Received: 03 Nov 2022 | Accepted: 30 Jan 2023 | Online: 04 Feb 2023

Electronic Journal of Plant Breeding

Research Note

Biochemical and morphological diversity of chickpea (*Cicer arietinum*. L)

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Abstract
Principal component analysis and hierarchical cluster analysis are the best tools to measure the degree of divergence and to suggest the parents for future crop improvement programmes. A study was done using 64 chickpea genotypes including *desi* and *kabuli* types provided from RARS, Nandyal. Research was conducted at Agricultural College Farm, Bapatla during *Rabi* 2021-22 in 8×8 square lattice design. Data was collected for 13 quantitative traits from five randomly selected and six biochemical traits were also estimated. Windostat version 9.3 statistical software was used for analysis of the data. Principal component analysis identified first six principal components with eigen value more than one and they accounted for 76.54 % of cumulative variance. Using ward's method, 64 genotypes were grouped into six clusters. Maximum inter cluster distance was found between cluster IV and cluster V followed by Cluster II and Cluster IV. Maximum intra cluster distance was observed within cluster IV followed by Cluster V. These studies revealed sufficient divergence among the genotypes for the traits studied.

Keywords: Chickpea, Genetic diversity, Hierarchical cluster, PCA

Chickpea (*Cicer arietinum* L.) is an important *rabi* pulse crop with several potential health benefits and provides an affordable alternative to animal protein. It is a self-pollinating crop with papilionaceous corolla and diploid chromosome number (2n = 2x = 16). With 116.2 lakh tonnes grown on 112 lakh hectares and a productivity of 1036 kg/hectare, India accounts for 70% of total global chickpea production. In India, chickpea is the major pulse crop grown followed by redgram. Andhra Pradesh is India's sixth largest producer of chickpea, producing 5.66 lakh tonnes on an area of 4.65 lakh hectares with a productivity of 1218 kg/hectare. (Annual report, All India Co-ordinated Chickpea Research Project, 2020-21). Chickpea seeds are the cheapest source of protein in developing countries, helping to reduce malnutrition. Chickpea seed proteins have received attention in recent years due to their anticancer, antidiabetic and antiHIV-1 reverse transcriptase effects (Bhagyawant *et al*., 2018). Chickpea seed, in comparison to other legumes, is relatively devoid of protein antinutrients such as lectins, but it does contain phytates, saponins and tannins, which are trypsin inhibitors that reduce seed protein bioavailability. Phytic acid binds with other minerals and ions thus reducing their bioavailability, but the phytate phosphorous aids in germination of chickpea seed thus it is essential for growth and

development. Phenols bind to proteins and minerals thus reducing biological functioning. The dark coloured seed coat in *desi* genotypes is due to difference in tannic acid concentration. Hence quantifying and understanding the mechanism of action of these non-nutritional compounds is an important challenge in future (Singh *et al.,* 2015).

Before conducting any hybridization, genetic variation within and between species is critical. Furthermore, genetic diversity aids in identifying diverse lines that can be included in crop improvement. To increase chickpea yield, it is necessary to improve the ability of associated seed yield characters and to take advantage of the diversity found in the germplasm. The study of genetic diversity in any gene pool aids in identifying superior parents for hybridization, which also aids in the development of improved varieties. Principal component analysis (PCA) is a non-parametric method for breaking down complex data into simple data sets. It is also known as canonical vector analysis, a multivariate technique that derives canonical vectors or roots that represent different axes of differentiation and the amount of variation accounted for by each of these axes, respectively (Rao, 1952). Principal component scores for genotypes were used as an input for clustering using Ward's minimum variance method (Ward, 1963). The present study aimed to study divergence in the selected germplasm of chickpea using principal component analysis and hierarchical cluster analysis.

Present study comprised of 64 chickpea genotypes including both *desi* and *kabuli*, provided by RARS, Nandyal, which included released and advanced breeding lines. Research was conducted at Agricultural College Farm, Bapatla during *Rabi* 2021-22 in 8×8 square lattice design. Data was recorded for three phenological traits (days to first flowering, days to 50% flowering and days to Maturity), 10 quantitative characters (plant height, number of primary branches per plant, number of secondary branches per plant, number of pods per plant, number of seeds per pod, total number of seeds per plant, biological yield, harvest index, 100 seed weight, seed yield per plot) and six quality parameters [protein content (Lowry *et al*. 1951), total free amino acid (Moore and Stein ,1984), phytic acid (Wheeler and Ferrel ,1971), tannic acid (Schanderl,1970), total phenolic content (Malik and Singh ,1980), total flavonoid content (Swain and Hillis ,1959)]. Sowing was done in black cotton soils with a row length of 4m and 3rows per genotype with a spacing of 30×10 cm. Data was collected from five randomly selected plants preferably in the middle row. Mean values were indicated for all the traits. For biochemical analysis, seed was ground into fine powder which is sieved and used for analysis. Data analysis for principal component analysis (PCA) was carried according to procedure described by Banfield (1978). Agglomerative hierarchical clustering technique was done as per Anderberg (1993).

The standardisation of columns in principal component analysis (PCA) on correlation matrix created 19 new variables for 64 genotypes without changing their relative positions. Each principal component is a linear combination of the 19 data matrix attributes. The loading values are scaled or standardised so that the sum of the squares of the loadings within a principal component equals one. The loadings are regarded as weights that define the contribution of characters in each principal component. Loadings, like regression coefficients, have a sign (+ / -) that indicates the direction of contribution. However, unlike regression, only the relative contributions matter, so all signs can be changed without affecting the analysis (Jackson, 1991).

In the present study ,PCA was used to validate the clustering pattern of 64 chickpea genotypes. **Table 1** indicates the contribution of the 19 characters towards the total divergence. Eigen values, variance (%) and cumulative variance (%) by the principal components (PC_s) in chickpea accessions evaluated are furnished in **Table 2**. The results suggested the importance of the first six PC_s with eigen values greater than or equal to one in discriminating the germplasm collection. The results revealed that six canonical roots accounted for 76.54 per cent of total divergence. PC_1 contributed maximum towards divergence (21.65%) with eigen value of 4.113. The second, third, fourth, fifth and sixth canonical vectors contributed 18.26%, 13.71%, 9.13%, 7.80% and 5.99% to total divergence, respectively (**Table 2**). First three principal components were considered as three axes X, Y and Z , and squared distance of each genotype, from these three axes was calculated.

The analysis identified maximum seed yield contributing characters *i.e*., number of secondary branches per plant, number of pods per plant, number of seeds per pod, total number of seeds per plant, harvest index in PC_1 and important quality parameters like protein content, total free amino acids, total flavonoid content in PC_3. It is important to study the variance as the relative contribution than the signs (indicative of direction) in PCA. The 2D plot (**Fig. 1**.) indicated that *desi* genotypes NBeG 452, NBeG 1639, NBeG1427, NBeG 1637, NBeG 1420and NBeG 1658 and *kabuli* genotypes NBeG 1706, ICCV 2, NBeG 1593, NBeG 1702, NBeG 1529, LBeG 7, NBeG 1554, NBeG 1532 and NBeG 1509 were divergent for yield and quality traits. Hence these diverse genotypes can be suggested for future crop improvement programmes. From the present study it was also observed that simultaneous selection for yield and quality traits may not be possible and balanced selection criteria should be followed depending on the objective. Similar studies confirming using PCA were reported by Samyuktha *et al.* (2017), Vijayakumar *et al.* (2017), Jain *et al.* (2020), Janghel *et al.* (2020), Rajani *et al.* (2020), Vishnu *et al*. (2020) and Jayalakshmi *et al*. (2022).

Table 1. Contribution of different characters towards genetic divergence in 64 genotypes of chickpea.

S. No.	Character	Times ranked 1st	Contribution %
1.	Days to first flowering	0	0.00%
2.	Days to 50% flowering	0	0.00%
3.	Days to maturity	37	1.84%
4.	Plant height (cm)	6	0.30%
5.	Number of primary branches per plant	1	0.05%
6.	Number of secondary branches per plant	0	0.00%
7.	Number of pods per plant	16	0.79%
8.	Number of seeds per pod	0	0.00%
9.	Total number of seeds per plant	117	5.80%
10.	Biological yield (g)	146	7.24%
11.	Harvest Index (%)	759	37.65%
12.	100 Seed weight (g)	341	16.91%
13.	Protein content (%)	0	0.00%
14	Total free amino acid (mg/g)	98	4.86%
15	Phytic acid (mg/g)	2	0.10%
16	Tannic acid (mg/g)	242	12.00%
17	Total phenolic content (mg/g)	137	6.80%
18	Total flavonoid content (mg/g)	106	5.26%
19	Seed yield per plot (g)	8	0.40%

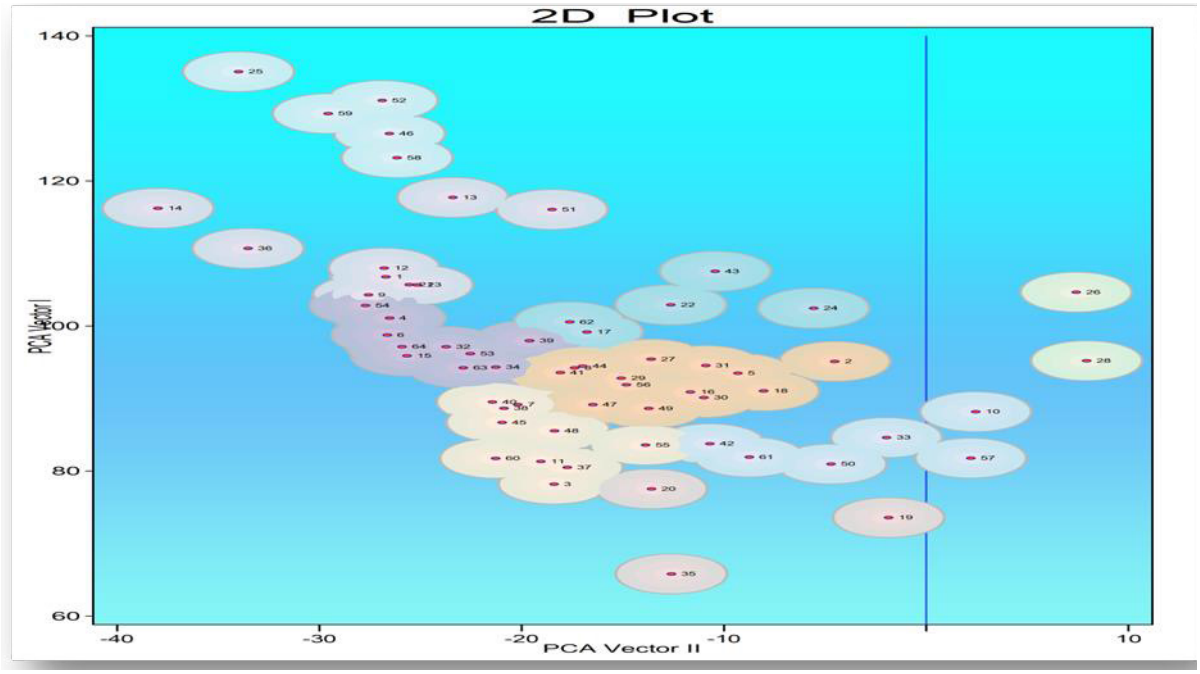

Fig. 1. Two dimensional (2D) graph based on PCA scores showing relative positions of 64 genotypes of chickpea.

Table 2. Canonical vectors for 19 characters in 64 genotypes in chickpea.

S. No.	Parameter	PC_1	PC_2	PC_3	PC_4	PC_5	PC_6
1.	Eigen Value (Root)	4.1134	3.4685	2.6052	1.7343	1.4824	1.1389
2.	% Var. Exp.	21.65	18.26	13.71	9.13	7.80	5.99
3.	Cum. Var. Exp.	21.65	39.90	53.62	62.74	70.55	76.54
S. No.	**Character**						
1.	Days to first flowering	0.2459	0.0833	0.1482	0.3701	0.0686	0.1508
2.	Days to 50% flowering	0.1748	0.1200	0.0469	0.0922	0.4605	0.4387
3.	Days to maturity	0.0850	-0.0096	-0.2210	0.3553	-0.3893	-0.2009
4.	Plant height (cm)	0.2431	0.1975	0.3612	0.2041	0.0483	-0.0418
5.	Number of primary branches per Plant	-0.0365	-0.0952	0.1129	-0.5132	0.1190	-0.2269
6.	Number of secondary branches per plant	0.2217	0.2496	0.2872	-0.1859	0.2460	0.0054
7.	Number of pods per plant	0.2292	0.3867	-0.2557	-0.0213	-0.0739	0.1027
8.	Number of seeds per pod	0.1420	0.0427	0.2062	0.1497	-0.0300	-0.6652
9.	Total number of seeds per plant	0.1535	0.3396	-0.3914	-0.1052	-0.0864	-0.0761
10.	Biological yield (g)	0.3948	-0.1768	0.0090	-0.1285	-0.2466	0.1042
11.	Harvest index (%)	0.4178	-0.1941	-0.0976	-0.1214	-0.0265	0.0424
12.	100 Seed weight (g)	0.1664	-0.0553	0.4056	0.1503	-0.4079	0.1479
13.	Protein content (%)	-0.0670	-0.3876	0.2073	0.0971	0.0720	0.0053
14.	Total free amino acid (mg/g)	0.2217	0.2378	0.1609	0.0381	0.1601	-0.2467
15.	Phytic acid (mg/g)	-0.3109	0.3022	0.0493	0.1886	-0.1817	0.1077
16.	Tannic acid (mg/g)	-0.0833	-0.1197	-0.1777	0.3785	0.4848	-0.2867
17.	Total phenolic content (mg/g)	0.2467	-0.3579	-0.0082	-0.0818	0.0130	-0.0075
18.	Total flavonoid content (mg/g)	-0.0141	0.2786	0.1369	-0.3202	-0.0670	-0.1845
19.	Seed yield per plot (g)	0.3456	-0.1066	-0.3801	0.0455	0.0974	-0.0953

The 64 chickpea genotypes were grouped into six clusters using ward's minimum variance method. Cluster means were computed for the 19 characters on pooled basis and same are presented in **Table 3**. Mean performance of top three clusters for future crop improvement programme is presented in **Table 4**. Cluster I registered high mean values for number of primary branches per plant and total phenol content. Cluster II recorded high mean values for protein content, phytic acid and tannic acid. Cluster III was observed to have the highest mean values for harvest index. Cluster IV recorded highest mean values for plant height, number of secondary branches per plant, number of pods per plant, number of seeds per pod, total number of seeds per plant, total free amino acid, total flavonoid content and seed yield per plot. Cluster V recorded minimal values for phenological traits, which have value in breeding programme with an objective of developing short duration varieties. Cluster VI was observed to record high mean value for days to first flowering, days to 50% flowering, days to maturity, biological yield, 100 seed weight. Thus, cluster IV showed highest mean values for important yield contributing traits. Cluster II exhibited high mean values for biochemical parameters like protein content and antinutritional factors like phytic acid and tannic acid. So, genotypes from these clusters can be used in future yield and quality improvement programmes. Dendrogram was constructed using ward's minimum variance method and the same is presented in **Fig. 2**. Detailed clustering pattern of genotypes based on dendrogram is presented in **Table 5**.

The inter cluster distance between Cluster IV (with highest mean for seed yield contributing characters and some quality traits) and Cluster V was maximum followed by cluster II (with highest mean for protein content) and Cluster IV (high mean for yield contributing characters). Average intra and inter-cluster eucledian^2 values among six clusters is presented in **Table 6**. Nearest and farthest cluster with respect to each cluster using ward's minimum variance method was calculated and the same is presented in **Table 7**. Therefore, selection for hybridization between genotypes of the above clusters can result in superior varieties. The genotypes from cluster IV *viz*; NBeG 1129 and NBeG 1639 of *desi*, NBeG 1509 and NBeG 1554 of *kabuli* types can be used for future yield improvement programme as this cluster recorded high mean values for major yield contributing characters.

Principal component analysis identified first six principal components with eigen value more than one and accounts for 76.54 % of cumulative variance. The 2D and 3D plots indicated that the *desi* genotypes *viz*; NBeG 452, NBeG

Table 3. Cluster means of six clusters estimated by ward's minimum variance method in 64 genotypes of chickpea.

Character	Cluster number					
	I	II	III	IV	V	VI
Days to first flowering	43.28	43.27	43.35	46.50	41.57	**47.07**
Days to 50% flowering	47.67	47.42	47.80	49.75	46.64	**51.21**
Days to maturity	89.74	90.42	90.25	91.13	84.21	**91.93**
Plant height (cm)	49.67	45.66	49.25	**60.70**	48.24	55.63
Number of primary branches per plant	**3.51**	3.37	3.42	3.48	3.40	3.45
Number of secondary branches per plant	12.55	10.91	12.28	**13.68**	11.10	12.16
Number of pods per plant	57.82	47.17	67.48	**76.58**	35.38	50.83
Number of seeds per pod	1.19	1.05	1.17	**1.25**	1.07	1.04
Total number of seeds per plant	70.80	49.45	79.11	**101.12**	37.25	51.87
Biological yield (g)	24.37	23.11	25.52	26.30	17.69	**32.18**
Harvest Index (%)	41.52	45.37	**49.12**	41.36	46.23	41.34
100 Seed weight (g)	26.97	27.36	23.07	31.09	25.40	**32.64**
Protein content (%)	18.84	**19.60**	18.83	19.15	18.58	19.02
Total free amino acid (mg/g)	9.75	8.64	8.49	**10.30**	8.61	8.45
Phytic acid (mg/g)	14.22	**14.17**	13.97	14.53	14.71	14.59
Tannic acid (mg/g)	3.91	**4.50**	4.38	3.96	4.01	4.00
Total phenolic content (mg/g)	**1.07**	1.03	1.06	0.98	0.95	0.92
Total flavonoid content (mg/g)	0.73	0.66	0.65	**0.82**	0.80	0.71
Seed yield per plot (g)	495.16	405.31	594.60	**830.38**	273.86	688.36

*Bold values indicate maximum values
Highlighted values indicate minimum values for phenological traits, recommendable for developing early maturing varieties.

Table 4. Promising characters in top three clusters using wards minimum variance method in chickpea.

S. No.	Cluster Number	Number of Promising Characters	Promising characters
1	II	3	Protein content, Phytic acid, Tannic acid.
2	III	1	Harvest Index.
3	IV	8	Plant height, Number of secondary branches per plant, Number of pods per plant, Number of seeds per pod, Total number of seeds per plant, Total free amino acid, Total flavonoid content, Seed yield per plot.

Table 5. Clustering pattern estimated by ward's minimum variance method in 64 genotypes of chickpea.

Cluster Number	Number of genotypes	Name of genotype(s)
I	23	ICCV 10, ICCV 37, JG 11, NBeG 690, NBeG 1377, NBeG 1430, NBeG 1487, NBeG 1506, NBeG 1667, NBeG 1674, NBeG 1679, NBeG 1688, NBeG 1689, NBeG 119, KAK-2, NBeG 1508, NBeG 1529, NBeG 1535, NBeG 1610, NBeG 1699, NBeG 1702, VIHAR, NBeG 47.
II	13	NBeG 452, NBeG 506, NBeG 1427, NBeG 1428, LBeG 7, NBeG 440, MNK-1, NBeG 1516, NBeG 1532, NBeG 1614, NBeG 1711, NBeG 3, NBeG 49.
III	10	NBeG 857, JAKI-9218, NBeG 699, NBeG 1296, NBeG 1426, NBeG 1445, NBeG 1496, NBeG 1658, NBeG 1537, NBeG 1706.
IV	4	NBeG 1129, NBeG 1639, NBeG 1509, NBeG 1554.
V	7	NBeG 1292, NBeG 1434, NBeG 1709, ICCV 2, Phule G 05107, NBeG 1627, NBeG 1629.
VI	7	NBeG 1420, NBeG 1423, NBeG 1637, NBeG 1642, NBeG 810, NBeG 1539, NBeG 1593.

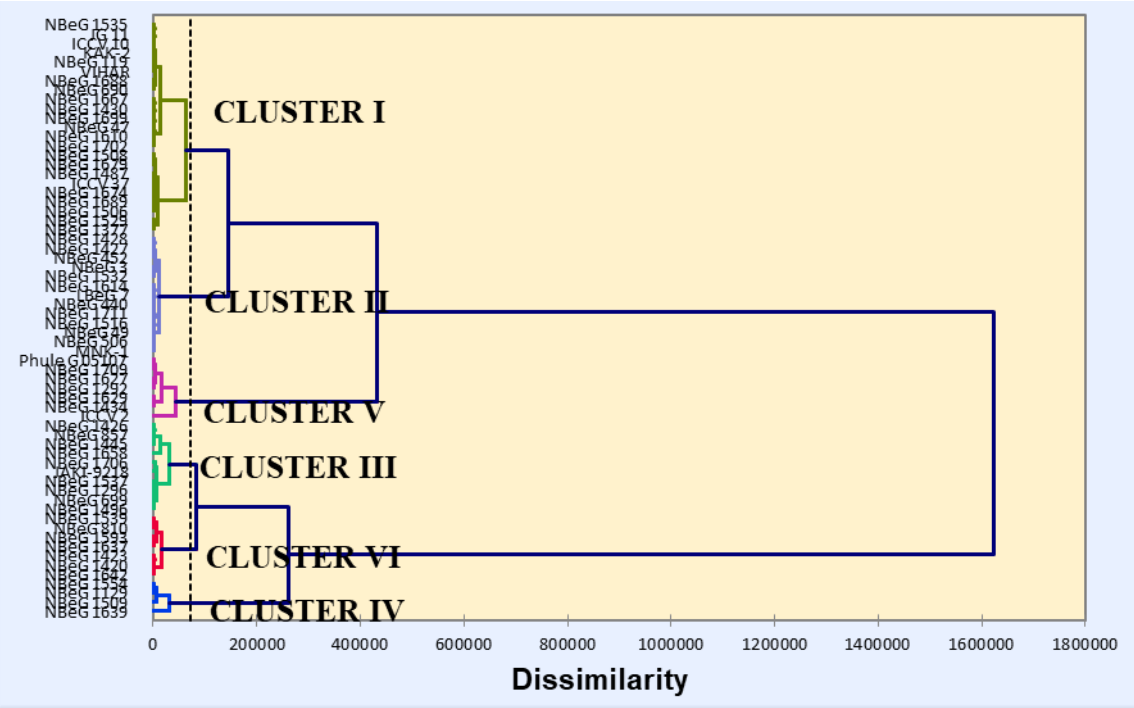

Fig. 2. Dendrogram showing relationship of six clusters based on Eucledian² distance in 64 genotypes of chickpea.

Table 6. Average intra and inter-cluster squared eucledian values among the six clusters in 64 genotypes of chickpea.

Cluster Number	I	II	III	IV	V	VI
I	45.865	93.174	100.631	337.341	225.201	194.661
II		29.406	192.816	429.548	132.858	283.541
III			48.757	237.558	325.235	100.375
IV				65.644	562.020	152.738
V					59.917	415.591
VI						43.257

Table 7. The nearest and the farthest cluster from each cluster based on squared eucledian values using ward's minimum variance method in 64 genotypes of chickpea

Cluster Number	Nearest cluster with Eucledian² values	Farthest cluster with Eucledian² values
I	II (93.174)	IV (337.341)
II	I (93.174)	IV (429.548)
III	VI (100.375)	V (325.235)
IV	VI (152.738)	V (562.020)
V	II (132.858)	IV (562.020)
VI	III (100.375)	V (415.591)

1639, NBeG1427, NBeG1637, NBeG1420 NBeG1658 and *kabuli* genotypes *viz*; NBeG 1706, ICCV 2, NBeG 1593, NBeG1702, NBeG1529, LBeG 7, NBeG 1554, NBeG 1532 and NBeG 1509 were divergent for yield and quality traits. Using ward's method 64 genotypes were grouped into six clusters. Maximum inter cluster distance was found between cluster IV and cluster V followed by Cluster II and Cluster IV. The promising genotypes from these clusters for various yield traits are NBeG 452, NBeG 1639, NBeG 1427, NBeG 1129 and NBeG 1428 of *desi* types and NBeG 1554, NBeG 1532, NBeG 1509 and NBeG 1614 of *kabuli* types. These clusters also had promising genotypes for quality traits like NBeG 452, NBeG 1639 and NBeG 1427 of *desi* type and NBeG 1554, NBeG 1509 and NBeG 1532 of *kabuli* type. These genotypes along with maximum inter cluster distance recorded high cluster means for most of the seed yield contributing characters and some quality traits. The aforesaid genotypes can be used in upcoming crop improvement programmes to produce superior varieties with yield advantage coupled with quality traits depending on the objective.

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