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RESEARCH ARTICLE

Evaluation of India's hottest chilli species (*Capsicum chinense* Jacq.) from various northeastern states to identify high-yielding lines with superior nutritional quality

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Abstract

The present study was conducted to assess the genetic diversity, association ship and identify stable lines of king chilli (*Capsicum chinense* Jacq.) at Horticulture Research Farm, ICAR Research Complex for NEH Region for three consecutive years (2021, 2022 and 2023) keeping in view the vast genetic diversity of king chilli present in the North Eastern Hill Region of India which are yet to explore to its fullest potential. The results of the study reveal that for the economic trait fruit yield per plant, the highest value was recorded in RCML5 (1003.40g) followed by RCML1 (949.85 g) and RCNL4 (898.46 g). Similarly, for quality parameters the highest dry matter (%), ascorbic acid content (mg/100g), total flavonoid (mg QE/g) and capsaicin (%) was recorded in RCML6 (19.78 %), RCMP6 (693.41 mg/100g), RCNL-1 (5.12 mg QE/g) and RCML16 (5.90 %), respectively. High heritability (>60 %) coupled with high GA (>20 %) was observed for desirable traits such as fruit yield per plant, no. of fruits per plant, no. of fruits per cluster and average fruit weight. Among the quality traits, ascorbic acid content exhibits high heritability (>60 %) and high GA (>20 %). This indicates the presence of additive gene action and the potential response of these traits towards selection. Cluster analysis revealed that cluster 6th, comprising of 9 accessions, was the largest, while the largest inter-cluster distance based on the Mahalnobis Euclidean Distance was recorded between cluster 8th and 6th (77.28). The Principal Component Analysis revealed that 7 PCs with eigenvalues more than 1 contribute 84.53 % of the total variability. Plant spread (E to W), no. of fruits per plant, ascorbic acid content and capsaicin content were depicted as the major predictors of total variability. The present study identifies accessions RCML5, RCML1 and RCNL4 as the best performers for fruit yield and most of its related traits. It exhibits the potential of giving higher yield in future improvement programmes, while accessions viz., RCML16, RCML5 and RCML10 are promising for capsaicin content. Based on stability analysis accession RCNL1, a moderate yielder and mid-maturity with average capsaicin content, was stable across environments. These identified accessions can served as a gene bank for future crop improvement programme in king chilli.

Keywords: *Capsicum chinense* Jacq.; diversity; principal component analysis; stability; variability; yield

Introduction

King chilli (*Capsicum chinense* Jacq.), is one of the hottest Capsicum species in the world. This chilli species is native to North Eastern Region of India and there is a wide diversity of the species in the region. It is locally known by various local names such as, Umorok, Naga Mircha, Bhot Jholokia etc (1). This species is reported to evolve through natural cross-pollination and occupies a taxonomic place between the two species *C. chinense* and *C. frutescens*, with more proximity to *C. chinense* group. It belongs to solanaceae family bears pendant flower white corollas, cymose or axillary inflorescence with conical to sub-conical fruits (2). The plant is semi-perennial and commonly cultivated as a vegetable and spice in North Eastern Region of India. This chilli species was also recorded in the Guinness Book as the hottest with exceptionally higher capsaicin content (2-4 times higher) than normal chilli. King chilli has an ethnic, traditional and economic importance in the life of tribal people of North Eastern Region. It was granted a Geographical Indication (GI) tag for Nagaland, one of North Eastern States of India (3). It is high in pungency, a palatable aroma and rich in nutritional quality. It is a rich source of vitamin C an important antioxidant compound and reported to be superior to tomato in this respect (4, 5). Capsaicin is one of chemical groups of most significant interest in the king chilli. These alkaloids contribute to the characteristic pungency in all the chilli species. The amount of capsaicin content in king chilli is variable and depends on the genetic makeup and the environment where it is cultivated (6). In general, the capsaicin content in most of the chilli species used for consumption in India is 1 % but it is reported to be about 2-4 % in *Capsicum chinense.* Capsaicin possesses diverse medicinal properties such as anti-inflammatory, analgesic, antimutagenic, anti-tumour, antioxidant, fungicide, bactericide, nematicide and insecticide (7). Thus, it has a huge potential in the pharmaceutical industry and even as a bioweapon for riot control (8). Moreover, the fresh fruit of king chilli has huge market potential, with prices ranging from Rs. 500-800 per kg and even up to Rs 1000-1300 per kg fresh fruit in the offseason (9, 10). Despite its huge potential, this particular chilli species has no improved variety or hybrid. For developing any varieties or hybrids germplasm collection, evaluation, diversity study, characterization and identification of indicator traits and potential lines are some of the essential pre-breeding works. Considering this, the present field experiment was conducted to evaluate, characterize and identify indicator traits for yield and quality and phenotypically stable lines for further improvement programme.

Materials and Methods

The study was conducted at the Experimental Farm, ICAR Research Complex for NEH Region, Umiam, Meghalaya, for three consecutive years, 2021, 2022 and 2023, during spring-summer. The experiment was laid out with 3 replications in Randomized Block Design. The treatments comprises of 30 accessions of *Capsicum chinense* Jacq. (Table 1) collected from different north eastern states of India viz., Manipur, Nagaland and Meghalaya. The location of the Experimental farm is at 950 m MSL altitude; 25°41'N latitude and 91°54'E longitude. The area have subtropical climate with average annual rainfall of 150-250 cm. The experimental plot is prepared after thorough ploughing keeping the bed at 20 cm height and 90 cm width. The accessions' nursery were raised in February and transplanted on the first week of April. The seedlings were transplanted at spacing of (1 × 1) m. The observations on yield, related traits and quality parameters were recorded from five randomly taken plants in each accession and replication followed by computing their means.

Quality parameters

Dry-matter content

Fully ripe fruits (100 g) of each accession were packed in paper bag and kept in oven at 60 °C till a constant weight was obtained. The per cent dry matter was calculated using following formula:

$$\text{Dry matter (\%)} = (\text{Dry weight} / \text{Fresh weight}) \times 100 \qquad \text{(Eqn. 1)}$$

Ascorbic acid (mg/100 g) and capsaicin content (%)

The ascorbic acid content was estimated using the standard method (11). The estimation of capsaicin content was carried out following the standard method by using a Shimadzu Double Beam Spectrophotometer (Model UV 1780) (12). Dry powdered sample (500 mg) was taken in a volumetric flask and mixed with 10 mL of dry was subjected to 3 hr of continuous shaking. The content was allowed to settle down and then centrifuged at 10000 rpm for 10 min and 1 mL of clear supernatant was taken out in a test tube and evaporated to dryness in a hot water bath. Residue was dissolved in 5 mL of 0.4 % sodium hydroxide solution, followed by the addition of 3 mL of 3 % phosphomolybdic acid. The content was shaken and allowed to stand for 1 hr. The solution was filtered and centrifuged at 5000 rpm for 10-15 min. Clear blue colored supernatant was read against blank solution in a UV-visible spectrophotometer at 650 nm. The concentration of capsaicin in the sample was calculated from the slope of the standard curve. The result was expressed as '%' of the capsaicin content of the sample.

Total flavanoids

The total flavonoid content was estimated per the standard method using Aluminum chloride ($AlCl_3$) colourimetric assay (13). The flavonoid content was calculated from standard curve of Quercetin (25-200 ug/mL) and expressed as Quercetin equivalents (QE) per gram of extract. Quercetin was used as a standard. Standard solutions of Quercetin of concentration 1.56-100 ug/mL were prepared in 80 % ethanol. 50 uL of extracts (1 mg/mL) or standard solution was added to 10 uL of 10 % aluminium chloride solution, followed by 150 uL of 95 % ethanol. 10 uL of 1M sodium acetate was added to the mixture in a 96 well plate. 80 % ethanol was used as reagent blank. All reagents were mixed and incubated for 40 min at room temperature and protected from light. The absorbance was measured at 415 nm with a microplate reader (Biotek, USA). The total flavonoids were calculated using the formula:

$$\text{Total flavonoid content (mg QE/g)} = \frac{\text{Conc of Q from std curve x volume of extract}}{\text{Weight of the sample}} \qquad \text{(Eqn. 2)}$$

Statistical analysis

The mean value of five randomly taken plants constituted one replication for each accession. These values were further subjected for analysis of variance (14). The PCV and GCV were estimated (15). Heritability and genetic advance (GA) were estimated using the formula suggested by previous studies (16, 17). The genotypic and phenotypic correlation coefficient and path coefficient were also estimated (18). Clustering, Stability Analysis and PCA were performed using Windostat Version 9.3 software.

Results

Variability studies for yield, related traits and quality parameters

The results of the variability study revealed the presence of wide variability amongst the accessions for all the traits taken. On a morphological basis fruit shape was categorized into 4 major groups *viz.,* elongated, triangular, blocky and companulate (Fig. 1) and the fruit surface as smooth and wrinkled (Fig. 1). The characterization based on fruit colour at different stages and fruit shape is given in Table 2. The result of the pooled data analysis reflects significant variability for days to 50 % flowering (50.17-68.03); days to 50 % harvesting (85.15-105.85), plant height (68.44-210.16 cm), stem diameter (0.92-1.75 cm), no. of main branches (1.66-2.44), no. of primary branch (2.77-4.78), plant spread E to W (94.22-180.42 cm), no. of fruits/cluster (2.44-3.33), no of fruits/plant (63.88-184.33); no. of seeds/fruit (25.66-60.33); fruit yield/plant (421.28-1003.40); average fruit wt. (3.54-9.05 g), fruit length (3.94-7.46 cm); fruit diameter (1.32-3.14 cm); dry matter (12.51 -19.78 %); ascorbic acid content (131.38-493.41 mg/100 g); total flavonoid (2.85-5.12 mg QE/g) and capsaicin content (3.13-5.90 %) (Table 3). Amongst the 30 accessions studied

Table 1. Description of accessions used in the study

Accession Code No	Accession	Pedigree		
		Origin		Breeding Centre
1	RCMP-1	Kumbi, Bishnupur District, Manipur	Advanced lines through recurrent selection of local landrace MP-1	ICAR Research Complex for NEH Region, Umiam, Meghalaya
2	RCMP-2	Sarikushi, Nongpoh District, Meghalaya	Advanced lines through recurrent selection of local landrace MP-2	ICAR Research Complex for NEH Region, Umiam, Meghalaya
3	RCMP-3	Yaingangpokpi, Imphal East District, Manipur	Advanced lines through recurrent selection of local landrace MP-3	ICAR Research Complex for NEH Region, Umiam, Meghalaya
4	RCMP-4	Sagang, Bishnupur District, Manipur	Advanced lines through recurrent selection of local landrace MP-4	ICAR Research Complex for NEH Region, Umiam, Meghalaya
5	RCMP-5	Nongrim Nongladaw, Ri Bhoi District, Meghalaya	Advanced lines through recurrent selection of local landrace MP-5	ICAR Research Complex for NEH Region, Umiam, Meghalaya
6	RCMP-6	Saikul, Senapati District, Manipur	Advanced lines through recurrent selection of local landrace MP-6	ICAR Research Complex for NEH Region, Umiam, Meghalaya
7	RCMP-7	Nambol, Bishnupur District, Manipur	Advanced lines through recurrent selection of local landrace MP-7	ICAR Research Complex for NEH Region, Umiam, Meghalaya
8	RCMP-8	Umsning, Ri Bhoi District, Meghalaya	Advanced lines through recurrent selection of local landrace MP-8	ICAR Research Complex for NEH Region, Umiam, Meghalaya
9	RCMP-9	Yaingangpokpi, Imphal East District, Manipur	Advanced lines through recurrent selection of local landrace MP-9	ICAR Research Complex for NEH Region, Umiam, Meghalaya
10	RCML-1	Pungba, Ukhrul District, Manipur	Advanced lines through recurrent selection of local landrace ML-1	ICAR Research Complex for NEH Region, Umiam, Meghalaya
11	RCML-2	Pungba, Ukhrul District, Manipur	Advanced lines through recurrent selection of local landrace ML-2	ICAR Research Complex for NEH Region, Umiam, Meghalaya
12	RCML-3	Kumbi, Bishnupur District, Manipur	Advanced lines through recurrent selection of local landrace ML-3	ICAR Research Complex for NEH Region, Umiam, Meghalaya
13	RCML-4	Kumbi, Bishnupur District, Manipur	Advanced lines through recurrent selection of local landrace ML-4	ICAR Research Complex for NEH Region, Umiam, Meghalaya
14	RCML-5	Pungba, Ukhrul District, Manipur	Advanced lines through recurrent selection of local landrace ML-5	ICAR Research Complex for NEH Region, Umiam, Meghalaya
15	RCML-6	Umshomatan, Ri Bboi District, Meghalaya	Advanced lines through recurrent selection of local landrace ML-6	ICAR Research Complex for NEH Region, Umiam, Meghalaya
16	RCML-7	Mawlasnai Village, Ri BHoi District, Meghalaya	Advanced lines through recurrent selection of local landrace ML-7	ICAR Research Complex for NEH Region, Umiam, Meghalaya
17	RCML-8	Khliehtem, Ri Bhoi District, Meghalaya	Advanced lines through recurrent selection of local landrace ML-8	ICAR Research Complex for NEH Region, Umiam, Meghalaya
18	RCML-9	Mynsong, Khweng, Ri Bhoi District, Meghalaya	Advanced lines through recurrent selection of local landrace ML-9	ICAR Research Complex for NEH Region, Umiam, Meghalaya
19	RCML-10	Nongrim Nongladaw, Ri Bhoi District, Meghalaya	Advanced lines through recurrent selection of local landrace ML-10	ICAR Research Complex for NEH Region, Umiam, Meghalaya
20	RCML-11	Jokiang, Maurong Road, Umsning, Ribhoi District, Meghalaya Meghalaya	Advanced lines through recurrent selection of local landrace ML-11	ICAR Research Complex for NEH Region, Umiam, Meghalaya
21	RCML-12	Dienglieng village, Mawkynrew, East Khasi Hills District, Meghalaya	Advanced lines through recurrent selection of local landrace ML-12	ICAR Research Complex for NEH Region, Umiam, Meghalaya
22	RCML-13	Nongjri village, Ranikor, West Khasi Hills, Meghalaya	Advanced lines through recurrent selection of local landrace ML-13	ICAR Research Complex for NEH Region, Umiam, Meghalaya
23	RCML-14	Madan Kynsaw, Jantia Hills District, Meghalaya	Advanced lines through recurrent selection of local landrace ML-14	ICAR Research Complex for NEH Region, Umiam, Meghalaya
24.	RCML-15	Madan Kynsaw, Jantia Hills District, Meghalaya	Advanced lines through recurrent selection of local landrace ML-15	ICAR Research Complex for NEH Region, Umiam, Meghalaya
25	RCML-16	Madan Kynsaw, Jantia Hills District, Meghalaya	Advanced lines through recurrent selection of local landrace ML-16	ICAR Research Complex for NEH Region, Umiam, Meghalaya
26	RCNL-1	Medziphema, Dimapur District, Nagaland	Advanced lines through recurrent selection of local landrace NL-1	ICAR Research Complex for NEH Region, Umiam, Meghalaya
27	RCNL-2	Jaluki1, Peren District, Nagalanad	Advanced lines through recurrent selection of local landrace NL-2	ICAR Research Complex for NEH Region, Umiam, Meghalaya
28	RCNL-3	Mon, Mon District, Nagaland	Advanced lines through recurrent selection of local landrace NL-3	ICAR Research Complex for NEH Region, Umiam, Meghalaya
29	RCNL-4	Mon, Mon District, Nagaland	Advanced lines through recurrent selection of local landrace NL-4	ICAR Research Complex for NEH Region, Umiam, Meghalaya
30	RCNL-5	Tsiepama, Dimapur, Nagaland	Advanced lines through recurrent selection of local landrace NL-5	ICAR Research Complex for NEH Region, Umiam, Meghalaya

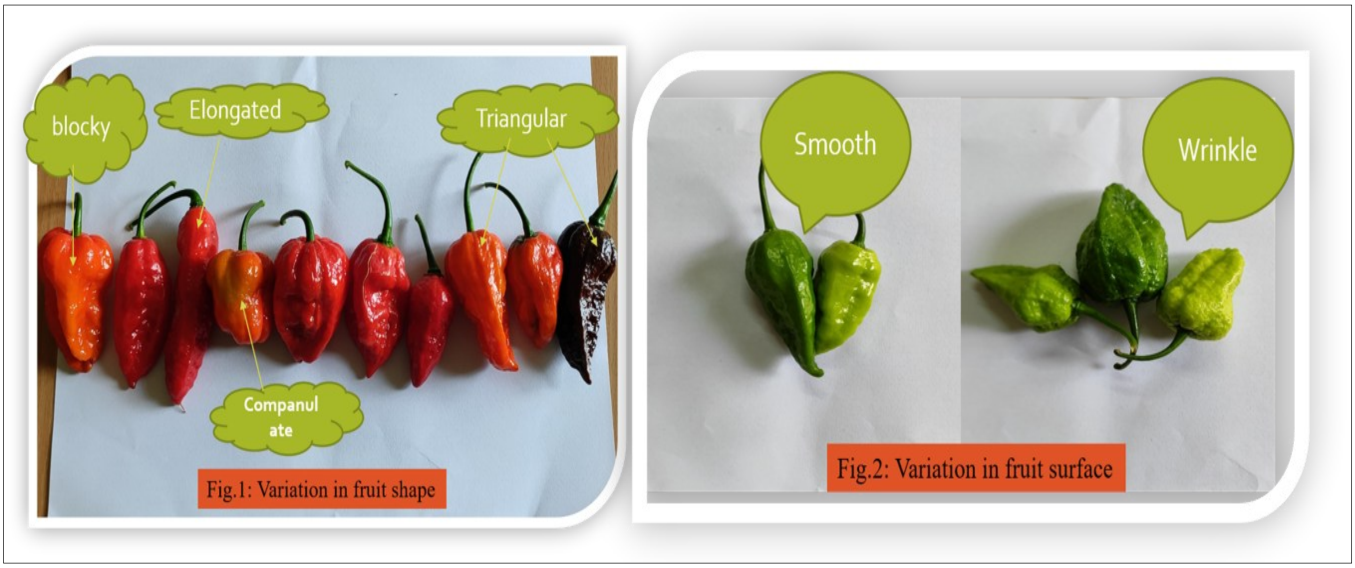


Fig. 1. Variation on fruit shape and surface in King Chilli (*Capsicum chinense* Jacq.).

Table 2. Morphological characterization based on fruit colour (Royal Horticulture Chart), surface and shape (visual)

S.No.	Accessions	Colour			Fruit	
		Immature stage	**Haft Mature Stage**	**Fully ripe stage**	**Surface**	**Shape**
1.	RCMP-1	Brilliant yellowish green B 142	Vivid reddish orange B. 40	Moderate Red A N45	Wrinkle	Blocky
2.	RCMP-2	Vivid yellowish green A 134	Vivid reddish orange C 44	Vivid red A 44	Wrinkle	Triangular
3.	RCMP-3	Strong green C N134	Vivid reddish orange C 44	Vivid red A 44	Wrinkle	Companulate
4.	RCMP-4	Strong green C N134	Vivid reddish orange C 44	Vivid red A 44	Wrinkle	Triangular
5.	RCMP-5	Deep yellowish green a 141	Strong reddish orange C 40	Vivid red A 44	Wrinkle	Blocky
6.	RCMP-6	Deep yellowish green a 141	Deep Yellowish pink D 44	Vivid redish orange B 44	Smooth	Triangular
7.	RCMP-7	Dark yellowish green A. N 134	Vivid reddish orange A 32	Vivid reddish orange A 42	Wrinkle	Triangular
8.	RCMP-8	Strong yellowish Green C 135	Vivid reddish orange C 44	Vivid reddish orange B 44	Wrinkle	Blocky
9.	RCMP-9	Vivid yellowish green A 134	Vivid reddish orange A 33	Vivid red A 44	Wrinkle	Blocky
10.	RCML-1	Vivid yellowish green A 134	Vivid redish orange B. N 30	Vivid redidsh orange A .34	Wrinkle	Triangular
11.	RCML-2	Vivid yellowish green A 134	Vivid reddish orange C 44	Vivid red A 44	Wrinkle	Triangular
12.	RCML-3	Strong yellow Green B 144	Strong yellow Green B 144	Moderate brown C 2000	Wrinkle	Triangular
13.	RCML-4	Moderate olive green A 137	Vivid reddish orange A 30	Vivid Red A 44	Wrinkle	Blocky
14.	RCML-5	Moderate olive green A 137	Vivid reddish orange A 30	Vivid Red B 45	Wrinkle	Elongated
15.	RCML-6	Brilliant yellowish orange B 142	Vivid reddish orange A 43	Vivid red A 44	Wrinkle	Triangular
16.	RCML-7	Brilliant yellowish orange B 142	Vivid reddish orange A 43	Vivid red A 44	Wrinkle	Triangular
17.	RCML-8	Moderate yellowish green C 138	Vivid reddish orange C 44	Vivid red A 44	Wrinkle	Triangular
18.	RCML-9	Strong green C N134	Vivid reddish orange C 44	Vivid red A 44	Wrinkle	Triangular
19.	RCML-10	Briliant yellowish green C 134	Vivid reddish orange B 43	Vivid reddish orange B 44	Wrinkle	Triangular
20.	RCML-11	Light yellowish green A 134	Vivid reddish orange C 44	Vivid red A 44	Wrinkle	Blocky
21.	RCML-12	Briliant yellowish green C 134	Vivid reddish orange C. N 30	Vivid red A 44	Wrinkle	Triangular
22.	RCML-13	Strong yellowish green B 134	Vivid redish orange B 43	Vivid reddish orange A 43	Wrinkle	Blocky
23.	RCML-14	Brilliant yellowish green B 142	Vivid reddish orange B. 40	Moderate Red A N45	Wrinkle	Blocky
24.	RCML-15	Strong yellowish Green C 135	Vivid reddish orange C 44	Vivid Red A 44	Wrinkle	Blocky
25.	RCML-16	Briliant yellowish green C 134	Vivid reddish orange B 43	Vivid Reddish Orange B 44	Wrinkle	Triangular
26.	RCNL-1	Moderate olive green A 137	Vivid reddish orange A 30	Vivid Red A 44	Wrinkle	Triangular
27.	RCNL-2	Brilliant yellowish orange B 142	Vivid reddish orange A 43	Vivid Reddish Orange B 44	Wrinkle	Triangular
28.	RCNL-3	Briliant yellowish green C 134	Vivid reddish orange B 43	Vivid Red B 45	Wrinkle	Triangular
29.	RCNL-4	Moderate olive green A 137	Vivid reddish orange A 30	Vivid Red B 45	Wrinkle	Blocky
30.	RCNL-5	Vivid yellowish green A 134	Vivid redish orange B. N 30	Vivid Reddish Orange B 44	Wrinkle	Blocky

the highest yield based on pooled data was recorded in RCML5 (1003.40 g) followed by RCML1 (949.85 g) and RCNL4 (898.46 g). Likewise for quality parameters the highest dry matter (%), ascorbic acid content (mg/100 g), total flavonoid (mg QE/g) and capsaicin (%) was recorded in RCML6 (19.78 %), RCMP6 (493.41 mg/100 g), RCNL-1 (5.12 mg QE/g) and RCML16 (5.90 %), respectively. The genetic parameter study (Table 3) reveals that the value of PCV is higher than the GCV, indicating the prevalence of environmental effects on the expression of these traits.

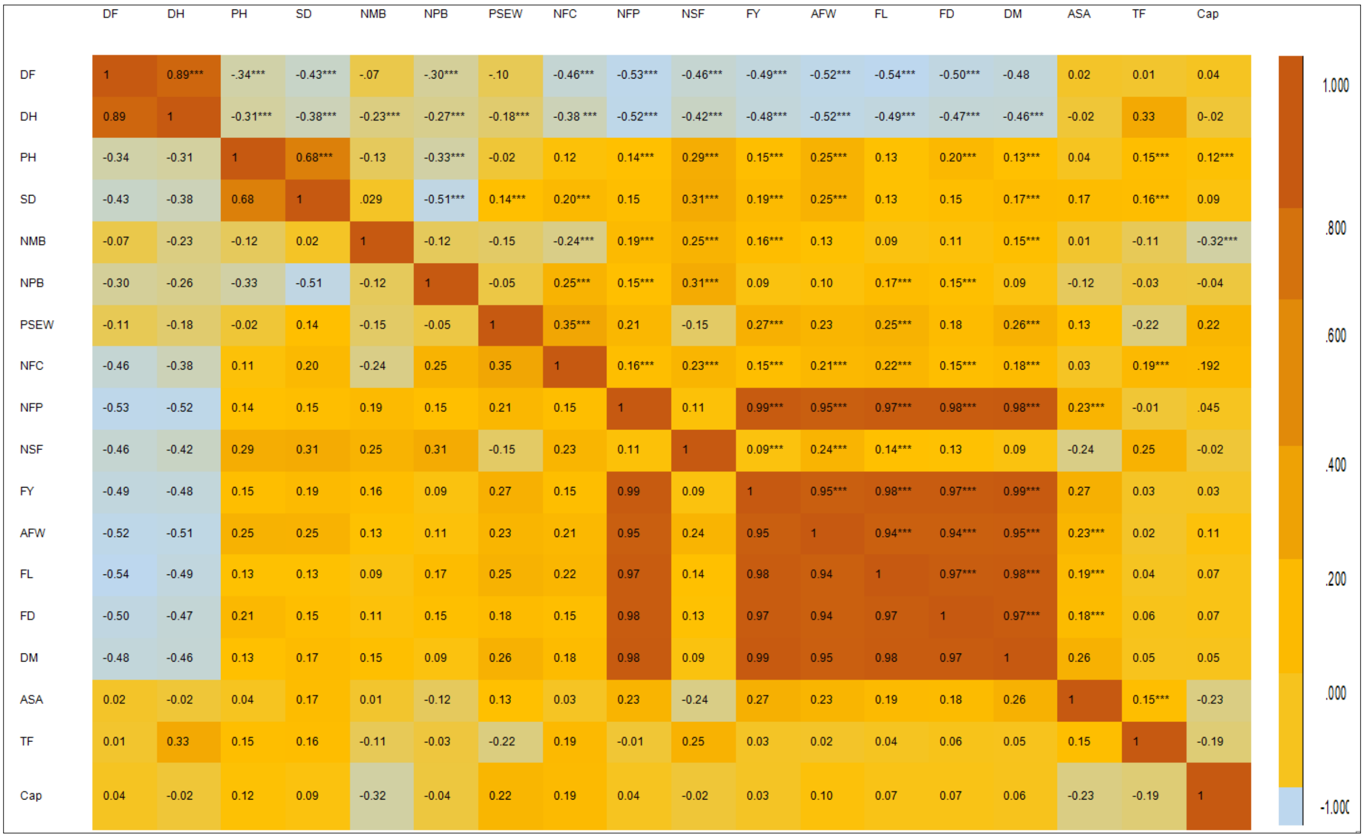
Character association studies

Correlation studies provide information that selection for one trait will improve all other positively correlated traits. Result from the correlation studies (Fig. 2) envisaged that at genotypic level the economic trait *i.e.,* fruit yield per plant, had the highest positive and significant correlation coefficient with no of fruits per plant (rg =0.99) followed by fruit diameter (rg =0.98), fruit length (rg =0.98), average fruit weight (rg =0.95), plant spread E to W (rg =0.27), stem diameter (rg =0.19) and no. of fruits per cluster (rg =0.15). The study also revealed a negative and significant association of fruit yield

Table 3. Genetic parameters (pooled over the year) of yield, related traits and quality parameters of King Chilli (*Capsicum chinense* Jacq.)

Components	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
ECV	5.72	5.96	11.66	10.26	16.21	13.81	14.21	9.89	14.58	13.49	12.63	13.39	10.18	12.08	8.01	8.13	7.76	11.14
GCV	7.77	6.87	25.65	16.50	8.82	13.34	17.79	6.52	20.75	23.30	17.47	17.94	13.77	15.57	9.47	22.48	15.02	13.73
PCV	9.65	9.09	28.18	19.43	18.46	19.19	22.77	11.85	25.36	26.93	21.55	22.39	17.13	19.71	12.40	23.9	16.90	17.68
h^2_{bs}	64.80	57.00	82.90	72.11	22.80	48.30	61.10	30.30	66.90	74.90	65.70	64.20	64.60	62.40	58.3	88.40	78.90	60.30
GA (% of mean at 5 %)	12.88	10.68	48.11	28.87	8.67	19.09	28.64	7.39	34.97	41.53	29.15	29.61	22.81	25.34	14.89	43.54	27.48	21.95
General Mean	59.41	94.91	111.17	1.37	2.05	3.49	132.65	2.95	121.64	42.08	720.90	6.084	5.48	2.22	16.13	326.12	3.95	4.24
Max.	68.03	105.85	210.16	1.75	2.44	4.78	180.42	3.33	184.33	60.33	1003.40	9.05	7.46	3.14	19.78	493.41	5.12	5.90
Min.	50.17	85.15	68.44	0.92	1.66	2.77	94.22	2.44	63.88	25.66	421.28	3.54	3.94	1.32	12.51	131.38	2.85	3.13
SE±(mean)	1.13	1.87	4.32	0.04	0.11	0.16	6.28	0.09	5.91	1.89	10.34	0.27	0.19	0.09	0.43	9.07	0.10	0.16
CD (5 %)	3.16	5.26	12.04	0.13	0.31	0.45	17.50	0.27	11.48	5.27	14.56	0.75	0.52	0.25	1.20	11.21	0.28	0.43

1-Days to 50 % flowering; 2-Days to 50 % harvesting; 3-Plant height (cm); 4-Stem Dia (cm); 5-No. of Main Branches; 6-No. of Primary Branch; 7-Plant spread E to W (cm); 8-No. of fruits/cluster; 9-No of fruits/plant; 10-No. of seeds/fruit; 11-Fruit yield/plant (g); 12-Av. frt wt (g);13-Fruit length (cm); 14-Fruit Diameter (cm); 15-Dry matter (%); 16-Ascorbic acid (mg /100g); 17-Total flavanoid (mg QE/g);18-Capsaicin content (%)



	DF	DH	PH	SD	NMB	NPB	PSEW	NFC	NFP	NSF	FY	AFW	FL	FD	DM	ASA	TF	Cap
DF	1	0.89***	-.34***	-0.43***	-.07	-.30***	-.10	-0.46***	-0.53***	-0.46***	-0.49***	-0.52***	-0.54***	-0.50***	-0.48	0.02	0.01	0.04
DH	0.89	1	-0.31***	-0.38***	-0.23***	-0.27***	-0.18***	-0.38 ***	-0.52***	-0.42***	-0.48***	-0.52***	-0.49***	-0.47***	-0.46***	-0.02	0.33	0-.02
PH	-0.34	-0.31	1	0.68***	-0.13	-0.33***	-0.02	0.12	0.14***	0.29***	0.15***	0.25***	0.13	0.20***	0.13***	0.04	0.15***	0.12***
SD	-0.43	-0.38	0.68	1	.029	-0.51***	0.14***	0.20***	0.15	0.31***	0.19***	0.25***	0.13	0.15	0.17***	0.17	0.16***	0.09
NMB	-0.07	-0.23	-0.12	0.02	1	-0.12	-0.15	-0.24***	0.19***	0.25***	0.16***	0.13	0.09	0.11	0.15***	0.01	-0.11	-0.32***
NPB	-0.30	-0.26	-0.33	-0.51	-0.12	1	-0.05	0.25***	0.15***	0.31***	0.09	0.10	0.17***	0.15***	0.09	-0.12	-0.03	-0.04
PSEW	-0.11	-0.18	-0.02	0.14	-0.15	-0.05	1	0.35***	0.21	-0.15	0.27***	0.23	0.25***	0.18	0.26***	0.13	-0.22	0.22
NFC	-0.46	-0.38	0.11	0.20	-0.24	0.25	0.35	1	0.16***	0.23***	0.15***	0.21***	0.22***	0.15***	0.18***	0.03	0.19***	.192
NFP	-0.53	-0.52	0.14	0.15	0.19	0.15	0.21	0.15	1	0.11	0.99***	0.95***	0.97***	0.98***	0.98***	0.23***	-0.01	.045
NSF	-0.46	-0.42	0.29	0.31	0.25	0.31	-0.15	0.23	0.11	1	0.09***	0.24***	0.14***	0.13	0.09	-0.24	0.25	-0.02
FY	-0.49	-0.48	0.15	0.19	0.16	0.09	0.27	0.15	0.99	0.09	1	0.95***	0.98***	0.97***	0.99***	0.27	0.03	0.03
AFW	-0.52	-0.51	0.25	0.25	0.13	0.11	0.23	0.21	0.95	0.24	0.95	1	0.94***	0.94***	0.95***	0.23***	0.02	0.11
FL	-0.54	-0.49	0.13	0.13	0.09	0.17	0.25	0.22	0.97	0.14	0.98	0.94	1	0.97***	0.98***	0.19***	0.04	0.07
FD	-0.50	-0.47	0.21	0.15	0.11	0.15	0.18	0.15	0.98	0.13	0.97	0.94	0.97	1	0.97***	0.18***	0.06	0.07
DM	-0.48	-0.46	0.13	0.17	0.15	0.09	0.26	0.18	0.98	0.09	0.99	0.95	0.98	0.97	1	0.26	0.05	0.05
ASA	0.02	-0.02	0.04	0.17	0.01	-0.12	0.13	0.03	0.23	-0.24	0.27	0.23	0.19	0.18	0.26	1	0.15***	-0.23
TF	0.01	0.33	0.15	0.16	-0.11	-0.03	-0.22	0.19	-0.01	0.25	0.03	0.02	0.04	0.06	0.05	0.15	1	-0.19
Cap	0.04	-0.02	0.12	0.09	-0.32	-0.04	0.22	0.19	0.04	-0.02	0.03	0.10	0.07	0.07	0.06	-0.23	-0.19	1

Fig 2. Correlation heat map of yield, its related traits and quality parameters in King Chilli (*Capsicum chinense* Jacq.).

***P<0.001

DF-Days to 50 % flowering; DH-Days to 50 % harvesting; PH-Plant height (cm); SD-Stem Dia (cm); NMB-No. of Main Branches; NPB-No. of Primary Branch; PSEW-Plant Spread E to W (cm); NFC-No. of fruits/cluster; NFP-No of fruits/plant; NSF-No. of seeds/fruit; FY-Fruit Yield per Plant (g); AFW -Average fruit weight (g); FL-Fruit length (cm); FD-Fruit Diameter (cm); DM- Dry Matter (%); ASA-Ascorbic Acid (mg/100g); TF-Total flavonoids (mgQE/g); Cap-Capsaicin (%).

per plant with days to 50 % flowering (rg=-0.49) and days to 50 % harvesting (rg =-0.48). This indicates that genotypes with early maturity could be an indicator for higher yield. Amongst the quality traits, dry matter % (rg=0.99) was significantly and positively correlated with fruit yield per plant. This indicates that importance should be given to these traits while improving fruit yield in king chilli.

Genetic divergence study

Knowledge of genetic divergence in the population is of utmost importance for any crop improvement programme because using genetically diverse parents within the same or different species results in wider variability. As observed from Fig. 3, the 30 king chilli accessions were grouped into 10 clusters. Amongst all the cluster group, cluster 6th, comprising of 9 accessions was the largest followed by cluster 1st (6 accessions); cluster 5th (4 accessions); cluster 2nd (3 accessions); cluster 8th and 10th (2 accessions each). It was observed that cluster 3rd, 4th, 7th and 9th are monogenotypic and comprise of brown colour, elongated, companulate shape and smooth surfaced accession, respectively. Based on the Mahalnobis Euclidean Distance (Fig. 4), the most significant inter-cluster distance (77.28) was recorded between cluster 8th and 6th, while the largest intra-cluster distance (18.45) was found between cluster 6th and 5th.

The percentage contribution of each trait towards the total genetic divergence of king chilli accessions was studied to rank the traits based on their contribution towards divergence observed. As depicted in Fig. 5, amongst all the traits studied plant height (cm), no. of main branches, plant spread E to W (cm) and no. of primary branch contribute maximum to the total genetic divergence amongst the accessions of king chilli. There was an insignificant contribution to total divergence by all the quality traits studied.

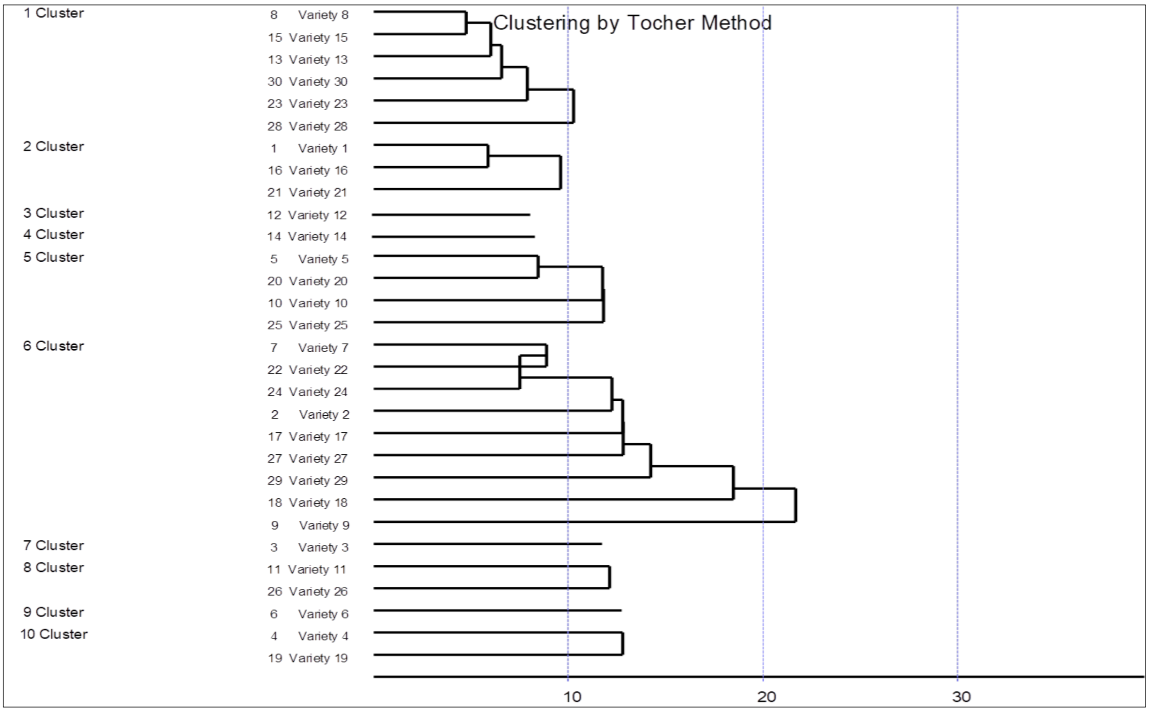


Fig. 3. Pattern of clustering in King Chilli (*Capsicum chinense* Jacq.) accessions.

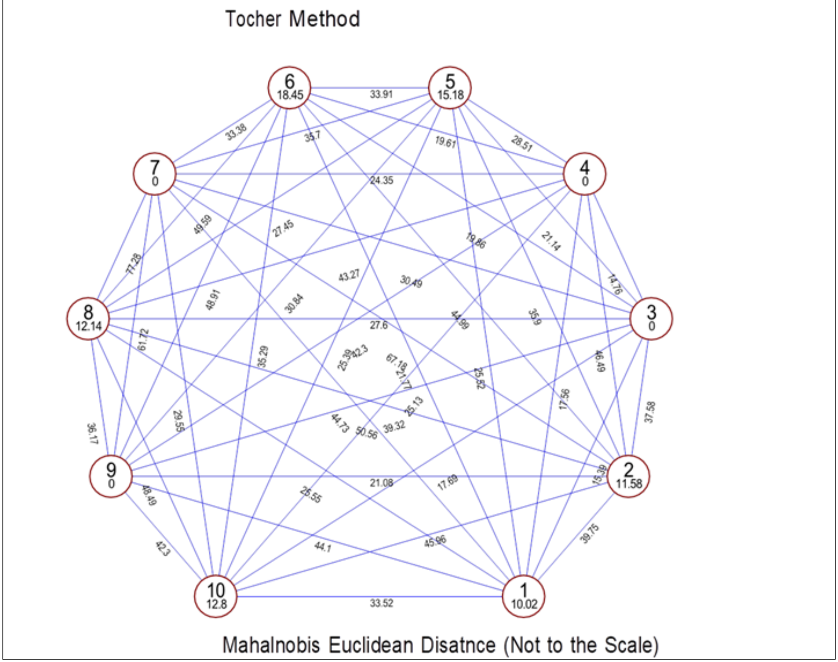


Fig. 4. Graphical representation of cluster distance of 30 genotypes of King Chilli (*Capsicum chinense* Jacq).

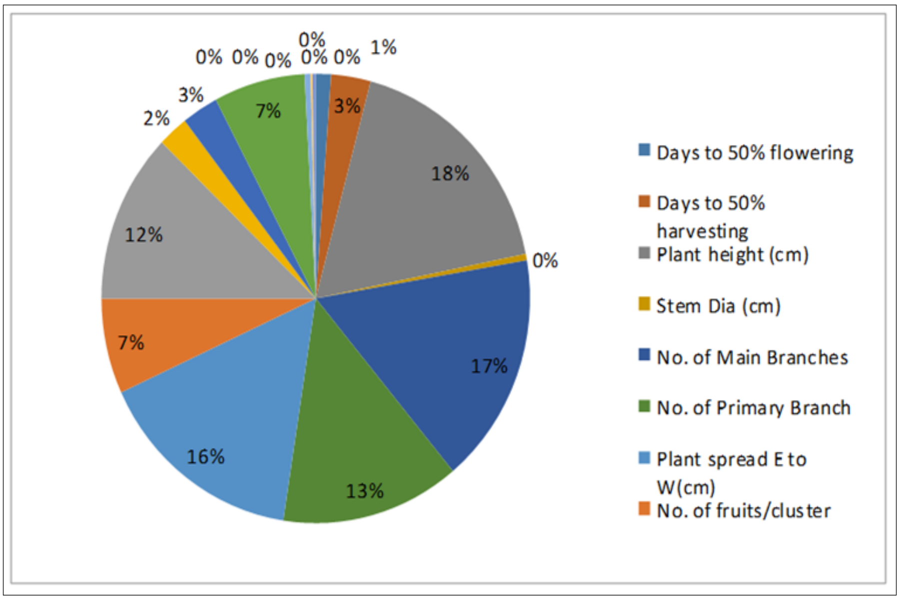


Fig. 5. Graphical representation of major traits contributing towards the total divergence of King Chilli (*Capsicum chinense* Jacq.).

Genotype × environment interaction

In case of often cross-pollinated crop such as king chilli where a high percentage of natural cross pollination is reported, the stability of selected promising accessions in its expression of phenotypic traits becomes indispensable. The performance of such accessions is mostly influenced by the environmental conditions where they are grown. Therefore, the Genotype × Environment interaction study is essential to identify stable accession for its utilization in future breeding programmes.

The pooled data was analyzed across the environments to estimate genotype × environment interaction (Table 4). The mean sum of square due to genotype × environment interaction was significant for no. of primary branch, ascorbic acid and capsaicin. The mean sum of square due to the environment linear was also significant for all the traits except stem diameter and no. of fruits per cluster. Environment × Genotype (Linear) mean sum of square were substantial for the traits viz., plant height, no. of primary branch, no. of seeds per fruit, dry matter (%), ascorbic acid content and capsaicin content. This indicated the linear contribution of environmental effects and additive environmental variance on these traits and the accessions significantly differing for their linear response to environments, respectively.

Stability parameters

The stability parameters *viz.,* mean, regression coefficient (bi) and deviation from linear regression (s^2di) of all the traits under study were calculated from the pooled data for 30 accessions to assess their stability across environments. A genotype is considered stable for the trait to diverse environmental conditions if it has high mean performance, a regression coefficient (bi=1) nearing unity and a non-significant deviation from linear regression ($S^2di=0$) (19). In the present study, the accession with lower mean value (days to 50 % flowering; days to 50 % harvesting) and higher mean value for the rest of the traits, regression coefficient around unity and low deviation mean squares nearing zero were regarded as stable. Similarly, accessions with regression coefficient (bi) < 1 and > 1 were considered above average and below average stable, respectively.

The stability parameters for yield, related and quality traits are enumerated in Table S1-S4. It was observed from the result that the environmental conditions primarily influence early maturity in king chilli and none of the accessions studied was stable across environments. Likewise, it was visible from the study that the environment affects the growth habit of king chilli in a significant way. Regarding fruit yield per plant (Table S3), the accessions RCNL1 was stable across environments as predicted by bi value = 1.02, mean value greater than population mean and non-significant linear regression. The promising genotypes for yield, related and quality traits based on stability study are listed in Table 5 & 6.

Principal component analysis

The PCA is a nonparametric approach to get the small number of factors which contribute the maximum variability to the total variability. From Table 7, it is observed that 7 PCs had more than 1 Eigen value, contributing maximum variability to the total variations with a diversity percentage of 84.53 %. 7 PCs *viz.,* PC 1 (6.14), PC 2 (4.37), PC 3 (3.38), PC 4 (2.35), PC 5 (1.87), PC 6 (1.14) and PC 7 (1.01) showed greater than 1 Eigen values. These PCs were utilized for an explanation of percentage of variation contributed to total variation. The PC 7contributed the maximum (84.53 %) followed by PC 6 (80.31 %), PC 5 (75.55 %), PC 4 (67.72 %), PC 3 (57.89 %), PC 2 (43.79 %) and PC 1 (25.58 %) of the total variation. The graphical representation of the seven PCAs is depicted in Fig. 6. The 7 PCs represent a significant degree of genetic variability and maximum contribution to genetic diversity amongst the accessions. The contribution of yield, its related and quality traits to the PC is shown in Table 7. Days to 50 % flowering (0.25), days to 50 % harvesting (0.23), no. of primary branch (0.18), no. of fruits per cluster (0.15), fruit length (0.24) and dry matter % (0.01) depicted maximum positive contribution to total divergence in PC 1. Likewise the individual positive and negative factor loading observed from each traits to all the PCs are shown in detail in Table 7.

Table 4. Pooled analysis of variance for stability of traits across environments in King Chilli (*Capsicum chinense* Jacq.)

Source of variation	df	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Genotypes	29	67.76 ***	138.02 ***	2495.80 ***	0.16 ***	0.13	0.72 ***	1789.27 ***	0.14	2016.02 ***	299.183***	50317.51 ***	3.79 ***	1.81 ***	0.381***	7.55***	41487.71***	1.08***	1.09 ***
Environment	2	62.56***	306.44***	3821.34***	0.01	0.01	4.69***	177.10	0.11	3315.11**	408.316***	82023.27**	3.92**	13.53***	2.470***	40.75**	366352.80***	16.03***	1.17***
Genotype × Environment	58	7.24	22.43	185.08	0.02	0.13	0.28 **	428.80	.09	384.42	38.238	9705.41	0.80	0.354	0.083	1.56	1722.98 ***	0.08	0.25 ***
Environment (Linear)	1	125.12 ***	612.88 ***	7642.69 ***	0.02	0.01	9.38 ***	354.20	0.22	6630.22 ***	816.632***	164046.50 ***	7.84 ***	27.06 ***	4.940***	81.50***	732705.60***	32.05***	2.35 ***
Environment × Genotype (Linear)	29	7.65	22.25	241.97*	0.02	0.15	0.45***	451.01	0.06	456.82	52.322*	7539.29	1.00	0.399	0.076	2.13*	2929.64***	0.05	0.47***
Pooled deviation	30	6.60 ***	21.86 ***	123.92 ***	0.02 ***	0.11 ***	0.10 ***	393.05 ***	0.12 ***	301.62 ***	23.34 ***	11475.8 ***	0.578 ***	0.29 ***	0.08***	0.96 ***	499.11 ***	0.11***	0.03 **
Pooled Error	174	2.73	6.75	12.98	0.00	0.00	0.01	14.88	0.01	11.83	1.59	448.81	0.02	0.02	0.00	0.22	217.82	0.01	0.02

*P < 0.05 ; **P< 0.01; ***P<0.001; 1-Days to 50 % flowering; 2-Days to 50 % harvesting; 3-Plant height (cm); 4-Stem Dia (cm); 5-No. of Main Branches; 6-No. of Primary Branch; 7-Plant spread E to W (cm); 8-No. of fruits/cluster; 9-No of fruits/plant; 10-No. of seeds/fruit; 11- Fruit yield/plant; 12- Av. Frt. Wt (g) 13 -Fruit length (cm); 14- Fruit Diameter (cm); 15-Dry matter (%); 16-Ascorbic acid (mg/100g); 17-Total flavonoids (mgQE/g); 18-Capsaicin content (%)

Table 5. Promising Accessions of King Chilli (*Capsicum chinense* Jacq.) based on stability for yield and related traits

Sno.	Traits	Accessions	Stability Analysis
1.	Days to 50 % flowering	RCML-1	Stable
2.	Days to 50 % harvesting	-	-
3.	Plant Height (cm)	RCMP3	Stable
4.	Stem Diameter (cm)	RCML-16	Responsive to favourable environmental conditions
5.	No. of main branches	-	-
6.	No of primary branch	RCML7, RCML8, RCML10, RCML9 and RCNL3	Responsive to unfavourable environmental conditions
7.	Plant Spread (E to W)	RCML12	Stable
8	Yield per plant (g)	a) RCMP1, RCML3, RCML11 and RCML1	Responsive to unfavourable environmental conditions
		b) RCMP4**,** RCNL4 and RCNL2	Responsive to favourable environmental conditions
		c) RCNL1	Stable
9.	No. of fruits per cluster	RCMP1 and RCML4	Responsive to favourable environmental conditions
10.	No. of fruits per plant	a) RCMP5, RCML3 RCML5 and RCML2	Responsive to unfavourable environmental conditions
		b) RCML1	Responsive to favourable environmental conditions
		c) RCNL1	Stable
11.	No. of seeds per fruit	a) RCMP4 and RCNL4	Responsive to favourable environmental conditions
		b) RCMP7, RCMP1 and RCML14	Responsive to unfavourable environmental conditions
		c) RCMP2	Stable
12.	Average fruit weight (g)	RCML5, RCMP9, RCMP1 and RCML1	Responsive to favourable environmental conditions
13.	Fruit Length (cm)	a) RCML3, RCNL3, RCML5, RCMP8, RCNL4 and RCML6	Responsive to favourable environmental conditions
		b) RCMP5, RCNL1, RC ML2 and RCML1	Responsive to unfavourable environmental condition
		c) RCMP4	Stable
14.	Fruit Diameter	a)RCMP8, RCML3, RCML6, RCNL5, RCML4, RCNL3 and RCNL4	Responsive to favourable environmental condition
		b)RCNL1, RCML12 and RCML15	Responsive to unfavourable environmental condition
		c) RCML16	Stable

Table 6. Promising accessions King Chilli (*Capsicum chinense* Jacq.) based on stability for quality parameters

SNo.	Traits	Accessions	Stability analysis
1.	Dry matter (%)	a)RCNL1, RCMP1, RCML5, RCMP5 and RCML12	Responsive to unfavourable environment condition
		b)RCNL4 and RCML3	Responsive to favourable environmental condition
		c) RCMP4, RCNL2	Stable
2.	Ascorbic acid content	a) RCMP5, RCML1 and RCNL5	Responsive to favourable environmental condition
		b)RCML11	Stable
3.	Total flavonoids (mgQE/g)	a)RCML7, RCML8 and RCML6	Responsive to unfavourable environment condition
		b)RCML12, RCNL4 and RCNL2	Stable
4.	Capsaicin (%)	-	-

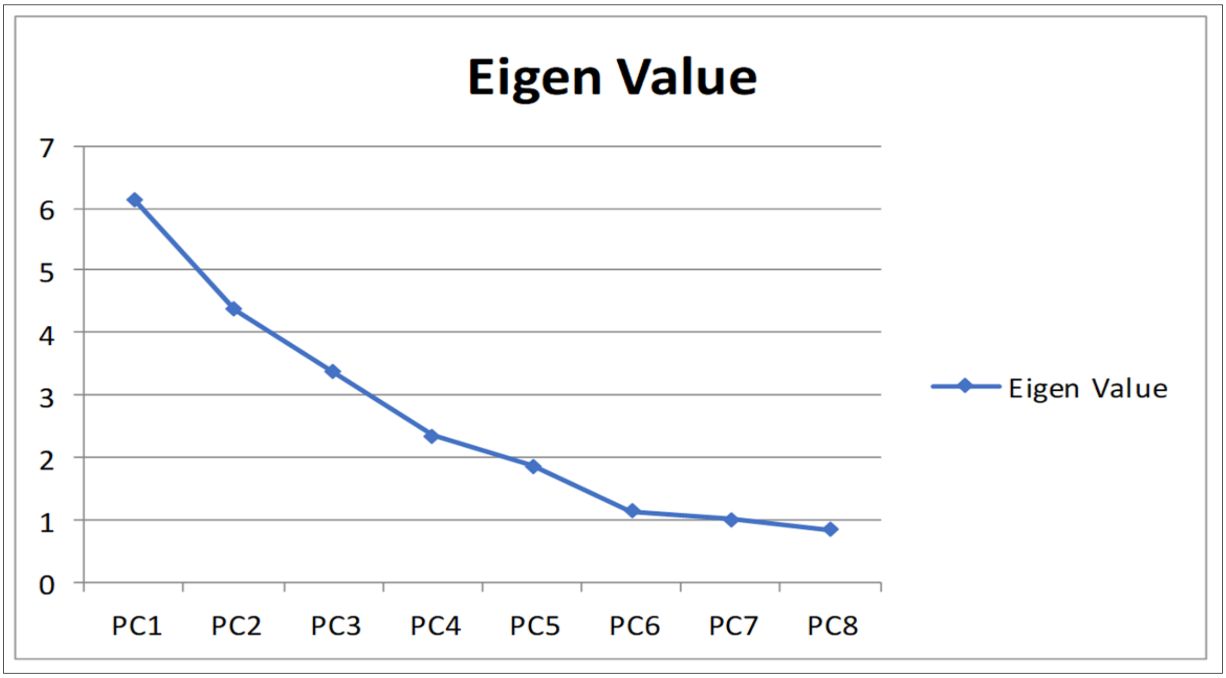


Fig 6. Principal component depicting the contribution to total variation of King Chilli (*Capsicum chinense* Jacq.).

Table 7. Eigen values and Eigen vectors for 18 quantitative and quality traits in King Chilli (*Capsicum chinense* Jacq.)

PCs	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
% Variance expected	25.58	18.21	14.09	9.83	7.82	4.76	4.21	3.57
Cumulative variance expected	25.85	43.79	57.89	67.72	75.55	80.31	84.53	88.10
Eigenvalue (root)	6.14	4.37	3.38	2.35	1.87	1.14	1.01	0.85
Days to 50 % flowering	0.25	0.06	0.28	0.01	0.22	0.03	0.13	0.28
Days to 50 % harvesting	0.23	-0.02	0.34	-0.17	0.08	-0.16	0.04	0.17
Plant height (cm)	-0.30	-0.05	0.17	0.01	0.11	-0.27	0.27	0.15
Stem Dia (cm)	-0.33	0.13	0.20	0.001	-0.09	-0.01	-0.11	-0.04
No. of main branches	-0.32	0.01	-0.02	0.12	-0.01	0.28	-0.05	-0.23
No. of primary branch	0.18	-0.19	-0.34	-0.10	-0.08	-0.05	0.26	0.15
Plant spread E to W (cm)	-0.06	0.43	-0.09	-0.10	0.01	-0.17	-0.09	-0.04
No. of fruits/cluster	0.15	0.01	-0.11	-0.22	-0.39	0.11	-0.15	0.23
No of fruits/plant	-0.05	-0.08	-0.23	-0.15	0.49	-0.11	0.10	-0.26
No. of seeds/fruit	-0.12	-0.18	-0.34	-0.11	-0.20	0.14	-0.11	0.19
Fruit yield per plant (g)	-0.28	-0.15	-0.14	-0.26	0.17	-0.13	-0.03	0.07
Av. Frtwt (g)	-0.32	0.10	-0.18	-0.11	-0.15	-0.14	0.04	0.11
Fruit length (cm)	0.24	-0.16	-0.29	-0.09	0.17	-0.11	-0.08	-0.07
Fruit diameter (cm)	-0.24	-0.27	-0.12	0.04	0.13	-0.27	0.17	0.12
Dry matter (%)	0.01	-0.38	0.22	0.11	0.14	0.19	-0.06	-0.03
Ascorbic acid content (mg/100g)	-0.15	0.09	0.01	-0.34	0.23	0.43	0.17	-0.17
Total flavanoids (mgQE/g)	-0.13	-0.12	0.23	-0.47	-0.18	-0.00	-0.03	-0.06
Capsaiscin (%)	-0.14	0.26	0.04	0.03	0.19	0.08	0.22	0.55

Discussion

For development of improved variety or hybrid through any breeding strategy pre-breeding study such as genetic parameters, diversity, association study, path analysis and information of satiability is essential. The results of the present study (Table 3) reflect wide variability amongst the 30 accessions for all the traits studied. Earlier findings also revealed significant variation in chilli germplasm's quantitative and qualitative traits (20-22). The genetic parameters study revealed higher PCV value than GCV for all the traits studied reflecting the environmental effects on the expression of these traits. The wider difference between PCV and GCV value indicates the substantial role of the environment and lesser difference indicates more influence of genetic makeup on the expression of the traits under study. High heritability is the measure of selection efficiency based on phenotypic performance but does not necessarily indicate high genetic gain for the specific trait. So high heritability, coupled with high values of genetic advance as a percentage of mean (> 20 %) is desirable for effective selection. The present study revealed (high heritability + high GA) for most of the traits studied except for few traits *viz.,* days to 50 % flowering, days to 50 % harvesting, no of main branches, no. of primary branch and no. of fruit per cluster indicating predominance of additive gene action and efficiency for effective selection in those traits. Amongst the quality parameters ascorbic acid content depicted high heritability (>60 %) with high GA (>20 %), while total flavonoid and capsaicin content exhibited high heritability with moderate GA (>10 %). This indicates the prevalent of additive gene hence selection of these traits would be effective for further improvement in *Capsicum chinense* Jacq. These findings aligned with the earlier report where high heritability with high Genetic Advance was reported in yield per plant, plant height, plant spread, fruit length, fruit diameter, number of fruits per plant, fruit weight and number of seeds per fruit (23-27).

The correlation studies of the pooled data reveal higher genotypic correlation coefficient than phenotypic correlation coefficient for most of the traits studied indicating the presence of inherent association between different traits at genetic level (28-30). The economic trait fruit yield per plant as a dependent variable was found to have high significant positive correlation with the independent variable *viz.,* no of fruits per plant, fruit diameter, fruit length, average fruit weight, plant spread, stem diameter and no. of fruits per cluster. The strong association of fruit yield with these traits indicated that these traits could indicate higher yield and priority should be given while selecting for improving fruit yield in future breeding programmes. Research indictes that positive correlation of fruit yield with the numbers of fruit per plant, fresh fruit weight, fruit length and fruit girth in chilli (31). A negative and significant association of fruit yield per plant was observed with days to 50 % flowering and days to 50 % harvesting. This indicates that accessions with early maturity could indicate higher yield. The result is justifiable as early maturity will lead to an extended harvest duration and hence more yield and result aligns with previous reports (32, 33).

The genetic divergent study revealed grouping of accessions into polygenotypic and monogenotypic clusters reflecting the heterogeneity of the accessions. Those accessions belonging to monogenotypic cluster have unique genetic characteristics that can be further utilized for the improvement of specific trait in future breeding programmes of king chilli. The result aligns with research findings (34). Among all the traits studied, plant height contributed the most to the genetic divergence. Research also reported plant height as the maximum contributor to diversity in chilli accessions he evaluated (35).

The stability analysis across three different years highlights the range of adaptability of all the 30 accessions studied. Very few accessions were found stable for each traits studied. This may be due to the heterogeneity cause by high percentage of natural cross-pollination in this particular capsicum species (10). The study results lead to identifying promising accessions with specific response to poor or favourable environments for each trait, thereby contributing to better breeding programs of king chilli. Amongst all the accessions, RCNL1 was stable across environments as predicted by bi value near unity, mean value greater than the population mean and a non-significant linear regression. For capsaicin content, one of the most important quality traits in king chilli, none of the accessions were stable or responsive to the environment. This indicates the enormous role of environmental factors in expressing this trait. Research also identified stable and responsive lines in chilli germplasm (36, 21).

The Principal Component Analysis (PCA) revealed that a trivial numbers of eigenvectors could apprehend the significant morphological variations among the accessions. The PCs with eigen values of 6.14, 4.37 and 3.38 resulted in 25.58 %, 18.21 % and 14.09 % of variance, respectively and would be of importance in framing selection criteria and identification of superior lines from a given breeding population. Similar findings were also reported by previous studies (37).

Conclusion

The result of the present study shows that the accessions RCML5, RCML1 and RCNL4 performed well for yield and most of its related traits and exhibited the nearest possibility of giving higher yield and could be utilized in future breeding programme. Similarly for developing early variety or hybrids accessions RCML5, RCML6 and RCML7 are at par and could be exploited. For capsaicin content the accession RCML16, RCML5 and RCML10 are promising. Based on stability analysis accession RCNL1, a moderate yielder and mid maturity with average capsaicin content, was stable across environments. This accession could be utilized to develop improved variety through recurrent selection in the future king chilli crop improvement programme. The farmers could also obtain higher and sustainable yield by cultivation of stable lines identified from the study.

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Authors' contributions

MBD was responsible for data collection, compilation, result interpretation, and writing the manuscript. VKV handled the documentation and interpretation of results and findings. HDT contributed to the documentation and reviewed the manuscript. HR assisted in interpreting the results. PR conducted the literature review. LJC carried out the laboratory analysis. HJS worked on data documentation and interpretation. YBK contributed to the documentation and interpretation of research data. AY performed the statistical analysis. NUS was also involved in statistical analysis. MBS contributed to the manuscript writing. SH supported the documentation and interpretation of the results. All authors read and approved the final version of the manuscript.

Compliance with ethical standards

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

Ethical issues: None

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