



RESEARCH ARTICLE

Multivariate analysis of genetic diversity in chili (*Capsicum annuum* L.) genotypes for yield and biochemical traits

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Abstract

India is a leading producer, user, and exporter of chili (*Capsicum annuum* L.), one of the world's most valuable vegetables and spice crops. However, India's future supremacy in chili production confronts several serious obstacles, such as the emergence of new pathogen races, selection-induced saturation, biotic and abiotic stresses caused by climate change and genetic drift in cultivars. So, to strike early, the available germplasm of 21 lines was analyzed for 12 morphological parameters, followed by a response to phytophthora fruit rot and biochemical traits such as capsaicin content, to identify diverse lines to be utilized in crop improvement, selection and hybridization breeding programs in the future. For all characteristics, the genotypic coefficient of variation (GCV) varied from 4.99 to 42.06%, while the phenotypic coefficient of variation (PCV) ranged from 5.19 to 42.11%. Heritability varied between 88.13 and 94.85%, whereas genetic gain was between 15.34 and 91.01%. Higher heritability and genetic gain for the number of fruits plant⁻¹, average fruit weight, fruit yield plant⁻¹, fruit breadth at the middle, fruit length, number of seeds fruit⁻¹ and total capsaicin content in dry chili suggest the presence of additive gene action and the reliability of selection for improvement of these traits in chili. A biplot showing principal component analysis contributed to the selection of superior accessions for fruit yield plant⁻¹ (g) and total capsaicin content (%) in dry chili.

Keywords: Diversity, GCV, Heritability, Multivariate, PCV.

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Introduction

Chili (*Capsicum annuum* L.; 2n=24), often known as pungent pepper, is an important vegetable crop of the Solanaceae family that is grown for its green, red, ripe or dried fruits (berries), depending on market demand. Chili is abundant in vital nutrients and bioactive compounds with antibacterial, antioxidant, antiviral, anti-inflammatory, and anticancer properties (Malik et al., 2022; Sushmitha et al., 2024; Brahmani et al., 2024). These features are mostly owed to the carotenoids and the strong flavor that makes them pungent. The presence of carotenoids, particularly capsanthin and capsorubin, which have significant oxygen scavenging characteristics and hence are antioxidants, adds to the red fruit color of the chili. In contrast, the presence of capsaicinoids causes pungency (Bal et al., 2022), which are a class of about twenty-five related alkaloids produced as secondary metabolites from the placenta's glandular epidermal cells and accumulate along the epidermis to form blisters (Stewart et al., 2007). In addition to these, chili is a good source of vitamins like vitamins A, B1, B2, B3, B6, C, K and minerals such as iron, calcium, magnesium, potassium, and thiamine (Chakrabarty et al., 2017).

The genus *Capsicum* has more than thirty-five species, but just five of them, namely *C. annuum*, *C. chinense*, *C.*

baccatum, *C. frutescens*, and *C. pubescens*, are commercially important and have been cultivated for thousands of years in various regions of the world (Pickersgill, 1997). This prolonged history of cultivation in many parts of the world has resulted in significant genetic variation in chili, which must be well studied in order to efficiently uncover better and more diverse chili lines (Pidigam *et al.*, 2019; Ahmed *et al.*, 2022). Genotypes must be examined for several physical and biochemical features in order to estimate diversity (Vishnu and Radhamany, 2021; Bhandari *et al.*, 2022). To improve assessment efficiency, data gathered for various characteristics must be analyzed for variance using the genotypic coefficient of variability (GCV) and phenotypic coefficient of variability (PCV) (Pandiyaraj *et al.*, 2017; Karim *et al.*, 2022). The more variability in a population, the more successful the selection of desirable kinds will be (Vavilov, 1951; Jyothi *et al.*, 2011). Heritability estimates quantify the transfer of traits from one generation to the next (Amit *et al.*, 2014; Vyas *et al.*, 2021). Since the environment may affect genotype response, phenotypic plant expression might not accurately reflect genotype genetic potential (Manjunathagowda and Anjanappa, 2021). Heritability and genetic advance can be used to assess the influence of the environment on character expression and the scope of improvement after selection. Furthermore, for studying genetic variability and the influence of variables on that variability, statistical techniques for multivariate analysis, such as hierarchical clustering and Principal Component Analysis (PCA), may be undertaken (Singh and Tiwari, 2020; Dutta *et al.*, 2022).

The Portuguese brought chili to India's southern regions (Do Rego *et al.*, 2011). It had spread throughout the country by the end of the nineteenth century. And now, India is the world leader in chili production, consumption, and export. The development of new pathogen races, selection-induced saturation, biotic and abiotic stresses brought on by climate change and genetic drift in cultivars are just a few of the significant obstacles that India's future dominance in the production of chilies must overcome (Tirupathamma *et al.*, 2021). To maximize production, it is, therefore, necessary to establish disease-free, high-yielding lines with high pungency. So, to strike early, the available germplasm was evaluated for various morphological parameters followed by a response to phytophthora fruit rot and biochemical traits such as capsaicin content to identify diverse lines to be used in a breeding program to promote crop improvement in the future.

Materials and Methods

The experiment was carried out at the Experimental Farm of Vegetable Science in Dr Yashwant Singh Parmar University of Horticulture and Forestry Nauni, Solan (HP), which is situated 1270 m above mean sea level at 35°5' N latitude and 77°11' E longitude. A nursery of 21 genotypes

was sowed in March 2018 and transplanted at a distance of 45×45 cm in the main field on a plot of 2.25×1.80 m with three replications per genotype in RCBD in May 2018. Out of 21 genotypes, 2 (Gundu-1, Gundu-2) were collected from the Professor Jayashankar Telangana State Agricultural University, Hyderabad, Telangana, 1 (DKC-8) from the Regional Horticultural Research training station Dhaulakuan, Sirmour, Himachal Pradesh and remaining 18 (UHF-CHI-1, 2, 3, 5, 7 to 19 and UHF Selection 04) from different district of Himachal Pradesh.

Observations were recorded at the red fruit stage for all the characters except days to 50% flowering. Other characters were days to green fruit maturity, days to red ripe fruit maturity, number of fruits plant⁻¹, average fruit weight (g), fruit length (cm), fruit breadth at the middle (cm), fruit yield plant⁻¹ (g), plant height (cm), dry yield as a percentage of fresh ripe, total capsaicin content (%) in dry chili and natural incidence of Phytophthora fruit rot.

The total capsaicin content in the red dried chili fruits was determined by the calorimetric method as described by Sadasivam and Manickam (1996). The capsaicin concentration was observed from the standard curve plotted by using a standard capsaicin solution (Fluka AG).

The observations were recorded on five randomly selected plants of each genotype in every replication for every trait. The mean values of all the ten traits were subjected to box plot analysis using R software. Another statistical analysis was done for GCV, PCV, heritability and genetic gain (%) using SPSS 16.0 software. GCV and PCV obtained were classified as low (0–10%), moderate (10–20%) and high (>20%) (Shivasubramanian and Menon, 1973) heritability as low (0–30%), moderate (31–60%) and high (>60%) (Burton and Devane, 1953) and genetic gain as low (0–25%), moderate (25–40%) and high (>40%) (Johnson *et al.*, 1955). In addition to this, to monitor the phylogeny relationships, a dendrogram based on the unweighted pair group method with arithmetic mean (UPGMA) algorithm using the Euclidean distance index was constructed through DARWin software (version 6.0.21) (Perrier *et al.*, 2003). To complement and verify the classification results of the phylogenetic tree, an additional multivariate analysis of PCA was applied in terms of the Mahalanobis distance matrix using PAST software (Hammer *et al.*, 2001).

Results and Discussion

Genetic variability

The mean performance of the genotypes for the traits showed adequate variation, which can be exploited further in the breeding program for crop improvement (Table 1). The genotype UHF-CHI-9 was the earliest to flower (35 days) and mature (89 days) and had the highest average fruit weight (8.05g). Genotype UHF-CHI-13 produced the highest number of fruits (194.52) and highest fruit yield

Table 1: Mean performance of genotypes

Genotypes	Days to 50 % flowering	Days to fresh ripe fruit maturity	Number of fruits plant ⁻¹	Fruit yield plant ⁻¹ (g)	Plant height	Number of seeds per fruit	Dry yield as percentage of fresh ripe	Average fruit weight(g)	Fruit length (cm)	Fruit breadth at the middle (cm)	1000 seed weight (g).
UHF-CHI-1	51.00	106.67	72.56	132.90	67.22	72.00	19.06	2.46	5.85	0.90	4.89
UHF-CHI-2	55.33	110.33	70.76	173.68	62.92	95.53	21.74	3.08	5.08	0.89	3.87
UHF-CHI-3	51.33	105.00	87.15	235.95	75.38	118.33	24.07	3.50	7.96	1.15	4.67
UHF-CHI-5	51.67	108.00	61.24	180.81	81.32	113.40	16.91	3.98	10.05	1.14	4.44
UHF-CHI-7	43.67	93.33	75.19	281.09	103.11	102.67	21.50	4.32	11.30	1.15	4.72
UHF-CHI-8	50.33	103.33	129.13	234.57	96.17	51.60	18.36	2.36	14.94	0.71	5.00
UHF-CHI-9	35.00	89.00	22.25	102.68	60.76	130.53	16.89	8.05	13.29	1.59	6.36
UHF-CHI-10	37.33	106.33	67.12	183.34	70.45	129.27	19.88	3.59	8.36	0.90	4.05
UHF-CHI-11	53.33	107.67	116.30	248.25	83.91	61.13	21.70	2.74	7.24	0.75	4.42
UHF-CHI-12	56.00	114.67	82.70	214.24	70.38	71.33	19.91	3.12	6.07	0.91	3.16
Gundu-1	42.00	95.67	42.26	92.85	80.96	137.13	16.29	2.23	2.18	2.19	4.49
Gundu-2	44.67	96.67	46.39	97.16	81.55	137.47	18.72	2.38	3.38	2.31	4.66
UHF-CHI-13	66.00	121.33	194.52	338.09	95.59	71.93	20.12	2.06	5.82	0.69	3.07
UHF-CHI-14	59.33	120.33	98.99	271.71	90.16	54.93	28.38	3.19	8.53	0.77	3.86
UHF-CHI-15	43.33	114.00	94.73	223.60	90.38	71.93	21.07	2.70	8.52	1.10	4.87
UHF-CHI-16	45.67	112.33	68.06	148.48	93.47	87.80	17.30	2.86	8.04	1.06	5.85
UHF-CHI-17	65.00	120.67	77.39	173.98	70.67	79.47	21.18	2.88	7.57	1.05	3.53
UHF-CHI-18	54.67	113.33	66.66	128.10	93.97	81.40	24.43	2.82	7.79	1.09	4.64
UHF-CHI-19	63.67	120.33	67.77	122.72	74.31	65.80	18.67	2.48	7.68	1.00	5.79
UHF Selection 4	43.67	92.67	34.71	115.94	99.06	74.00	24.16	4.46	10.26	1.54	5.33
DKC-8 (check)	61.33	107.00	86.47	189.32	67.79	75.12	19.29	3.06	6.61	0.88	4.96
Mean	51.16	107.56	79.16	185.21	89.66	4.60	81.41	3.25	7.93	1.13	20.46
CD_{0.05}	3.94	3.38	2.57	4.37	1.83	0.13	1.63	0.11	0.54	0.08	1.26
SE(m)±	1.38	1.18	0.90	1.53	0.64	0.05	0.57	0.04	0.19	0.03	0.44
C.V.	4.66	1.90	1.96	1.43	1.24	1.76	1.21	2.13	4.13	4.07	3.74

Table 2: Eigen values and percentage of variation for principal components

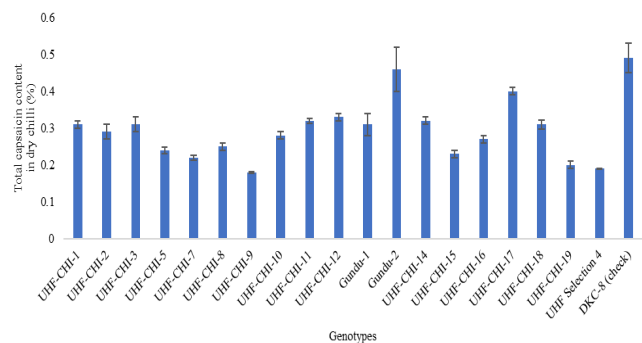
Principal component	Eigen value	Percentage of variation	Cumulative percentage of variation
PC1	5.54	42.64	42.64
PC2	2.45	18.87	61.50
PC3	1.26	9.71	71.21
PC4	1.13	8.67	79.88
PC5	0.99	7.59	87.46
PC6	0.48	3.67	91.13
PC7	0.34	2.64	93.78
PC8	0.27	2.09	95.87
PC9	0.19	1.46	97.33
PC10	0.12	0.96	98.29
PC11	0.11	0.86	99.15
PC12	0.08	0.58	99.73
PC13	0.03	0.27	100.00

plant⁻¹ (338.09) among all the genotypes. The highest fruit length was found in genotype UHF-CHI-8 (14.94 cm) and the highest fruit breadth in the middle was found in Gundu-2 (2.31 cm). Gundu-2 also had the highest number of seeds per fruit (137.47). 1000 seed weight was found to be highest in UHF-CHI-9 (6.36) and dry weight as a percentage of fresh ripe for UHF-CHI-14 (28.38). UHF-CHI-7 (103.11) was found to be the tallest among all the genotypes. Whereas DKC-8 had the highest total capsaicinoid content at dry fruit stages (0.49) (Fig. 1). These findings are in agreement with the study by Shrishat *et al.* (2007), Kumari *et al.* (2010) and Amit *et al.* (2014).

The fact that PCV was bigger than GCV (Table 2) showed that the apparent variation in the characteristics was caused not just by genotype but also by environmental influences. However, the difference in PCV and GCV values was small, which revealed that the environment has little influence on phenotypes and that phenotypic variability can be leveraged for improvement. Furthermore, the variances ranged from low to high, with high GCV and PCV for the number of fruits plant⁻¹, average fruit weight, fruit breadth at middle, fruit length, fruit yield plant⁻¹, number of seeds plant⁻¹ and total capsaicin content in dry chili indicating towards availability of significant variation for genetic improvement of chili, despite the fact that PCV was greater than GCV. This level of variation in these characteristics allows for successful genotype selection and development. (Amit *et al.*, 2014, Pandiyaraj *et al.*, 2017). Aside from these characteristics, PCV and GCV were moderate in days to 50% flowering, 1000 seed weight (g), and dry yield as a percentage of fresh ripe and plant height (cm), but low in Days to fresh

Table 3: Major contributing traits towards PC's taken into consideration

Characters	PC 1	PC 2	PC 3	PC 4
Days to 50 % of flowering	0.342	-0.129	-0.318	0.042
Days to fresh ripe fruit maturity	0.333	-0.085	-0.373	0.119
Number of fruits plant ⁻¹	0.374	0.086	0.211	-0.197
Fruit yield plant ⁻¹ (g)	0.325	0.228	0.288	-0.133
Plant height	0.098	0.290	0.585	0.347
Number of seeds fruit ⁻¹	-0.301	-0.257	0.255	-0.137
Dry yield as percentage of fresh ripe	0.161	0.161	-0.033	0.724
Average fruit weight(g)	-0.269	0.284	-0.149	-0.157
Fruit length (cm)	-0.066	0.582	-0.051	-0.165
Fruit breadth at the middle	-0.311	-0.279	0.329	0.151
1000 seed weight (g)	-0.284	0.240	-0.161	-0.065
Total capsaicin content in dry chili (%)	0.214	-0.426	0.159	-0.088

**Fig.1:** Variation in genotypes for total capsaicin content in dry chili (%)

ripe maturity. Because genotypes exhibit minimal genetic diversity for these features, therefore cannot be utilized for selection. Further, to estimate the inheritance of characters, heritability was estimated, which ranged from 88.13–94.85%, offering a high probability of fixing a character by selection (Table 2). Heritability alone is not sufficient to determine the effectiveness of selection as it includes both fixable and non-fixable variance; thus, it is combined with the estimates of genetic gain to increase the significance from the point of expected gain and the type of selection method to be followed. High heritability with a high genetic gain was observed for the number of fruits plant⁻¹, average fruit weight, fruit yield plant⁻¹, fruit breadth at the middle, fruit length, number of seeds fruit⁻¹ and total capsaicin content in dry chili (Table 2). High heritability with high genetic gain contributes to the additive gene effect of these traits suggesting the suitability of selection for improvement in contrast to low genetic gain observed for days to maturity (green stage), days to red ripe maturity and dry yield as a

percentage of fresh ripe (%) similar to previous reports by Amit *et al.* (2014) who reported low genetic gain for days to green and red fruits maturity and Kumar *et al.* (2018) for dry yield as a percentage of fresh ripe.

Clustering and PCA

To measure the level of diversity across all the lines, the pair-wise genetic dissimilarity indices based on Euclidean distance were computed. With an average of 29.13, the total germplasm dissimilarity index varied from 2.04 (Gundu 1 and Gundu 2) to 84.73 (UHF-CHI-13 and UHF-CHI-7). A dendrogram based on the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method was created using the dissimilarity matrix from the morphological and biochemical data, which separated the twenty-one genotypes into two primary clusters (Figure 1).

Only one genotype (UHF-CHI-13) belonged to Cluster I. The remaining twenty genotypes fell into Cluster II and were further separated into two subclusters having 5 and 15 genotypes, respectively (Figure 2). The inter-subcluster dissimilarity index varied from 58.10 (UHF-CHI-14 and Gundu 1) to 10.48 (UHF-CHI-11 and 12). Within subclusters, genotypes showed broad dissimilarity, with subcluster I genotype having a maximum dissimilarity index of 24.7 (UHF-CHI-7 and 8) and a minimum dissimilarity index of 7.36 (UHF-CHI-8 and 11), and subcluster II genotypes having a maximum dissimilarity index of 43.76 (UHF-CHI-15 and Gundu-1) and a minimum dissimilarity index of 2.04 (Gundu 1-Gundu-2).

Further, in order to analyze multi-dimensional relationships that characterize the amount of genetic variation in the sample, PCA was also used. Four principal components (PCs) in all were taken into account since they showed eigenvalues larger than one and a cumulative variation of 79.88%; therefore, the traits falling in these PCs may be given due importance in chili breeding (Table 2). PC1 accounted for the greatest variance (42.64%), followed by PC2 (18.87%). PC1 was mostly due to the number of fruits plant⁻¹, days to 50% flowering, days to fresh ripe fruit maturity, fruit yield plant⁻¹ (g) and total capsaicin content (%) in dry chili (Table 3). The fruit length (cm) and average

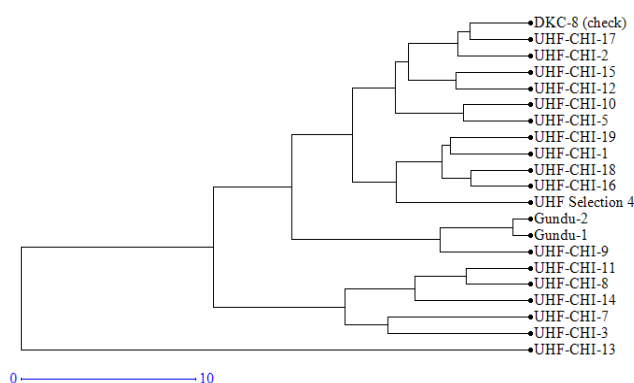


Fig. 2: Dendrogram based on morphological and biochemical data

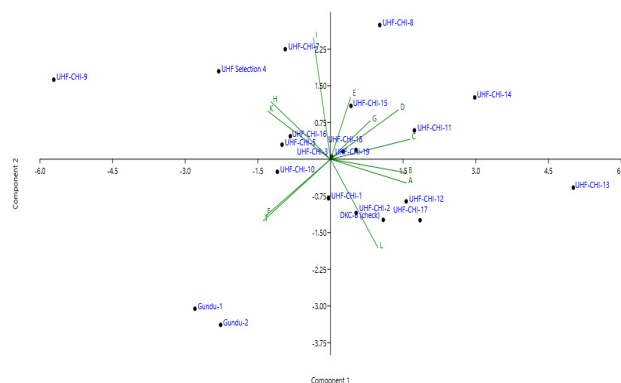


Fig. 3: Principal coordinate analysis (where A= days to 50 % flowering, B= days to fresh ripe fruit maturity, C=number of fruits plant⁻¹, D=fruit yield plant⁻¹ (g), E=Plant height, F=number of seeds per fruit, G=dry yield as percentage of fresh ripe, H=average fruit weight(g), I= fruit length (cm), J= fruit breadth at the middle (cm), K= 1000 seed weight (g), L=total capsaicin content (%) in dry chili

fruit weight (g) dominated the second major component, PC2, whereas PC3 comprises plant height (cm) and fruit breadth in the middle (cm). PC4 was influenced by dry yield as a percentage of fresh ripe. PC1 and PC2 accounted for the greatest variance (42.64 and 18.87%); therefore, it can be concluded that number of fruits plant⁻¹, days to 50% flowering, days to fresh ripe fruit maturity, average fruit weight, total capsaicin content (%) in dry chili and number of seeds fruit⁻¹ were the important factors contributing towards 79.88% variability in the chili genotypes. The biplot representing 21 genotypes depicted that high PC1 and PC2 are required for the selection of genotypes for fruit yield plant⁻¹ (g), whereas for total capsaicin content (%) in dry chili, high PC1 and low PC2 are required (Figure 3).

Therefore, based on these selection criteria, genotypes like UHF-CHI-11, UHF-CHI-14 and UHF-CHI-8 are suitable and superior for fruit yield plant⁻¹ (g) and genotypes DKC-8, UHF-CHI-16 and UHF-CHI-2 are suitable for total capsaicin content (%) in dry chili whereas, UHF-CHI-13 was superior for both the traits. The current findings are comparable to those of Rana *et al.* (2014), who discovered that fruit length, fruit width, average fruit weight, and fruit yield per plant had the highest positive values. Lahbib *et al.* (2012) found similar results, with the first principal component (PC1) contributing the most to overall variability (25.059%).

Conclusion

The mean performance, GCV, PCV, heritability, genetic gain, dissimilarity matrix and PCA have all contributed to a better understanding of the fundamental characteristics responsible for genetic variability and the production of superior breeds of chili. A biplot showing principal component analysis contributed to the selection of superior accessions for fruit yield plant⁻¹ (g) and total capsaicin content (%) in dry chili that might be used in crop improvement, selection and hybridization breeding programs in the future.

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सारांश

भारत अधिकतम मिर्च (*Capsicum annuum* L.) के उत्पादक, उपभोक्ता, और निर्यातकों में से एक है, जो दुनिया की सबसे मूल्यवान सब्जियों और मसाला फसलों में से एक है। हालांकि, भारत की भविष्य में मिर्च उत्पादन में सर्वाधिकता का सामना कई गंभीर बाधाओं से करता है, जैसे कि नए पैथोजन रस का उदय, चयन-उत्प्रेरित संतुष्टि, जैविक और अजैविक तनाव जो जलवायु परिवर्तन और जीनेटिक ड्रिफ्ट द्वारा फसलों में होता है। इसलिए, समय पर हमला करने के लिए, भविष्य में फसल की सुधार, चयन, और हाइब्रिडीकरण प्रजनन कार्यक्रमों में उपयोग किए जाने वाले विविध पंक्तियों की उपलब्ध जिनशास्त्रीय संभावनाओं को पहचानने के लिए, 12 मोफोलॉजिक पैरामीटरों के लिए 21 उपलब्ध मिर्च जीनोटाइप का विश्लेषण किया गया था, जिसके बाद पाइथोफोरा फ्रूट रॉट के प्रतिसाद और बायोकेमिकल गुणों की विशेषताएं जैसे कि कैप्सेसिन सामग्री, को पहचानने के लिए। सभी विशेषताओं के लिए मिर्च जीनोटाइपिक परिवर्तन सम्बंध (GCV) 4.99% से 42.06% तक बदला, जबकि फेनोटाइपिक परिवर्तन सम्बंध (PCV) 5.19% से 42.11% तक था। संवर्धता 88.13% से 94.85% तक थी, जबकि आनुवंशिक लाभ 15.34% से 91.01% तक था। पौधे प्रति फल की संख्या, औसत फल का वजन, पौधे प्रति फल का उत्पादन, मध्य में फल की चौड़ाई, फल की लंबाई, बीज प्रति फल की संख्या, और सूखी मिर्च में कुल कैप्सेसिनोइड्स की सामग्री के लिए उच्च संवर्धता और आनुवंशिक लाभ इस बात का सुझाव देते हैं कि मिर्च में इन गुणों की सुधार के लिए सहायक जीन क्रिया की उपस्थिति और चयन की विश्वसनीयता है। प्राध्यापक घटक विश्लेषण को उत्तम पहुंच के लिए उत्तम पहुंच संख्या प्रति पौधा (ग्राम) और सूखी मिर्च में कुल कैप्सेसिन की सामग्री (%) के लिए उत्तम प्रवेशों का चयन करने में योगदान किया।