad genetic diversity across morphological and yield parameters. Traits such as fruit width (98.77 %), ascorbic acid content (99.83 %) and fruits per plant (99.83 %) showed high heritability and genetic advance, underscoring the predominance of additive genetic effects and highlighting their potential for improvement through selection. Correlation analysis revealed strong positive associations between fruit yield per plot and traits such as fruit weight (0.805 and 0.711), fruit length (0.593 and 0.462) and fruit width (0.676 and 0.572), emphasizing their collective contribution to yield. Path coefficient analysis showed significant direct effects of traits like fruit width, fruit weight and hundred seed weight on fruit yield. Additionally, principal component analysis (PCA) revealed that the first component was the most influential in explaining the variance. This study stands out by providing detailed insights into the genetic variability of chilli genotypes, offering a more comprehensive understanding compared to previous research. These findings have essential breeding implications, specifically recommending that breeders focus on traits with high heritability and genetic advance, such as fruit width, fruit weight and ascorbic acid content, to enhance yield and quality. Breeding programs should prioritize these traits for selection to develop high-yielding, nutrient-rich chilli cultivars with improved resilience and consistency. This approach will help accelerate the development of improved chilli varieties, benefiting both commercial production and consumer health.

Keywords: additive; breeding; correlation; genotypes; path analysis; yield

Introduction

Chilli (Capsicum annuum) is an essential vegetable and spice crop, valued for its heat, flavour and significant nutritional content. It plays a crucial role in global cuisines, agriculture and medicinal applications, making it economically scientifically important. This research explores its cultivation, genetic diversity and potential applications, highlighting its significance in global food systems and local economies (1). In India, chilli contributes substantially to both domestic consumption and export markets (2). Historically, chilli was domesticated around 5000 BC in Latin America, with Mexico and Guatemala recognized as the primary centers of origin. Chilli is rich in L-ascorbic acid and it also contains vital nutrients such as thiamine, magnesium, iron, potassium and calcium, making it a valuable dietary inclusion (3). Its pungency is primarily due to capsaicin, a volatile crystalline alkaloid found in the fruit's placenta. Chilli thrives under frost-free conditions, with optimal temperatures ranging between 15 to 35 °C and a growth period of 130-150 days (4).

Understanding genetic variability and its impact on crop performance is crucial for enhancing chilli breeding. Traits exhibiting high heritability and genetic advance are especially effective for selection, which is why they are considered key targets for crop improvement programs (5). Genetic variation plays a pivotal role in determining the efficacy of breeding programs by revealing the potential for improvement in both yield and quality. Correlation and path analyses are critical for understanding the relationships between traits and their direct or indirect effects on yield, providing valuable insights for selecting optimal genotypes (6). In this context, this study aims to identify the critical factors influencing chilli yield and quality by evaluating genetic diversity, heritability and genetic advance, as well as the interrelationships between traits through correlation, path analysis and principal component analysis (7). These methodologies will enhance our understanding of how genetic traits influence chilli performance, facilitating more informed and effective breeding decisions.

Materials and Methods

The experiment was conducted at the Department of Vegetable Science, College of Horticulture and Forestry, Neri, Hamirpur, during the summer season of 2022-23. Fifteen chilli genotypes, including one check variety, were evaluated for various horticultural traits, with three replications. Each replication consisted of 10 plants, ensuring a sufficient sample size for robust data analysis. The plants were transplanted in a Randomized Complete Block Design (RCBD), with planting space of 45 cm × 45 cm and plot size of 1.25 m × 1.25 m. The soil used was loamy, well-drained, with a pH of 6.5 and an organic matter content of 1.2 %. Fertilizer application followed the standard procedure for chilli cultivation, including 50 kg/ha of nitrogen, 40 kg/ha of phosphorus and 30 kg/ha of potassium. Additionally, micronutrient applications were made based on the soil test recommendations. Irrigation was provided through drip irrigation, ensuring a uniform water supply to the plants. The temperature during the growing season ranged from 18 °C to 35 °C, with an average rainfall of 600 mm distributed throughout the season. These agronomic conditions provided optimal growth conditions for the chilli genotypes.

Traits evaluated

Thirteen different yield-attributing traits and biochemical parameters were documented. The traits contributing to yield were days to 50 % flowering, number of primary branches, number of secondary branches, number of fruits per plant, fruit weight (g), fruit length (cm), fruit width (cm), stem girth (cm), plant height (cm), hundred seed weight (g), ascorbic acid content, capsaicin content (mg/100 g) and fruit yield per plot (kg).

Statistical analysis

The analysis of variance (ANOVA), phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV), heritability in a broad sense, genetic advance as a percentage of the mean, correlation coefficients and path coefficient analysis were calculated using R-based tool (7-12). The principal component analysis was carried out using the correlation matrix method (13).

Results and Discussion

The violin plot presented provides a comprehensive visualization of the distribution of 12 key agronomic and biochemical traits in chilli peppers, highlighting both variability and consistency across the samples. Traits such as fruit yield per plot (kg), capsaicin content (mg/100 g) and fruit weight (g) exhibit narrow distributions, indicating a high degree of consistency across chilli pepper genotypes (Fig. 1). This suggests that these traits are less influenced by environmental conditions or genetic variations, making them reliable targets for breeding programs aiming at uniformity. On the other hand, traits such as ascorbic acid content (mg/100 g) and fruits per plant show broader distributions, revealing significant variability within the samples. The broad range for ascorbic acid indicates substantial differences in vitamin C content, which both genetic variation and environmental factors could influence. This variability in ascorbic acid content is biologically significant as it suggests a potential for selecting genotypes with higher vitamin C content for improved nutritional value. Similarly, the variability in fruit production per plant highlights

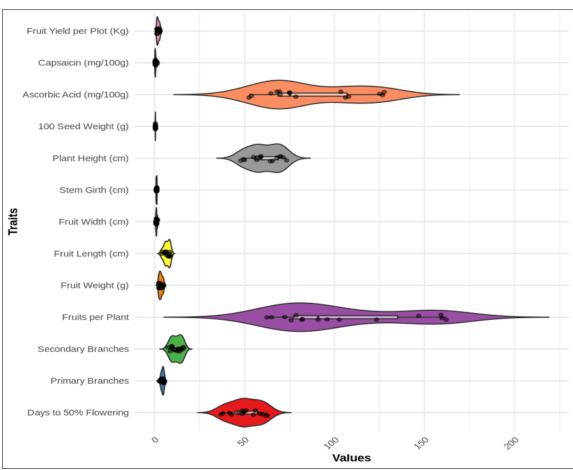


Fig. 1. The violin plot for different traits in chilli.

differences in plant vigour or reproductive success, which are critical factors for improving yield. Traits like 100 seed weight, plant height and days to 50 % flowering exhibit moderate variability with tighter clustering, indicating that these traits are more consistently expressed across genotypes. The violin plot effectively distinguishes stable traits from those with considerable variability, providing valuable insights for breeding programs aimed at enhancing specific traits in chilli peppers. This evenness is important for developing varieties with predictable growth and flowering patterns, which is crucial for large-scale cultivation and commercial farming.

Estimation of variability and its components

In terms of genetic variability, assessing heritable components is a critical factor in crop improvement programs. Genetic variability is crucial for identifying superior genotypes that can yield high-quality progeny. In this study, traits with the highest phenotypic and genetic coefficients of variation (PCV and GCV) (Table 1) include fruit width (30.64 % and 30.45 %), fruit yield per plot (35.27 % and 30.14 %), ascorbic acid content (30.56 % and 30.53 %), fruits per plant (35.07 % and 35.04 %) and capsaicin content (41.68 % and 35.83 %). These traits demonstrate high variability, which is crucial for breeding efforts aimed at improving these characteristics. For example, the high variability in fruit yield per plot (35.27 %) and capsaicin content (41.68 %) supports the notion that these traits are more likely to respond positively to selection in breeding programs. Conversely, traits like plant height (13.91 % and 13.75 %) and hundred-seed weight (11.79 % and 11.13 %) exhibit low PCV and GCV, suggesting that they are genetically stable and may not provide significant improvements through selection-based breeding programs. Notably, the PCV values for all traits exceed the GCV, indicating the prominent influence of environmental factors on phenotypic expression. However, the small difference between GCV and PCV suggests that environmental factors have a minimal impact on the expression of these traits. This is consistent with previous studies (which highlight that for traits with low variability, breeding improvements may be limited (14-16).

Heritability and genetic advance

Heritability is another crucial aspect in selecting genotypes for breeding programs. Traits with high heritability are considered ideal candidates for further selection, as they are more likely to pass their traits on to future generations. In this study, most traits exhibited high heritability, ranging from 43.38 % to 99.83 % (Table 1), indicating a substantial genetic contribution to trait expression. For instance, the high heritability of ascorbic acid content (99.83 %) and fruits per plant (99.83 %) suggests that these traits are strongly influenced by genetic factors, making them ideal for selection in breeding programs aimed at improving nutritional value and yield. The traits with the highest heritability include ascorbic acid content (99.83 %), fruits per plant (99.83 %), fruit width (98.77 %), days to 50 % flowering (98.26 %), plant height (97.81 %), fruit length (95.64 %), hundred seed weight (89.16 %), number of secondary branches (88.72 %), number of primary branches (88.46 %) and fruit weight (88.36 %). These results align with previous studies revealing high heritability (17). Capsaicin content (73.90 %) and fruit yield per plot (73.05 %) had moderate heritability, confirming the results of earlier studies (18). The traits, namely, fruits per plant (72.12 %), capsaicin content (63.45 %), ascorbic acid content (62.84 %), fruit width (62.33 %) and fruit yield per plot (53.07 %) showed high genetic advance. These findings are consistent with those of previous studies on chilli, which emphasize the importance of genetic factors in determining breeding outcomes (19-21).

Correlation analysis

The associations between different traits of chilli genotypes were examined and illustrated in Fig. 2 & 3. Genotypic correlation coefficients had superior values than phenotypic correlation coefficients. A substantial positive correlation was observed both at the genotypic and phenotypic levels for yield per plot with traits, namely fruit length (0.593 and 0.462), number of secondary branches (0.508 and 0.446), fruit weight (0.805 and 0.711), hundred-seed weight (0.644 and 0.522) and fruit width (0.676 and 0.572). Traits *viz.*, fruit width, fruit weight and fruit length had a positive association with yield per plot,

Table 1. Estimation of phenotypic and genotypic coefficients of variation, heritability and genetic advance as percent of mean for various traits in chilli

	Coefficient of variation (%)							
Characters	Mean	Range		- Phenotypic	-	Heritability (%)	Genetic	
		Maximum	Minimum				advance as % mean	
Days to 50 % flowering	50.56	37.00	62.67	16.66	16.51	98.26	33.72	
Number of primary branches	4.41	2.58	5.58	19.17	18.03	88.46	34.94	
Number of secondary branches	12.23	7.43	16.31	24.16	22.76	88.72	44.16	
Number of fruits per plant	103.92	62.47	161.97	35.07	35.04	99.83	72.12	
Fruit weight (g)	3.46	2.21	4.88	27.12	25.49	88.36	49.36	
Fruit length (cm)	6.91	4.15	8.74	22.97	22.46	95.64	45.25	
Fruit width (cm)	1.08	0.60	1.62	30.64	30.45	98.77	62.33	
Stem girth (cm)	1.13	0.89	1.38	19.87	13.09	43.38	17.76	
Plant height (cm)	61.11	47.88	73.37	13.91	13.75	97.81	28.02	
Hundred seed weight (g)	0.454	0.363	0.537	11.79	11.13	89.16	21.65	
Ascorbic acid (mg/100g)	86.91	52.44	127.64	30.56	30.53	99.83	62.84	
Capsaicin content (mg/100g)	0.95	0.21	1.47	41.68	35.83	73.90	63.45	
Fruit yield per plot (kg)	2.04	1.27	3.19	35.27	30.14	73.05	53.07	

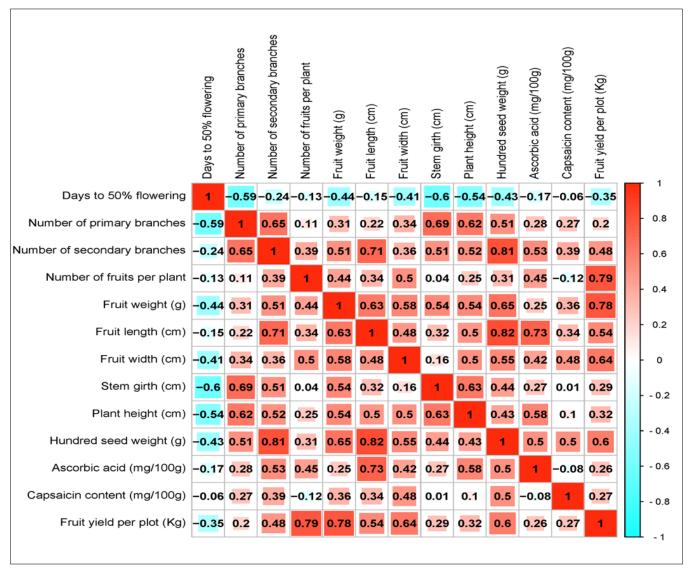


Fig. 2. Estimates of phenotypic (P) and genotypic (G) correlation coefficients for different traits in chilli.

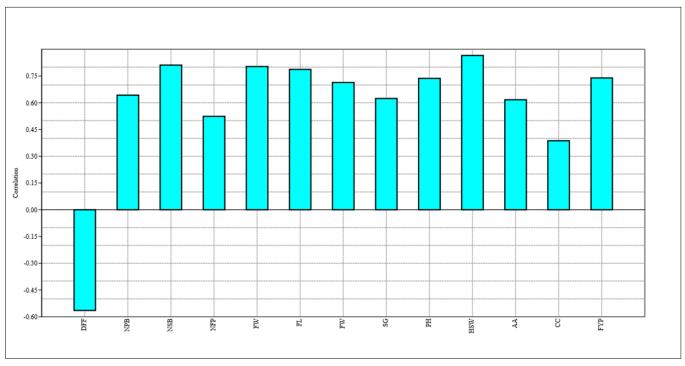


Fig. 3. Correlation among different horticultural traits in chilli. DFF=Days to 50 % flowering; NPB=Number of primary branches; NSB=Number of secondary branches; NFP=Number of fruits per plant; FW=Fruit weight (g); FL=Fruit length (cm); FW=Fruit width (cm); SG=Stem girth (cm); PH=Plant height (cm); HSW=Hundred seed weight (g); AA=Ascorbic acid content (mg/100g); CC=Capsaicin content (mg/100g); FYP=Fruit yield per plot (kg).

whereas a negative correlation was reflected for days to 50 % flowering (-0.367 and -0.304) with yield. Early flowering could lead to lower yield due to reduced time for fruit development, which hinders the plant's overall productivity. These findings suggest that optimizing such traits can notably enhance the overall yield per plant (22-27).

Path coefficient analysis

The path coefficient analysis findings are illustrated in Table 2. It helps enhance crop improvement programs by pinpointing essential yield-contributing traits and offering accurate insights to implement indirect selection strategies efficiently. Hundred seed weight (1.727) had the most significant positive direct effect on fruit yield per plot, followed by fruit width (0.704), number of secondary branches (0.459), number of fruits per plant (0.346), days to 50 % flowering (0.181) and plant height (0.157). Breeders should focus on traits such as fruit weight, but the selection process must also consider the balance between fruit width and weight to optimize yield. Focusing on fruit weight alone might not be sufficient, as fruit width also plays a critical role in yield optimization. All these outcomes are in close compliance with other studies (28-31).

Principal component analysis (PCA)

Principal Component Analysis (PCA) is a valuable technique that provides a clear understanding of complex multi-trait

relationships between variables by reducing the dimensionality of a dataset. PCA helps group traits based on similarity and importance, thereby reducing complexity in the selection process. PCA was performed on the genotypes across all evaluated traits and thirteen principal components were identified, as presented in Table 3. The eigenvalue cutoff (> 1) is commonly used in PCA because components with eigenvalues greater than one account for a significant portion of the variance, while those with eigenvalues less than one are considered to contribute less to the overall data structure and are often discarded in order to reduce complexity. PC1 had eigenvalues exceeding one and accounted for a cumulative variance of 80.81 % (Table 4). PC1 imparted maximum variability at 25.99 %, followed by PC2 at 20.81 %, PC3 (12.94 %), PC4 (11.34 %) and PC5 (9.73 %). The variation of various principal components was plotted on a screen plot and is shown in Fig. 4. The variability decreases with each successive principal component.

Similarly, an examination of the factor loadings for principal components revealed distinct patterns. The PCA biplot effectively visualizes the relationships between different chilli genotypes and traits, capturing 61.9 % of the overall variation through PC1 and PC2. Traits such as fruits per plant, fruit length and fruit yield per plot, represented by longer arrows, significantly contribute to the variation in these genotypes, indicating that they play a significant role in distinguishing

Table 2. Genotypic path coefficients analysis for direct and indirect effects of component characters on fruit yield per plot in chilli

Traits	DFF	NPB	NSB	NFP	FW	FL	FWd	SG	PH	HSW	AA	СС	FYP
DFF	0.181	0.283	-0.114	-0.045	0.005	0.169	-0.290	0.206	-0.084	-0.798	0.110	0.010	-0.367 [*]
NPB	-0.110	-0.468	0.311	0.037	-0.004	-0.259	0.250	-0.238	0.100	0.920	-0.183	-0.132	0.225
NSB	-0.045	-0.317	0.459	0.139	-0.006	-0.840	0.261	-0.176	0.083	1.409	-0.347	-0.112	0.508**
NFP	-0.024	-0.050	0.184	0.346	-0.005	-0.393	0.352	-0.012	0.039	0.533	-0.289	0.151	0.832**
FW	-0.079	-0.150	0.237	0.158	-0.012	-0.737	0.404	-0.186	0.087	1.155	-0.175	0.103	0.805**
FWd	-0.027	-0.107	0.340	0.120	-0.008	-1.135	0.342	-0.106	0.079	1.455	-0.474	0.112	0.593**
FL	-0.075	-0.166	0.170	0.173	-0.007	-0.551	0.704	-0.055	0.079	0.966	-0.270	-0.292	0.676**
SG	-0.131	-0.391	0.283	0.015	-0.008	-0.420	0.136	-0.286	0.117	1.003	-0.211	0.241	0.348*
PH	-0.098	-0.298	0.243	0.086	-0.007	-0.575	0.355	-0.213	0.157	0.739	-0.373	0.325	0.341*
HSW	-0.084	-0.249	0.375	0.107	-0.008	-0.956	0.394	-0.166	0.067	1.727	-0.326	-0.237	0.644**
AA	-0.031	-0.133	0.247	0.155	-0.003	-0.834	0.295	-0.094	0.091	0.874	-0.645	0.351	0.274
СС	-0.002	-0.068	0.057	-0.058	0.001	0.139	0.226	0.076	-0.056	0.450	0.249	-0.909	0.105

^{*, **} Significant at 5 and 1 % levels respectively. Residual effect= 0.06285. DFF=Days to 50 % flowering; NPB=Number of primary branches; NSB=Number of secondary branches; NFP=Number of fruits per plant; FW=Fruit weight (g); FL=Fruit length (cm); FWd=Fruit width (cm); SG=Stem girth (cm); PH=Plant height (cm); HSW=Hundred seed weight (g); AA=Ascorbic acid content (mg/100g); CC=Capsaicin content (mg/100g); FYP=Fruit yield per plot (kg)

Table 3. Eigenvalues, percentage of variance and cumulative variance associated with the principal components analyzed for various traits in chilli

Sr. No.	Principal components	Eigenvalue	Percent variance	Cumulative variance
1	PC1	6.1949	47.6530	47.65
2	PC2	1.8473	14.2100	61.86
3	PC3	1.4017	10.7820	72.65
4	PC4	1.2374	9.5186	82.16
5	PC5	0.7643	5.8793	88.04
6	PC6	0.6477	4.9822	93.03
7	PC7	0.4451	3.4241	96.45
8	PC8	0.1874	1.4413	97.89
9	PC9	0.1036	0.7969	98.69
10	PC10	0.0764	0.5880	99.28
11	PC11	0.0652	0.5015	99.78
12	PC12	0.0288	0.2215	100.00
13	PC13	0.0002	0.0017	100.00

Table 4. Factor loadings of various principal components for various traits in chilli

Traits	PC 1	PC 2	PC 3	PC 4
DFF	-0.227	0.371	0.058	0.397
NPB	0.258	-0.442	0.072	0.011
NSB	0.326	-0.022	0.082	0.322
NFP	0.210	0.399	-0.404	-0.246
FW	0.323	0.093	0.084	-0.273
FL	0.316	0.215	0.015	0.392
FW	0.287	0.206	0.106	-0.236
SG	0.251	-0.461	-0.104	-0.068
PH	0.296	-0.260	-0.209	0.046
HSW	0.348	0.060	0.217	0.181
AA	0.248	0.093	-0.402	0.459
cc	0.156	0.103	0.734	0.034
FYP	0.297	0.327	-0.039	-0.380

DFF=Days to 50 % flowering; NPB=Number of primary branches; NSB=Number of secondary branches; NFP=Number of fruits per plant; FW=Fruit weight (g); FL=Fruit length (cm); FWd=Fruit width (cm); SG=Stem girth (cm); PH=Plant height (cm); HSW=Hundred seed weight (g); AA=Ascorbic acid content (mg/100g); CC=Capsaicin content (mg/100g); FYP=Fruit yield per plot (kg)

between them. This clustering of fruit yield, fruit weight and fruit length on the biplot is likely because these traits are closely related in terms of their genetic basis and phenotypic expression; for example, larger fruits typically result in higher yields, so they exhibit similar patterns in genotype differentiation. In contrast,

traits like plant height (cm) and stem girth (cm) are clustered near the origin, suggesting they contribute less to genotype differentiation in this analysis. The genotypes are spread across the plot, with some, like EC-362911 and IC-265003, positioned far from the origin, indicating that they exhibit extreme values. Genotypes like Him Palam Mirch-2 and Him Palam Mirch-1 are closely grouped, suggesting similar trait profiles. Similar results were also found from the previous research (32-34).

Scree plot

The variance of every principal component (PC) is illustrated using scree plot in the analysis of chilli genotypes, offering insights into the key traits driving genetic diversity (Fig. 5). PC1 explains the most significant proportion of variance at 47.65, followed by PC2 (14.21), PC3 (10.78) and PC4 (9.52). The sharp decline in variance across components suggests that the first few principal components (PCs) capture the majority of the variability, while the later components contribute minimally to the overall variance. As the principal components progress, traits like fresh weight (FW), plant height (PH), head seed weight (HSW) and ascorbic acid content (AA) contribute less variance, reflecting their relatively lower impact on genetic differentiation. The cumulative variance explained by the PCs highlights the importance of focusing on the traits that contribute heavily to the first few components. These traits are critical for breeding and selection programs, as they capture the majority of the genetic diversity within the studied genotypes and are likely to play a key role in improving chilli yield and other desirable characteristics. These results are in accordance with previous studies (35-37).

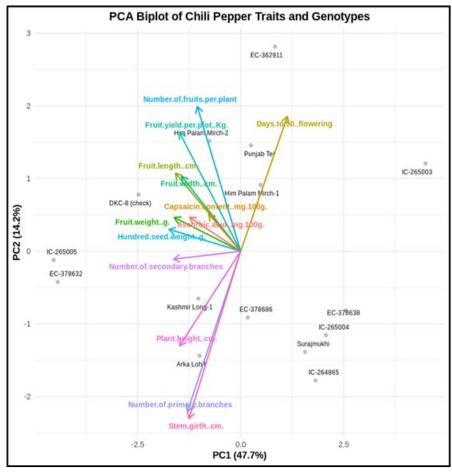


Fig. 4. Principal component biplot for first two principal components.

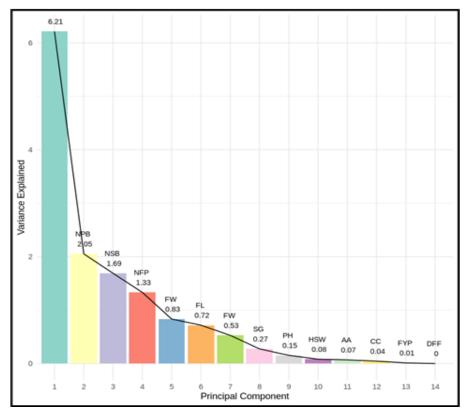


Fig. 5. Scree plot illustrating variance distribution across principal components for different traits. DFF=Days to 50 % flowering; NPB=Number of primary branches; NSB=Number of secondary branches; NFP=Number of fruits per plant; FW=Fruit weight (g); FL=Fruit length (cm); FWd=Fruit width (cm); SG=Stem girth (cm); PH=Plant height (cm); HSW=Hundred seed weight (g); AA=Ascorbic acid content (mg/100g); CC=Capsaicin content (mg/100g); FYP=Fruit yield per plot (kg).

Conclusion

This study highlights the significant genetic variability and heritability of various traits in chilli genotypes, with key characteristics such as ascorbic acid content, fruit width and fruits per plant showing high heritability and promising potential for selection in breeding programs. Among the genotypes evaluated, EC-378632, IC-275005 and EC-362911 emerged as the best performers, demonstrating superior yield and quality traits. These genotypes are particularly promising due to their high fruit yield per plot and consistent performance across the studied traits, making them excellent candidates for further breeding efforts. Future research should focus on multienvironment trials to assess the stability of these genotypes across diverse environmental conditions. Genome-wide association studies (GWAS) could also provide deeper insights into the genetic basis of key traits and help identify specific genes responsible for high yield and disease resistance. Additionally, conducting field trials in different climates will help evaluate the adaptability of the top-performing genotypes, further refining breeding strategies for improved chilli cultivars.

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Authors' contributions

NT conceptualized the research work, designed the experiments, contributed to data analysis and interpretation, and participated in manuscript preparation. DS¹ co-conceptualized the study and assisted in designing the experimental framework. SH conducted field and laboratory experiments, collected data, and contributed to data analysis and interpretation. DS² was involved in executing field and lab experiments and supported data collection. JK analyzed and interpreted the data and drafed the manuscript. All authors read and approved the final version of the manuscript. [DS¹ stands for Deepa Sharma and DS² stands for Diksha Sharma]

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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