





RESEARCH ARTICLE

Integrating genetic diversity and principal component analysis for trait-based selection in niger (*Guizotia abyssinica* (L.f.) Cass.) genotypes

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Received: 27 August 2025; Accepted: 05 October 2025; Available online: Version 1.0: 30 October 2025

Cite this article: Aruna DA, Rajani B. Integrating genetic diversity and principal component analysis for trait-based selection in niger (*Guizotia abyssinica* (L.f.) Cass.) genotypes. Plant Science Today (Early Access). https://doi.org/10.14719/pst.11503

Abstract

Niger (*Guizotia abyssinica* (L.f.) Cass.) is an important oilseed crop cultivated in rainfed areas of India, yet its productivity remains low due to limited genetic improvement. The present study evaluated 42 breeding lines for morphological diversity and trait contribution using principal component analysis (PCA). Cluster analysis using Tocher's method grouped the genotypes into six distinct clusters, indicating substantial genetic variability. The highest inter-cluster distance was observed between Clusters IV and VI, suggesting wide divergence suitable for hybridization. PCA revealed that the first 5 principal components accounted for 92.80 % of the total variation, with PC1 (57.93 %) associated with yield-related traits (seed yield, branches and biological yield) and PC2 (16.51 %) with phenological traits (days to flowering, days to maturity and plant height). Mahalanobis D² analysis grouped the genotypes into 7 distinct clusters, with oil content contributing the most to genetic divergence (87.92 %), followed by biological yield and thousand grain weight. Maximum inter-cluster distances were observed between Clusters II and VI (164.99) and Clusters IV and VI (131.93), indicating these combinations as potential sources for generating superior recombinants. Integration of PCA and cluster results identified JCN-29, JCN-30, JCN-34 and JCN-31 as promising genotypes for both high yield and adaptability. This combined approach provides a reliable basis for parent selection in niger breeding programs.

Keywords: cluster analysis; D² statistics; genetic diversity; Guizotia abyssinica; niger; principal component analysis

Introduction

Oilseed crops play a crucial role in global agriculture, with world production exceeding 600 million metric tons annually and serving as a major source of edible oils, protein-rich feed and industrial raw materials. Among them, niger (Guizotia abyssinica (L.f.) Cass.) is an ancient oilseed crop of Ethiopia and India. It holds significant importance due to its adaptability to marginal environments and tolerance to drought. The crop also produces high-quality edible oil, which is rich in polyunsaturated fatty acids (75-80 %) (1, 2). Despite its economic and nutritional value, niger has remained underutilized compared to other oilseed crops, primarily due to limited research and improvement programs (3). Genetic improvement of niger depends largely on the extent of variability present within its germplasm and the ability of breeders to identify and utilize superior genotypes. Assessment of genetic diversity provides essential insights into population structure, breeding potential and gene pool utilization (4, 5). Traits, particularly those associated with yield and its components, such as seed yield per plant, number of capitula per plant, thousand grain weight (TGW) and biological yield, remain the most widely used descriptors in diversity studies, owing to their direct relevance to breeding programs and ease of evaluation under field conditions.

However, reliance on individual traits often limits the ability to capture the complex interrelationships among traits. Multivariate statistical approaches such as principal component analysis (PCA) and cluster analysis are widely used as powerful tools for summarizing large datasets, identifying patterns of variation and determining key traits contributing to genetic divergence (6). PCA reduces dimensionality by grouping correlated traits into a few components, thereby highlighting traits with maximum discriminatory power, while cluster analysis classifies genotypes based on similarity, facilitating the identification of distinct and promising breeding lines (7). These approaches have been effectively used in several oilseed crops, including sunflower, sesame and linseed, for dissecting variability and guiding parent selection (8, 9). Given the crop's importance for rainfed and resource-poor farmers in India, systematic characterization of diversity and trait interrelationships is urgently needed to broaden the genetic base and accelerate breeding efforts (10).

This study assesses niger breeding lines using PCA and cluster analysis to identify key traits, classify genotypes and highlight potential parents for crop improvement.

ARUNA & RAJANI 2

Materials and Methods

Experimental setup

The study was conducted at the Zonal Agricultural Research Station, AICRP on niger, Jawaharlal Nehru Krishi Vishwavidyalaya (JNKW), Chhindwara, Madhya Pradesh (latitude 22.0574°N, longitude 78.9382°E, altitude 683 m above mean sea level), during the 2021 and 2022 Kharif seasons under three environments (EI: 5 August 2021 and 2022, EII: 20 August 2021 and 2022, EIII: 5 September 2021 and 2022). The region experiences a subtropical climate with an average annual rainfall of 1200–1300 mm, mean maximum temperatures ranging between 28-32 °C and mean minimum temperatures of 18-22 °C during the crop season. Fortytwo niger genotypes, including 2 checks (JNS-9 and JNS-28), were evaluated in a randomized block design (RBD) with 3 replications across three sowing environments (early, mid and late planting). Each plot consisted of four rows, 1.5 m long, with 30 cm row-to-row and 10 cm plant-to-plant spacing.

Data collection and statistical analysis

Eleven phenological and yield traits were recorded, including days to DF - days to 50 % flowering; DM - days to maturity; PH - plant height (cm); BP - branches per plant; CP - capitula per plant; SC - seeds per capitula; TGW - thousand grain weight (g); BY - biological yield (g); HI - harvest index (%) was calculated as the ratio of seed yield to biological yield multiplied by 100; oil % - oil content was estimated using Soxhlet extraction with petroleum ether and YP - seed yield per plant (g).

PCA was carried out on the correlation matrix of standardized data, considering components with eigenvalues >1 as significant and trait loadings were examined to identify the most discriminating traits (11, 12). Genetic divergence was assessed using Mahalanobis' D² statistics and genotypes were grouped into clusters using Tocher's method based on inter-

cluster distances (13). Cluster means were computed to identify superior clusters and promising genotypes for yield and stability. All statistical analyses were carried out using R software version 3.6.0.

Results and Discussion

Principal component analysis

PCA was employed to assess the contribution of various phenological and yield-related traits to the total variability among niger genotypes based on pooled data. The first 5 PCs, each with eigenvalues >1, explained a substantial proportion of the variation, justifying their retention. Specifically, PC1 accounted for 57.93 %, PC2 for 16.51 %, PC3 for 8.83 %, PC4 for 6.05 % and PC5 for 4.29 % of the total variance, cumulatively explaining 92.80 % of the overall variability (Table 1) (14). This indicates that most of the phenotypic diversity in the niger germplasm could be captured by a relatively small number of components.

The trait loadings revealed differential contributions of traits across components. In PC1, the highest positive loadings were observed for seed yield per plant (0.99), CP (0.95) and BY (0.77), suggesting that this axis largely represented yield potential and its associated traits (Table 2). PC2 was strongly influenced by DF (0.97) and DM (0.56), indicating its relevance for phenological traits. PC3 was characterized by SC (0.62) and oil percentage (0.61), showing the highest positive loadings, demonstrating its importance in seed-related traits. PC4 was associated mainly with PH (0.62) and TGW (0.54), representing architectural and reproductive traits. PC5 was most strongly associated with HI (0.57), indicating its relationship with biomass partitioning and yield efficiency (Table 3) (15).

Table 1. Eigenvalues and percentage contribution of principal components

| S. No. | Traits | Principal component | Eigen values | Variability % | Cumulative % |
|--------|------------------------------|---------------------|--------------|---------------|--------------|
| 1 | Days to flowering | PC1 | 7.12 | 57.93 | 57.93 |
| 2 | Days to maturity | PC2 | 2.02 | 16.51 | 74.45 |
| 3 | Plant height | PC3 | 1.08 | 8.83 | 83.28 |
| 4 | Branches per plant | PC4 | 0.74 | 6.05 | 89.33 |
| 5 | Number capitula per plant | PC5 | 0.52 | 4.29 | 93.63 |
| 6 | Number of seeds per capitula | PC6 | 0.38 | 3.15 | 96.78 |
| 7 | Thousand grain weight | PC7 | 0.14 | 1.15 | 97.94 |
| 8 | Biological yield | PC8 | 0.12 | 0.99 | 98.94 |
| 9 | Harvest index | PC9 | 0.06 | 0.48 | 99.43 |
| 10 | Oil % | PC10 | 0.04 | 0.33 | 99.76 |
| 11 | Seed yield per plant | PC11 | 0.02 | 0.21 | 99.98 |

Note: PC - Principal component

Table 2. Principal components for 11 yield contributing traits of niger genotypes

| Traits | PC1 | PC2 | PC3 | PC4 | PC5 |
|------------------------------|-------|-------|--------|-------|--------|
| Days to flowering | -0.03 | 0.97 | 0.01 | -0.17 | -0.03 |
| Days to maturity | 0.02 | 0.56 | 0.002 | -0.14 | 0.20 |
| Plant height | 0.49 | 0.63 | 0.24 | 0.62 | 0.06 |
| Branches per plant | 0.92 | -0.28 | 0.02 | -0.24 | 0.03 |
| Number capitula per plant | 0.95 | -0.06 | -0.006 | -0.16 | -0.20 |
| Number of seeds per capitula | 0.40 | -0.20 | 0.62 | -0.03 | 0.003 |
| Thousand-grain weight | 0.24 | 0.008 | 0.24 | 0.54 | 0.03 |
| Biological yield | 0.77 | 0.03 | -0.11 | 0.23 | 0.31 |
| Harvest index | 0.40 | -0.09 | 0.11 | -0.19 | 0.57 |
| Oil % | -0.01 | 0.04 | 0.61 | 0.001 | -0.002 |
| Seed yield per plant | 0.99 | 0.02 | 0.34 | -0.01 | 0.027 |

Note: PC – Principal component

Table 3. Rotated component matrix of trait loadings (pooled analysis)

| Principal component | Traits with high loadings | | | | | |
|-----------------------|--|--|--|--|--|--|
| PC1 (57.9 % variance) | Number of branches per plant, number of capitula per plant, biological yield, seed yield per plant | | | | | |
| PC2 (16.5 % variance) | Days to 50 % flowering, days to maturity, plant height | | | | | |
| PC3 (8.8 % variance) | Number of seeds per capitula, oil percentage | | | | | |
| PC4 (6.0 % variance) | Thousand-grain weight | | | | | |
| PC5 (4.3 % variance) | Harvest index | | | | | |

Note: PC - Principal component

PCA biplot analysis

The biplot derived from PC1 and PC2 (explaining 74.40 % of the variance) provided insights into genotype distribution and trait associations (Fig. 1). In PC1, the highest positive scores were recorded for JNS-28 (4.166), JCN-32 (3.965), JCN-34 (3.897), JCN-30 (3.811) and JCN-29 (3.694), these genotypes were positioned near the yield-related vectors, indicating their superiority for seed yield and biological productivity. On the other hand, JCN-17 (2.375), JCN-15 (1.923), JCN-19 (1.906) and JCN-40 (1.259) clustered around the phenological vectors, suggesting a tendency toward early flowering and maturity, which may be beneficial under late planting or moisture-stress conditions. Oil content was orthogonal to most yield components, suggesting relative independence and genotypes like JCN-9 (2.635), JCN-37 (2.462), JCN-23 (1.983), JCN-29 (1.439) and JCN-14 (1.340) showed association with this trait. PC4 was dominated by JCN-14 (1.888), JCN-12 (1.363), JCN-19 (1.342), JCN-11 (1.301) and JCN-29 (1.193). Finally, JCN-23 (1.436), JCN-13 (1.422), JCN-12 (1.249), JCN-16 (1.113) and JCN-1 (1.045) scored positively for HI.

These findings are consistent with earlier reports in oilseed crops where PCA efficiently identified the key traits explaining variability and facilitated genotype differentiation (16). The identification of yield-associated traits in PC1 highlights their

predominant role in discriminating high-performing lines, while the segregation of phenological and seed quality traits in PC2 suggests opportunities for simultaneous selection of earlymaturing and high-oil genotypes (17).

Overall, the biplot patterns demonstrated that different sets of genotypes dominated across various PCs, highlighting the presence of diverse genetic profiles. These results highlight the potential utility of high-scoring genotypes, such as JNS-28, JCN-32, JCN-34, JCN-17 and JCN-23, for breeding programs. Conversely, negatively scoring genotypes could serve as contrasting parents to enhance variability in hybridization programs. Moreover, the independence of oil content from yield traits implies that simultaneous improvement of both is feasible through targeted breeding.

Genetic diversity analysis

Pooled data analysis for genetic divergence, based on Mahalanobis' D² statistics, grouped 42 niger breeding lines into 7 clusters using Tocher's method. This indicated the presence of substantial variability exploitable in breeding programs. Cluster formation was not strictly associated with the geographical origin of the genotypes, suggesting that adaptive divergence and selection pressure contributed more to diversity than physical distance (18).

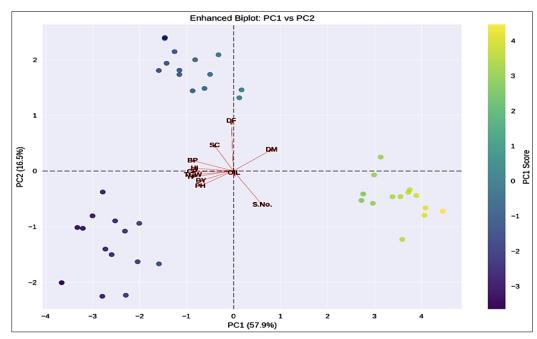


Fig. 1. PCA biplot of 42 niger genotypes based on 11 morphological and yield traits.

Note: The biplot of the first two principal components (PC1 = 57.90% and PC2 = 16.50%) explained 74.40% of the total variation. Vectors represent the contribution of traits: DF – days to 50% flowering; DM – days to maturity; PH – plant height (cm); BP – branches per plant; CP – capitula per plant; SC – seeds per capitulum; TGW – thousand grain weight (g); BY – biological yield (g); HI – harvest index (%), calculated as the ratio of seed yield to biological yield \times 100; Oil (%) – oil content, YP – seed yield per plant (g). Genotypes distributed along the positive side of PC1 were associated with yield-related traits, while those along PC2 were more influenced by phenological traits, indicating differential adaptation and trait associations.

ARUNA & RAJANI 4

Among these, Cluster II was the largest, comprising 18 genotypes, followed by Cluster IV with 9, Cluster III with 5, Cluster V with 4, Cluster I with 3, Cluster VI with 2, while Cluster VII contained only one genotype (Fig. 2). This distribution indicates wide genetic variability among the genotypes, with a tendency of several accessions to cluster together, reflecting shared genetic backgrounds.

The contribution of individual traits to genetic divergence revealed that oil content (%) was the most influential, accounting for 87.92 % of the total divergence (Table 4). Other traits contributed comparatively less, including BY (3.14 %), TGW (2.67 %), HI (1.39 %), CP (1.16 %), PH and BP (1.05 % each). Traits such as number of SC and DM contributed marginally (0.70 %). These results highlight oil %, BY and seed weight as key drivers of diversity and important criteria for selection.

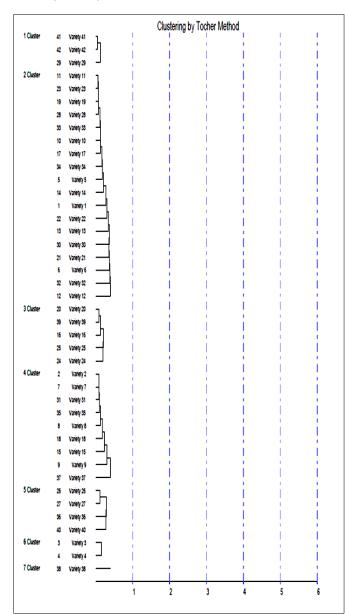


Fig. 2. Clustering of niger genotypes using Tocher's method based on Mahalanobis D² statistics.

Note: The dendrogram illustrates the genetic relationships among 42 niger breeding lines, which were grouped into seven distinct clusters. Cluster II was the largest with 18 genotypes, followed by Cluster IV with 9 genotypes, while Cluster VII contained a solitary genotype. The clustering pattern indicates substantial genetic divergence, highlighting opportunities for selecting divergent parents for recombination breeding.

Table 4. Percent contribution of quantitative traits towards divergence

| S. No. | Traits | Times ranked 1st | Contribution towards divergence % | | |
|--------|------------------------------|---------------------|---|--|--|
| 1 | Days to flowering | 2 | 0.23 | | |
| 2 | Days to maturity | 6 | 0.7 | | |
| 3 | Plant height | 9 | 1.05 | | |
| 4 | Number of branches per plant | 9 | 1.05 | | |
| 5 | Number of capitula per plant | 10 | 1.16 | | |
| 6 | Number of seeds per capitula | 6 | 0.7 | | |
| 7 | Thousand-grain weight | 23 | 2.67 | | |
| 8 | Biological yield | 27 | 3.14 | | |
| 9 | Harvest index | 12 | 1.39 | | |
| 10 | Oil % | 757 | 87.92 | | |

Inter- and intra-cluster divergence

The intra-cluster distances varied from 0.37 (Cluster I) to 0.93 (Cluster V), suggesting that genotypes within Cluster V were more diverse than those in Cluster I. Inter-cluster distances were generally higher than intra-cluster values, indicating substantial diversity between clusters (Table 5). The maximum inter-cluster distance was observed between Clusters II and VI (164.99), followed by Clusters IV and VI (131.93), suggesting that genotypes from these clusters are highly divergent and may serve as promising parents in hybridization programs. Conversely, the lowest inter-cluster distance was recorded between Clusters I and VII (1.38), indicating close genetic similarity. The clustering pattern and divergence values suggest that crossing distant clusters, such as Cluster II and VI, Cluster IV and VI, could generate superior recombinants due to the wide genetic base. The predominance of oil content and yield-related traits as major contributors to diversity underlines their importance as key selection criteria for niger improvement (19). These findings corroborate earlier reports in niger and oilseed crops like rapeseed and mustard, where oil percentage and yield components were dominant contributors to divergence (20, 21).

Cluster mean performance

The cluster mean analysis revealed trait-specific superiority across different groups (Table 6). For phenology, DF ranged from 50.33 (Cluster VI) to 51.87 (Cluster III), while DM ranged from 98.85 (Cluster I) to 100.46 (Cluster V). Morphological traits also varied substantially: PH was highest in Cluster I (93.31 cm) and lowest in Cluster VII (85.39 cm); BP was highest in Cluster VII (10.06) and lowest in Cluster I (7.54). Yield component traits also showed significant variation. Number of CP ranged from 25.50 (Cluster I) to 28.06 (Cluster V), while number of SC was highest in Cluster VII (38.33) and lowest in Cluster I (28.43). TGW ranged from 3.71 g (Cluster I) to 4.63 g (Cluster VII). For yield attributes, BY was maximum in Cluster VII (34.89 g) and minimum in Cluster III (27.10 g); HI ranged from 11.54 % (Cluster I) to 17.49 % (Cluster VII); and seed yield per plant ranged between 2.92 g (Cluster I) and 6.23 g (Cluster VII). Importantly, oil % was highest in Cluster VI (45.31 %) and lowest in Cluster II (30.78 %). The analysis revealed wide genetic variability, with Cluster VII excelling in yield traits and Cluster VI in oil %, underscoring their potential as key parental sources. Strategic hybridization among divergent clusters could enable the development of high-yielding, oil-rich and earlymaturing niger varieties (22, 23).

Table 5. Intra and inter distances between 42 niger genotypes in different clusters

| clusters | I | II | Ш | IV | V | VI | VII |
|----------|------|-------|-------|-------|-------|--------|-------|
| I | 0.37 | 14.16 | 3.8 | 5.93 | 12.32 | 84.52 | 1.38 |
| II | | 0.85 | 30.67 | 2.62 | 51.04 | 164.99 | 10.74 |
| III | | | 0.59 | 17.63 | 3.22 | 54.55 | 6.31 |
| IV | | | | 0.74 | 33.6 | 131.93 | 4.08 |
| V | | | | | 0.93 | 33.64 | 16.7 |
| VI | | | | | | 0.4 | 93.98 |
| VII | | | | | | | 0 |

Table 6. Cluster means value for 11 traits in 42 genotypes of niger

| | DF | DM | PH | NBPP | NCPP | NSPC | TGW | ВҮ | HI | Oil% | ΥP |
|-------------|-------|--------|-------|-------|-------|-------|------|-------|-------|-------|------|
| Cluster I | 50.8 | 98.85 | 93.31 | 7.54 | 25.5 | 28.43 | 3.71 | 27.46 | 11.54 | 34.95 | 2.92 |
| Cluster II | 50.96 | 99.83 | 91.91 | 7.9 | 26.77 | 33.45 | 4.06 | 30.79 | 14.78 | 30.78 | 4.29 |
| Cluster III | 51.87 | 100.29 | 91.21 | 7.76 | 27.04 | 33.39 | 3.97 | 27.1 | 15.14 | 37.01 | 4.01 |
| Cluster IV | 50.91 | 99.2 | 91.67 | 7.71 | 25.74 | 31.51 | 3.95 | 32.86 | 13.15 | 32.33 | 3.97 |
| Cluster V | 51.11 | 100.46 | 92.54 | 7.56 | 28.06 | 33.18 | 3.89 | 27.69 | 14.16 | 38.84 | 3.68 |
| Cluster VI | 50.33 | 99.47 | 85.89 | 9.00 | 26.47 | 36.28 | 4.24 | 32.67 | 16.98 | 45.31 | 5.2 |
| Cluster VII | 51.11 | 100.39 | 85.39 | 10.06 | 25.89 | 38.33 | 4.63 | 34.89 | 17.49 | 34.29 | 6.23 |

Note: DF – days to 50 % flowering; DM – days to maturity; PH – plant height (cm); BP – branches per plant; CP – capitula per plant; SC – seeds per capitulum; TGW – thousand grain weight (g); BY – biological yield (g); HI – harvest index (%), calculated as the ratio of seed yield to biological yield × 100; Oil (%) – oil content, YP – seed yield per plant (g).

Conclusion

The present study revealed substantial genetic variability among the 42 niger genotypes evaluated across multi-environment trials. The multivariate evaluation further revealed substantial variability that can be exploitable for crop improvement. PCA showed that the first 5 PCs explained 92.80 % of the total variation, with PC1 (57.93 %) capturing yield-related traits such as seed yield, CP and BY, while PC2 (16.51 %) represented phenological variation. High-scoring genotypes, such as JNS-28, JCN-32 and JCN-34, were superior for yield traits, whereas JCN-17 and JCN-23 were notable for their phenological adaptation and oil content. In contrast, JCN-17 and JCN-23 were notable for their phenological adaptation and oil, respectively. Importantly, the independence of oil content from yield traits indicated the feasibility of achieving simultaneous improvement in both yield and quality. Mahalanobis' D² analysis grouped the genotypes into 7 clusters, with oil % contributing most to divergence (87.92 %), followed by and seed weight, while maximum inter-cluster distances between Clusters II and VI (164.99) and Clusters IV and VI (131.93) suggested these combinations as promising for recombination breeding. Cluster means further identified Cluster VII as superior for seed yield (6.23 g), BY (34.89 g), HI (17.49 %) and TGW (4.63 g), while Cluster VI excelled in oil % (45.31 %). Collectively, these results underscore the importance of strategic hybridization among divergent clusters and the selection of highscoring genotypes to accelerate the development of highyielding, early-maturing and oil-rich niger varieties, thereby providing a strong foundation for future breeding programs.

Acknowledgements

The authors express their sincere gratitude to the All India Coordinated Research Project on niger (AICRP-Niger), Zonal Agricultural Research Station, JNKW, Chhindwara (Madhya Pradesh), for providing the necessary facilities and field support to carry out the research. We also thank the technical and field staff for their assistance during the experimental work.

Authors' contributions

ADA conceived and designed the experiment, conducted fieldwork, recorded and analyzed the data and led the manuscript writing and revision. RB supervised the research, provided technical guidance throughout the study and critically reviewed the manuscript for scientific content and accuracy. All authors read and approved the final manuscript.

Compliance with ethical standards

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

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used "Quillbot" in order to improve the clarity, grammar and formatting of the manuscript text, without altering the scientific content, results, or conclusions. After using this tool/ service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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ARUNA & RAJANI 6

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Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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