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GENETIC DIVERGENCE OF LOCAL YELLA (*BRASSICA JUNCEA* L. CZERN & COSS) LANDRACES OF MANIPUR INDIA USING D² STATISTICS

Vineet Kumar Tiwari[1*], Pushparani Senjam[2], Thokchom Renuka Devi[1], Nabakishor Nongmaithem[3], Naorem Gopimohan Singh[4] and Nongthombam Surbala Devi[5]

[1]Department of Genetics and Plant Breeding, College of Agriculture, Iroisemba, Central Agricultural University, Imphal, Manipur, India.
[2]All India Coordinated Research Project on Rapeseed-Mustard, College of Agriculture, Iroisemba Central Agricultural University, Imphal, Manipur, India.
[3]Department of Plant Pathology, College of Agriculture, Iroisemba, Central Agricultural University, Imphal, Manipur, India.
[4]Department of Basic Science, College of Agriculture, Iroisemba, Central Agricultural University, Imphal, Manipur, India.
[5]Department of Soil Science and Agricultural Chemistry, College of Agriculture, Iroisemba, Central Agricultural University, Imphal, Manipur, India.
[*]Corresponding author E-mail: vineettiwari0527@gmail.com

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ABSTRACT

Local Yella [*Brassica juncea* (L.) (Czern and Coss)], a popular Indian mustard landrace of Manipur is a highly adapted *rabi* season oilseed crop grown popularly in certain pockets of mustard growing belt of Manipur. They are cultivated for vegetable as well as oil purpose. Tender shoots are widely consumed as "Chamfut" a popular local delicacy of the Manipuris. At present very scarce information is available about the genetic diversity and nutritional profile of local yella as such studies are over looked. Proper characterization and documentation is required for better crop improvement programme of the local yella landraces. In the present study, 20 genotypes of local yella along with one check collected from different parts of Manipur were evaluated for genetic diversity using Mahalanobis D² statistics for 19 morphological and nutritional traits. The analyses of variance revealed highly significant differences among the genotypes which, in turn, indicated the presence of genetic variability among them. The 21 genotypes were grouped into 8 clusters using Tocher's method. Inter-cluster distance revealed high scope of obtaining transgressive segregants in the advanced generations through intermating the genotypes, CAULC-19, CAULC-23 and NRCHB-101. Cluster mean identified crosses between CAULC-19 with NRCHB-101, could give progenies with higher yield, early maturity, higher oil content with high zinc and iron content, CAULC-19 with CAULC-23, could result in progenies with high yield, earliness and high protein, zinc and ascorbic acid content and CAULC-23 with NRCHB-101 could give progenies with higher yield, oil content along with high protein, iron and ascorbic acid content. Traits, phosphorus content, Zinc content, days to 50% flowering and ascorbic acid content could be considered during selection of genetically diverse parents for hybridization programme based on their contribution towards genetic divergence in local yella. Among the 21 genotypes, CAULC-5 was identified to be highly promising in terms of yield (21% higher over the check, NRCHB-101) and early maturity (97 days), while, CAULC-9, CAULC-11, CAULC-16, and CAULC-23 were identified to be highly nutritive genotypes. Hence, these genotypes may be utilized as desirable donors for their respective identified characters in future crop improvement programme of local yella.

***Keywords* :** Local yella, Genetic diversity, D^2 statistics.

Introduction

Indian mustard [*Brassica juncea* (L.) Czern and Coss, 2n-36, AABB genome], an allopolyploid species is a popular *rabi* season oilseed crop grown primarily in India's Northern and Eastern regions. It is basically self-pollinated, however, an average of 7.5% - 30% out-crossing does occur under natural field conditions due to honey bee activities (Yadav *et al.*, 2021; Yengkokpam *et al.*, 2024). Rapeseed mustard is the third most important oilseed crop of the world and also the first most important oilseed crop of India (*Meena et al*., 2020). With the production of 13.2 million tons during 2023-24, rapeseed-mustard became the first largest oilseed crop in India. National average yield of rapeseed-mustard is 1443 kg/ha compared to the combined oilseeds crops average of 1314 kg/ha (2023-24) (ICAR-IIRMR, 2024). Although rapeseed-mustard is cultivated in majority of states of the country, bulk of the production comes from Rajasthan (45.40 %), Madhya Pradesh (13.28%), Uttar Pradesh (14.24 %), Haryana (10.78%) and West Bengal (6.0 %) during (2023-24) (ICAR-IIRMR, 2024). Among rabi oilseed, rapeseed and mustard plays an important role in North (NEH) Eastern Hill region. Rapeseed and mustard occupies the most important position as edible oilseed crop in Manipur. It has occupies an area of 31 thousand hectares and average productivity of 7.2 ton / hectare (DoA,GoM, 2023). In Manipur, it is mainly cultivated for oil as well as a vegetable. Mustard oil is rich in monounsaturated and polyunsaturated fatty acids, has a significant amount of omega- 3 fatty acids, and contains a very little amount of saturated fatty acids, which makes it quite useful from a health point of view (Singh *et al.,* 2022). After oil extraction, the remaining part of the seed is used to produce rapessed/mustard meal, an important component of cattle and poultry feed. The leaves of young plants are used as green vegetables (Gupta *et al.,* 2024).

In Manipur, rapeseed-mustard is the only oilseed crop grown commercially during rabi season, which contributes about 80-90% to the total oilseed production of the state. The state is marginally deficient in cereals and highly deficient in oilseeds and pulses. Apart from growing rapeseed-mustard as an oilseed crop, it is also grown for its tender shoots. Although mustard is not a new crop in this region, yet farmers are growing local cultivars or genotype with low productivity and low oil content and the state is still facing oilseed deficient of about 12 thousand metric tonnes at the present level of per capita consumption (NFSM-2022). The tender shoots of Local Yella, a popular Indian mustard landrace of Manipur shoots are widely consumed as “chamfut” a popular local delicacy of the Manipuris.

The utilization of a species into any crop improvement breeding programme depends not only on the degree of genetic diversity it holds, but also on the precise information regarding genetic divergence and relatedness among breeding material (Gupta *et al.*, 2024). Genetic diversity in crop species is essential to sustain levels of high productivity. As a result of narrowing genetic base of commercially important varieties, crops today are in imminent danger of serious losses from the ravages of major diseases or insects or both when uniformity becomes the cause of genetic vulnerability hence, genetic divergence is the only insurance against it. Furthermore, genetic diversity could help breeders and geneticists to understand the structure of germplasm, predict which combinations would produce the best offsprings (Hu *et al*., 2007), and facilitate to widen the genetic basis of breeding material for selection (Qi *et al*., 2008).

Understanding the extent of genetic diversity in these landraces can provide insights into their potentials for breeding programs and help in developing new varieties with higher yield, oil content with enchanced nutritional property.

Materials and Methods

Experimental Details

The experiment consisting of 21 genotypes including 1 check was obtained from AICRP on Rapeseed and mustard, CAU, Imphal center was conducted at CAU Research Farm, Andro, Imphal East during *rabi* 2024-2025. The Andro research farm is geographically located in Imphal East District of Manipur at 24°45.89’N latitude and 94°03.46’E longitudes with an elevation of 808m above Mean Sea Level which comes under the Eastern Himalayan region (II) and agro-climatic Sub-Tropical zone (NEH-4) of Manipur (Agro meterological observatory, ICAR-RC NEH Region, Manipur Centre, Lamphelpat, Imphal). Each genotypes were raised in a RBD design with 3 replications, row to row distance 30 cm and plant to plant distance 10 cm respectively.

Collection of Data

Sampling method ten plants were selected randomly from each genotypes for all replication and observation for the following morphological traits, days to 50% flowering, Number of primary branches, Number of secondary branches, Plant height (cm), days to 80% maturity, Number of Siliqua per plant, Number of seed per siliqua, Siliqua length (cm), 1000 seed weight (g), Seed yield (Kg/ha), and oil content (%) and for nutritional content tender shoots were collected at 42-45 days after showing for Protein content (%), Phosphorus content (mg/100g), Sulphur content (mg/100),Potassium content (mg/100g), Iron content (mg/100g), Manganese content (mg/100g), Zinc content (mg/100g) and Ascorbic acid content (mg/100g) were recorded.

Statistical Analysis

The mean values were subjected to statistical analysis. The multivariate analysis of genetic divergence using Mahalanobis's D^2 statistics was first suggested by C.R. Rao (1952).

Mahalanobis' D^2 statistic

Mahalanobis (1936) defined the distance between two populations as:

$$D^2 = \sum_{i=1}^{p}\sum_{j=1}^{p} Wijdidj$$

Where,

W_{ij} is the (ij^{th}) element in the inverse of estimated with population variance-covariance matrix

Group constellation of genotypes based on D^2 values

The genotypes were grouped using tocher's method (Rao, 1952). The average intra- cluster was calculated by using the formula

Average intra-cluster distance is estimated as:

$$\sum_{i=1}^{n} \frac{Di^2}{n}$$

Where,

$\Sigma D_i^2/n$ is the sum of distance between all possible combinations of the i=1 population include in the cluster, 'n' is the total number of all possible combinations.

Average inter-cluster distance

Clusters were taken one by one and their distance from other clusters was calculated. The distance between two clusters is the sum of D^2 values between the numbers of one cluster to each of the number of other clusters divided by the product of number of genotypes in both the clusters under consideration.

Average inter-cluster distance is estimated as:

$$\sum \frac{D^2}{ninj}$$

Where,

$\Sigma D^2/n_i n_j$ is the sum of distance between the populations of i^{th} and j^{th} clusters. n_i and n_j are the number of populations in the i^{th} and j^{th} clusters.

Cluster Mean

The cluster means for a particular trait were calculated by dividing the summation mean values of the strains included in a cluster by the number of strains in the cluster.

Cluster Diagram

With the help of D^2 values between and within the cluster, a cluster diagram has been drawn that showed the relationship between the different populations.

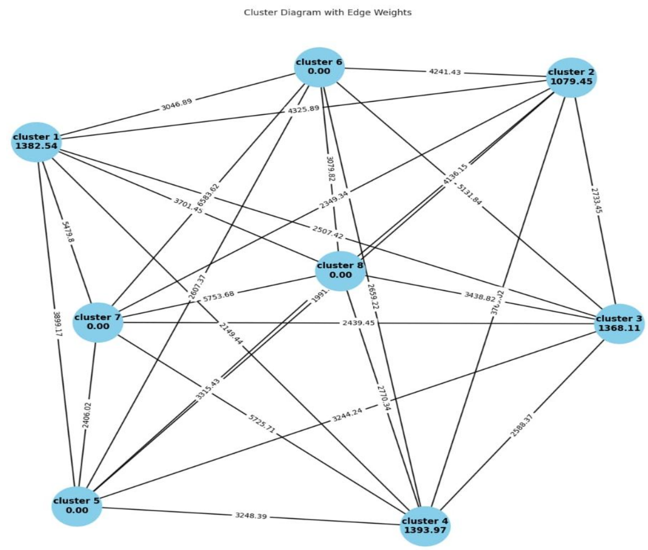

Fig. 1: Cluster Diagram showing relationships among different populations

Contribution of individual character towards divergence

The characters contributing maximum to divergence in order of descending magnitude were phosphorus content, zinc content, days to 50% flowering, ascorbic acid, number of secondary branches, iron content, potassium content, number of seed per siliqua, manganese content, number of siliqua per plant, oil content, sulphur content, days to 80% maturity, number of primary branches, siliqua length, seed yield, 1000 seed weight, plant height, and protein content. The traits phosphorus content, zinc content, days to 50% flowering, and ascorbic acid content might be considered during selection of diverse parents for hybridization programme to obtain good recombinant lines in segregating generation.

Table 1 : Percent contribution of 19 different traits towards divergence

S. No.	Traits	Propor-tion	Cumula-tive propo-rtion
1	Phosphorus content	16.26	16.26
2	Zinc content	13.54	29.81
3	Days to 50% flowering	10.82	40.63
4	Ascorbic acid	10.06	50.69
5	Number of secondary branches per plant	7.80	58.50
6	Iron content	7.70	66.20
7	Potassium content	6.90	73.11
8	Number of seed per siliqua	4.31	77.43
9	Manganese content	3.95	81.38
10	Number of siliqua per plant	3.29	84.68

11	Oil content	3.12	87.80
12	Sulphur content	2.97	90.78
13	Days to 80% maturity	1.97	92.75
14	Number of primary branches per plant	1.68	94.44
15	Siliqua length	1.60	96.04
16	Seed yield	1.41	97.46
17	1000 seed weight	1.36	98.83
18	Plant height	0.98	99.81
19	Protein content	0.18	100

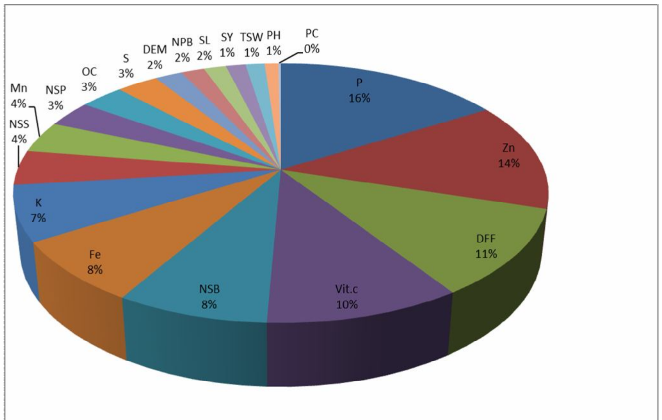

Fig. 2 Pie chart showing Percent contribution of 19 different traits towards divergence

Grouping of genotypes into different clusters using Tocher's method

The 21 genotypes of local yella were grouped into 8 clusters based on the relative magnitude of D^2 values using the Tocher's method (Rao, 1952) of cluster formation with the criterion that the intra-cluster average D^2 values should be less than the inter-cluster D^2 values. Cluster I consisted of maximum number of genotypes (10) out of the 21 genotypes of local yella studied. Cluster II consisted of (3) genotypes, Cluster III of (2) genotypes, Cluster IV of (2) genotypes. While, Cluster V, cluster VI, cluster VII and cluster VIII was monogenotypic. Adhikari (2022) studied genetic diversity in 22 genotypes of *Brassica juncea* subspecies *rugosa* and grouped them into 4 clusters. Dev (2022) also studied genetic diversity in 34 genotypes of leafy mustard and grouped them into 4 clusters.

A random distribution pattern was observed for genotypes from diverse geographical region in the cluster distribution. It might be due to free and frequent exchange of genetic materials among the farmers of different regions. Differential selection pressure according to regional preference also produced greater uniformity in the germplasm. The absence of relationship between genetic diversity and geographical distance indicated that forces other than geographical origin such as exchange of genetic stock, genetic drift, spontaneous mutation, natural and artificial selection were responsible for genetic diversity. As a result, the selection of parents for hybridization should be based on genetic divergence rather than geographic diversity.

Table 2 : Cluster classification of 21 genotypes of local Yella mustard

Cluster	Number of Genotypes	Name of Genotypes	Source/Site of Collection (District & State)
Cluster 1	**10**	**CAULC-1**	SAPAM
		CAULC-10	KEIKOL
		CAULC-11	YAIRIPOK YAMBEN
		CAULC-12	AWANG POTSANGBAM KHUNOU
		CAULC-15	KAKCHING KHUMAN
		CAULC-16	TEKCHAM
		CAULC-20	PAPAL
		CAULC-21	HUIKAP
		CAULC-22	KABOWAKCHING
		CAULC-6	LEIMARAM
Cluster 2	**3**	**CAULC-14**	KAKCHING MAYAI LEIKAI
		CAULC-5	SHAJIROK
		CAULC-7	KAKCHING NINGTHOU LEIKAI
Cluster 3	**2**	**CAULC-18**	KABOWAKCHING KHUNOU
		CAULC-9	IRENGBAMD
Cluster 4	**2**	**CAULC-17**	KHA POTSANGBAM
		CAULC-8	SHAJIROK
Cluster 5	**1**	**CAULC-13**	AWANG POTSANGBAM
Cluster 6	**1**	**CAULC-19**	NUNGBRANG
Cluster 7	**1**	**CAULC-23**	PAPAL
Cluster 8	**1**	**NRCHB-101**	RAJASTHAN

Average intra and inter cluster distance

In the present study, inter-cluster distance was maximum between cluster VI and cluster VII (6583.62) followed by cluster VII and cluster VIII (5753.68). Maximum inter-cluster distance indicates wide diversity among the genotypes of cluster VI and cluster VII, cluster VII and cluster VIII. Hence, genotypes belonging to these clusters can be used in hybridization programmes as magnitude of heterosis largely depends on the degree of genetic diversity of the parental population. Intermating between genotypes included in these clusters will have high potential of obtaining transgressive segregants in the advanced generation for all the traits studied. Accordingly, two separate crossing programmes, wherein cluster VI genotypes (CAULC-19) may be used as common parent for crossing with genotype in cluster VIII (NRCHB-101) and with genotypes in cluster VII (CAULC-23) could be suggested. The minimum inter-cluster distance was found between cluster II and cluster V (44.63), which indicates close relationship between the genotypes in these clusters. Bhandari (2021) and Adhikari (2022) also found results justifying the above results.

The maximum intra-cluster distance was found in cluster IV (1393.97), which shows that considerable amount of heterogeneity is present among the genotypes of this cluster (CAULC-17 and CAULC-8). Intra cluster heterogeneity among constituent genotypes might serve as a guideline to choose heterogenous parents for recombination breeding programme.

Table 3 : Average intra and inter cluster D^2 values of 21 genotypes of local yella

Cluster Number	cluster.1	cluster.2	cluster.3	cluster.4	cluster.5	cluster.6	cluster.7	cluster.8
cluster 1	**1382.54 (37.18)**	4325.88 (65.77)	2507.41 (50.07)	2149.43 (46.36)	3899.17 (62.44)	3046.88 (55.19)	5479.80 (74.02)	3701.44 (60.83)
cluster 2		**1079.45 (32.85)**	2733.45 (52.28)	3769.01 (61.39)	1991.85 (44.63)	4241.43 (65.12)	2349.33 (48.46)	4136.15 (64.31)
cluster 3			**1368.10 (36.98)**	2588.37 (50.87)	3244.24 (56.95)	5131.83 (71.63)	2439.44 (49.39)	3438.81 (58.64)
cluster 4				**1393.97 (37.33)**	3248.39 (56.99)	2659.22 (51.56)	5725.70 (75.66)	2770.33 (52.63)
cluster 5					**0**	2607.36 (51.06)	2406.02 (49.05)	3315.42 (57.57)
cluster 6						**0**	6583.62 (81.13)	3079.82 (55.49)
cluster 7							**0**	5753.68 (75.85)
cluster 8								**0**

(D values are in parenthesis)

Cluster means

Highest cluster mean values for number of primary branches (8.66), number of secondary branches (11.86), plant height (173.4),number of siliqua per plant (371.8), number of seeds per siliqua (19.73), siliqua length (5.5), 1000 seed weight (4.9), oil content (35.93), and iron content (4.19 mg/100g) was found in cluster VIII. Similarly, cluster VII exhibited maximum cluster mean values for protein content (2.59) and ascorbic acid content (26.81). Cluster VI recorded maximum cluster mean for seed yield (1500.26), zinc content (0.96) and minimum days to 50% flowering (46) along with minimum days to 80% maturity (97). However, other clusters exhibited maximum cluster mean values for single character each Cluster V recorded maximum cluster mean value for Poshphorus content (99.96), Cluster III recorded maximum cluster mean value for Sulphur content (141.23), Cluster II recorded maximum cluster mean for Manganese content (0.98) and Cluster I recorded maximum cluster mean for Potassium content (234.95) respectively. Hence, genotypes which belonged to Cluster VIII will exhibit maximum primary branches, secondary branches, plant height, number of of siliqua per plant, number of seed per siliqua, siliqua length, 1000 seed weight, oil content, and iron content while that in cluster VII will exhibit high protein content with high ascorbic acid content and those in cluster VI with high seed yield, high zinc content along with early maturity. Thus, three crosses i.e., genotypes in cluster VI (CAULC-19) with genotypes in cluster VII (CAULC-23) for higher yield, followed by earliness, high content of zinc, high protein content along with ascorbic acid, another for genotype in cluster VI (CAULC-19) with genotype in cluster VIII (NRCHB-101) for higher yield, earliness, high content of zinc and for high oil content, and along with high content of iron content and another for genotype in cluster VII (CAULC-23) with genotype in cluster VIII (NRCHB-101) for high protein content, high ascorbic acid content and with high oil content, along with high iron content may be suggested for future research programmes. Bhandari (2021) also reported similar results. Sharma *et al* (2020) and Dev *et al* (2022) also reported similar results.

Table 4 : Cluster Means for 19 different traits in 21 genotypes of Local Yella.

Trait	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII
Days to 50% Flowering	62.3	62.83	47	50.83	52.77	46	61	58.66
No. Primary Branches per Plant	5.59	7.86	4.8	7.78	6.26	4.86	6.93	8.66
No. Secondary Branches per Plant	4.01	2.7	6.4	4.18	6.32	9.76	5.3	11.86
Plant Height (c.m.)	125.55	121.81	84.16	105.3	114.62	82.83	95.96	173.4
Days to 80% Maturity	108.5	109.16	97.66	101.16	100.33	97	105	114
No. of Siliqua per Plant	168.63	151.33	368.33	248.16	198	316.66	141.33	371.8
No. of Seeds per Siliqua	11.02	14.63	14.2	11.26	9.15	15.66	19.6	19.73
Siliqua Length (c.m.)	3.06	2.98	4.86	4.6	3.68	3.92	3.8	5.5
1000 Seed Weight (g)	3.33	4.53	4.5	4.56	2.97	4.36	4.33	4.9
Seed Yield (Kg/ha)	700.65	730.51	1020.56	730.05	626.62	1500.26	391.76	1242.83
Oil Content (%)	26.73	25.90	32.56	28.63	24.53	27.63	26.96	35.93
Protein Content (%)	2.06	1.90	1.77	1.71	2.06	2.04	2.59	1.94
Phosphorus (mg/100g)	43.83	57.59	43.16	52.71	99.96	41.81	79.65	34.74
Sulphur (mg/100g)	113.80	120.52	141.23	107.83	116.41	111.12	105.79	129.06
Potassium (mg/100g)	234.95	210.5	137.83	205.45	199.95	203.55	147.6	173.4
Iron (mg/100g)	2.47	3.85	1.46	3.59	2.61	1.46	2.69	4.19
Manganese (mg/100g)	0.94	0.98	0.83	0.86	0.93	0.27	0.68	0.9
Zinc (mg/100g)	0.95	0.62	0.46	0.91	0.54	0.96	0.35	0.82
Ascorbic Acid	11.76	11.67	24.96	9.12	18.79	13.44	26.81	9.76

Conclusion

- The 21 genotypes of local yella under study were grouped into 8 clusters based on Mahalanobis D^2 statistics using Tocher's method. Cluster I had the maximum number of genotypes (10), followed by cluster II (3), cluster III (2), cluster IV (2), while cluster V, cluster VI, cluster VII and cluster VIII was monogenotypic.
- Based on the inter cluster distance, genotypes from cluster VI and cluster VII as well as genotypes from cluster VII and cluster VIII can be selected as parents for crossing programs to obtain transgressive segregants in advanced generations as they have maximum divergence for the traits studied.
- The maximum intra-cluster distance was observed in cluster IV (37.33) which indicates that there is considerable heterogeneity present among the genotypes within the cluster.
- Cluster mean analysis revealed three crosses i.e., genotype in cluster VI (CAULC-19) with genotypes in cluster VII (CAULC-23) for higher seed yield, earliness, high content of zinc, high protein content along with high ascorbic acid. and another for genotypes in cluster VI (CAULC-19) with genotype in cluster in cluster VIII (NRCHB-101) for higher yield, earliness, high content of zinc and for high oil content, phosphorus and along with high content of iron and another for genotype in cluster VII (CAULC-23) with genotype in cluster VIII (NRCHB-101) for high protein content, high ascorbic acid content and with high oil content, along with high iron content may be suggested for future research programmes.
- Selection of genetically diverse parents can be done based on the traits viz., days to 50% flowering, number of secondary branches, number of seed per siliqua, number of siliqua per plant, along with oil content, phosphorus content, zinc content and ascorbic acid content which were significant contributors towards divergence.
- From the clustering pattern, it was observed that there is random pattern of clustering of the genotypes collected from different geographical area, suggesting that selection should be based on genetic divergence rather than geographical diversity.

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