

Genetic Variability, Character Association and Phytochemical Analysis in Okra Genotypes, *Abelmoschus Esculentus* (L.) Moench

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Abstract

*This study was conducted on the "Genetic variability, character association and phytochemical analysis in okra genotypes, *Abelmoschus esculentus* (L.) Moench". The study aimed to determine the extent of variability among the okra genotypes using phenotypic variation, correlation analysis and phytochemical analysis with a view of identifying the outstanding okra genotypes that could be utilized in future okra breeding program. The 20 okra genotypes used for this study were obtained from the okra germplasm bank of National Center for Genetic Resources and Biotechnology (NACGRAB), Ibadan, Oyo State, Nigeria. The experiment was carried out at the Teaching and Research Farm, Federal University of Technology, Akure. The experiment was laid out in a randomized complete block design with three replications. Data were collected on eleven agronomic characters. The statistical analysis was carried out using SAS version 9.2. The analysis of variance revealed that the okra varieties recorded significant differences for characters considered at 1% and 5% probability. The analysis of variance showed significant differences among the varieties for number of fruits, total fruit weight and individual fruit weight, indicating the presence of genetic variability for these characters. The phenotypic variance and phenotypic coefficient of variation were higher than the genotypic variance and genotypic coefficient of variation. The correlation analysis showed significant positive associations between fruit yield and plant height (0.772), number of fruits (0.843), number of branches (0.619), fruit weight (0.483) and fruit length (0.384) indicating that they are potential characters to target and select for in okra breeding programs. Phytochemical analysis also showed differences in phytate (21.01-32.96mg/g), tannin (0.03-0.08mg/g), saponin (5.80-10.20mg/g), oxalate (1.80-3.69mg/g), phenols (0.01 – 0.46mg/g) and flavonoids (9.90 -11.30mg/g). It can be concluded that the okra genotypes were from diverse origins indicating hybridization among them would result in good progenies. The okra genotypes also exhibited diversity in agronomic characters, association between the different agronomic characters and nutritional profile providing valuable information for okra characterization and future breeding programs in okra improvement.*

Key Words: Genetic Variability, Correlation, Phytochemical, Phenotypic, Genotypic

Introduction

Okra (*Abelmoschus esculentus* L.), a widely cultivated vegetable in tropical and subtropical regions, is valued for its high nutritional content and medicinal properties (Ogunbor, 2020). It is an important crop in both the agricultural and food industries, contributing significantly to the diets of people. Okra is known for its high fiber content, vitamins, and antioxidant properties, making it a vital component of a healthy diet (Axe, 2021). Okra is rich in bioactive compounds, including flavonoids, alkaloids, polyphenols, and saponins, which are known for their antioxidant, anti-inflammatory, and antimicrobial properties (Ajao *et al.*, 2020). Okra is also an excellent source of vitamins A, C, and K, as well as folate, calcium, and magnesium, all of which contribute to its nutritional benefits (Olayanju *et al.*, 2021). Genetic variability is a fundamental concept in plant breeding, providing the basis for improving agronomic traits such as yield, disease resistance, and quality (Kumar *et al.*, 2018). In okra, there is considerable genetic diversity, both within and between different varieties which can be harnessed to enhance desirable traits (Mishra *et al.*, 2021). To increase yield in okra, assessment of genetic variability is prerequisite since it is the source of variation in order to perform selection in any breeding programme (Kumar *et al.*, 2018). Presence of large amount of variability in any genetic material indicates the scope for further improvement of the crop. Characters association studies aim to understand the relationships between various traits or characters in okra (Durazzo *et al.*, 2018). Knowledge of correlation between yield and other characters is helpful and essential in selection of suitable plant type (Samiksha *et al.*, 2021). Character association studies help breeders understand the relationships between various agronomic traits, which can inform selection criteria in breeding programs (Melaku *et al.*, 2020). Phytochemical analysis involves identifying and quantifying bioactive compounds in plants (Ravindran and Samman, 2002). These compounds are known for their antioxidant, anti-inflammatory, and anticancer properties. Phytochemical analysis in diverse okra genotypes is essential to identify varieties with high levels of beneficial compounds. This research is embarked upon to determine: i) the extent of genetic diversity among the okra genotypes ii) identify the different characters associated with yield in okra; iii) the phytochemical composition of the various okra genotypes

MATERIALS AND METHOD

The experimental materials for this project consist of twenty (20) varieties of Okra, *Abelmoschus esculentus* (L.) Moench. The okra varieties were obtained from the germplasm bank of National Center for Genetic Resources and Biotechnology (NACGRAB), Department of Plant Genetic Resources, Ibadan, Oyo – State, Nigeria. The names of the 20 Okra genotypes, *Abelmoschus esculentus* (L) Moench are: NGB00245, NGB00297, NGB00298, NGB00304, NGB00305, NGB00322, NGB00323, NGB00338, NGB00339, NGB00342, NGB00380, NGB00416, NGB00438, NGB00466, NGB00535, NGB02212, NGB02423, NGB02428, NGB02433, NGB07726.

Field Evaluation

The experimental field was located at the Teaching and Research Farm, Federal University of Technology, Akure, Ondo – State, Nigeria. The field was manually cleared and was laid out in a randomized complete block design (RCBD). A single row plot was adopted for each of the varieties

with two replications. Each replication was made up of 20 rows comprising of 7 plants per row at a planting distance of 60cm and 45cm between and within the rows respectively. Data was collected on 5 competitive plants on each of the okra genotypes on the following agronomic characters: Days to first flowering (DTF), Days to maturity (DTM), Plant height at flowering (PHTF), Plant height at maturity (PHTM), Number of branches per plant (NBP), Number of fruits per plant (NFP), Fruit width (FW), Fruit length (FL), Number of ridges (NRF), Individual fruit weight (g) (IFWT), Total fruit weight (TFWT). All agronomic practices were carried out as when due which include the application of insecticides at 100mls/15 litres of water on fortnight basis to prevent damages by insect pests. NPK 15:15:15 fertilizer was applied at the rate of 60kg/Hectare from flowering till maturity on fortnight basis. Weeding was done thrice on the field manually to guide against unnecessary competition between the okra plants and the weeds.

Phytochemical Analysis

Determination of Tanin : About 0.2g of finely ground sample was weighed into a 50ml sample bottle. 10ml of 70% aqueous acetone was added and properly covered. The bottles were put in an ice bath shaker and shaken for 2hours at 30⁰C. Each solution was then centrifuge and the supernatant store in ice. 0.2ml of each solution was pipetted into the test tube and 0.8ml of distilled water was added. Standard tannin acid solutions were prepared from a 0.5mg/ml of the stock and the solution made up to 1ml with distilled water. 0.5ml of Folin ciocateau reagent was added to both sample and standard followed by 2.5ml of 20% Na₂CO₃ the solutions were then vortexed and allow to incubate for 40minutes at room temperature, its absorbance was read at 725nm against a reagent blank concentration of the same solution from a standard tannic acid curve was prepared (Makkar and Goodchild. 1996).

Determination of Total Phenol: This was determined according to the method of Makkar *et al.*, (1993). 0.2 millilitre of tannin containing extract was pipette and the volume made up to 1.0 ml with distilled water, 0.5ml of folin reagent was added to the mixture followed by 2.5ml of sodium carbonate reagent. The mixture was properly mixed with magnetic stirrer and allowed to stand for 40 minutes. The absorbance was read at 700nm and extrapolated in the standard curve.

Determination of Saponin : Saponin was determined by methods described by Sofowora(1993); Obadoni and Ochuko (2002).Five grams the sample was soaked in 100ml of 20% ethanol and allowed to stand for 4hours, the content was heated with continuous stirring over a hot water bath at a temperature of 55⁰C , the residue was re-extracted after filtration and heated with continuous stirring over a hot water at a constant temperature to 40cm² over water bath at 900.To the concentrate,20cm³ of dietyl ether was added in a separating funnel of about 250cm³ strongly agitated to recover the aqueous layer and ether layer was discarded. The purification process was repeated two times. N- butanol (60cm³) was added to 5% sodium chloride (10cm³) and extracted .The sodium layer was discarded while the remaining solution was heated in a water bath for 30minutes.The solution was transferred into a petri dish and oven dried to a constant weight. The saponin content was expressed in percentage as follows:

$$\% \text{ Saponin} = \frac{\text{weight of saponin}}{\text{Weight of sample}} \times 100$$

Determination of Oxalate :Oxalate was determined according to the method of Day and Underwood (1986). One gram of the finely powdered flour was mixed with seventy-five mililitre of 1.5N H₂SO₄ in a conical flask. The mixture was magnetically stirred for about 3hours and filtered through Whatmann No 1 filter paper. 25ml of the filtrate was titrated hot (80-90°C) on a water bath against 0.1N KMnO₄ solution to the point when a faint pink colour appeared that persisted for at least 30seconds. The titre value was obtained and calculation made as shown below

$$\text{Oxalate} = X * 0.9004\text{mg}/100\text{g}$$

Where X is the titre value(ml)

Determination of Flavonoid : Analytical measurement of flavonoid was carried out using the method reported by Ejikeme et al (2014) and Bohann and Kocipai (1974). 50cm³ of methanol was added to 2.5g of sample in a 250cm³ conical flask, covered and allowed to stand for 24hours. The supernatant was discarded followed by the re extraction of the residue for three times. The filtrate was transferred into a petri dish and oven dried to a constant weight, allowed to cool in a dessicator and weighed.

$$\% \text{ Flavonoid} = \frac{\text{weight of flavonoid}}{\text{Weight of sample}} \times 100$$

Determination of Phytate: Phytate was determined according to Young and Greaves (1940). Four grams of powdered sample was soaked in 200ml of 2% of Hcl for 3hours. This was then filtered through two layers of whatman No 1 filter paper. 50ml of the filtrate was taken into 400ml beaker while while 10cm³ of 0.3% ammonium thiocyanate solution (NH₄SCN) was added as an indicator, 170ml of distilled water was then added. The resulting solution titrated with ferric chloride solution that contained 0.00195g of Fe per ml of FeCl₃ used and the titre value was obtained and the calculation made: Phytate= X*564.11mg/100

Where X = titre value (ml)

Statistical Analysis: The analysis of variance (ANOVA) for different characters was carried out using the mean of the data in order to partition variability due to different sources by following the procedure of Panse and Sukhatme (1961). All statistical analysis was performed using SAS version 9.2 (SAS, 2008) to examine the presence of statistically significant differences among the genotypes for characters studied.

RESULT

The estimates of the mean square of all the characters studied in the okra genotypes are presented in Table 1. the analysis revealed that the okra genotypes were highly significant for almost all the characters with the exception of the number of leaves per plant at flowering and fruit length which recorded significant differences among the okra genotypes at 17.45 and 13.04 respectively.

TABLE 1: Analysis of Variance of the Characters studied in the Okra genotypes

SOV	DF	DTF (Days)	PHTF (cm)	DTM (Days)	PHTM (cm)	NBP	NLPF	FW (cm)	FL (cm)	NFP	NRF	TFWT (g)	IFWT (g)
REP	1	38.03	1345.60*	38.03	164.00	1.60	0.03	12.66	14.40	0.23	0.63	11.12	0.01
VARTY	19	22.45	283.80*	22.45	916.1**	6.75	17.45*	15.63**	13.04*	9.34**	4.91	1469.05**	25.77*
ERROR	19	16.39	166.1	16.39	587.8	2.15	6.24	9.05	6.86	2.96	0.94	944.13	10.86

*, ** indicate significance at 1% and 5% level of probability

DF= Degree of freedom; DTF= Days to flowering; PHTF= Plant height at flowering(cm); DTM= Days to maturity(days); PHTM=Plant height at maturity(cm); NBP= Number of branches per plant; NLPF= Number of leaves per plant; FW= Fruit width(cm); FL= Fruit length(cm); NFP= Number of fruits per plants; NRF= Number of ridges per fruit; TFWT= Total fruit weight (g); IFWT= Individual fruit weight (g).

The estimates of the genetic components among the okra genotypes are presented in Table 2. The phenotypic variance were higher than the genotypic variance implying that the expression of the characters were under the influence of the environmental factors. The genotypic variance varied between 1.985 and 262.460. The highest genotypic variance was recorded in total fruit weight (262.460) followed by plant height at maturity (164.150) followed by plant height at flowering (58.850) whereas the lowest value was recorded in number of ridges per fruit (1.985). As regards the phenotypic variance, it ranged from 4.450 to 1206.590 being maximum in individual fruit weight (1206.590) followed by plant height at maturity (751.950) followed by plant height at flowering (224.95) whereas it was minimum in number of branches per plant (4.450). The genotypic coefficient of variation varied between 2.170 and 44.931. Number of branches per plant, number of leaves per plant at flowering, number of fruits per plant, total fruit weight and individual fruit weight recorded a high level of genotypic coefficient of variation 30.646%, 23.036%, 44.931%, 38.1385 and 26.518% respectively. Days to flowering and days to maturity recorded a low level of genotypic coefficient of variation of 3.334% and 2.170% respectively. The phenotypic coefficient of variation ranged from 5.493% to 81.770%. All the characters recorded a very high level of phenotypic coefficient of variation with the exception of days to flowering (8.438%) days to maturity (5.493%). The heritability estimates ranged between 15.602% and 67.863%. The heritability estimates were moderately high for number of branches per plant (51.685%), number of leaves per plant (47.320%), fruit length (31.055%), number of fruits per plant (51.870%) and individual fruit weight (40.704%). The heritability estimates were generally low for days to flowering (15.604%), plant height at flowering (26.161%), days to maturity (15.6025), plant height at maturity (21.830%), fruit weight (26.661%) and total fruit weight (21.752%).

The genetic advance were generally very high for all the characters ranging from 141.646 % to 1233.112%. The genetic advance of mean % ranged from 1.7666 to 66.665%. The genetic advance of mean % estimates were generally high for almost all the characters with the exception

of days to flowering (2.712%), plant height at flowering (19.835%), days to maturity (1.766%), plant height at maturity (16.125%) and fruit width (15.860%).

TABLE 2: Estimates of Genetic Components in the Okra genotypes

Character	Mean	Genotypic variance	Phenotypic variance	GCV%	PCV%	HB%	GA%	GAM%
DTF (days)	52.225	3.030	19.420	3.334	8.438	15.602	141.646	2.712
PHTF (cm)	40.750	58.850	224.950	18.825	36.805	26.161	808.279	19.835
DTM (days)	80.225	3.030	19.420	2.170	5.493	15.602	141.646	1.766
PHTM (cm)	76.470	164.150	751.950	16.752	35.859	21.830	1233.112	16.125
NBP	4.950	2.300	4.450	30.646	42.626	51.685	224.656	45.385
NLPF	10.275	5.605	11.845	23.036	33.499	47.320	335.520	32.654
FW(cm)	12.162	3.290	12.340	14.915	28.877	26.661	192.887	15.860
FL(cm)	9.650	3.090	9.950	18.218	32.684	31.055	201.774	20.909
NFP	3.975	3.190	6.150	44.931	62.390	51.870	264.993	66.665
NRF	7.675	1.985	2.925	18.358	22.280	67.863	239.055	31.147
IFWT (g)	10.295	7.455	18.315	26.518	41.574	40.704	358.882	34.860
TFWT (g)	42.480	262.460	1206.590	38.138	81.770	21.752	1556.505	36.641

DTF= Days to flowering; PHTF= Plant height at flowering(cm); DTM= Days to maturity(days); PHTM=Plant height at maturity(cm); NBP= Number of branches per plant; NLPF= Number of leaves per plant; FW= Fruit width(cm); FL= Fruit length(cm); NFP= Number of fruits per plants; NRF= Number of ridges per fruit; TFWT= Total fruit weight (g); IFWT= Individual fruit weight(g); GCV= Genotypic coefficient of variation; PCV= phenotypic coefficient variation; HB= Heritability; GA= Genetic advance; GAM= Genetic advance as percentage of mean %.

The estimates of the correlation between the fruit yield and the other characters are presented in Table 3. days to flowering exhibited a positive and significant correlation with days to maturity (1.000) whereas it exhibited a negative and significant correlation with plant height at maturity (-0.425), number of fruits per plant (-0.481) and number of ridges per fruit (-0.463). plant height at flowering displayed a positive and highly significant correlation with plant height at maturity (0.559), fruit width (0.623), fruit length (0.560), number of ridges per fruit (0.729) and total fruit weight (0.657) but a positive and significant correlation with number of branches per plant (0.389). plant height at maturity exhibited a positive and highly significant correlation with number of branches per plant, number of leaves per plant, fruit width, fruit length, number of fruits, number of ridges per fruit, individual fruit weight and total fruit weight at 0.547, 0.667, 0.712, 0.622, 0.818, 0.590 and 0.772. number of leaves at flowering displayed a positive and significant correlation with fruit width, number of fruits, individual fruit weight and total fruit weight at 0.455, 0.492, 0.491 and 0.505 respectively. Fruit width also exhibited a positive and highly significant correlation with fruit length, number of fruits, number of ridges and total fruit weight at 0.931,

0.660, 0.845 and 0.483 respectively. Fruit length also exhibited a positive and highly significant correlation with number of fruits, number of ridges and total fruit weight at 0.635, 0.728 and 0.384 respectively. Number of fruits exhibited number of ridges and total fruit weight at 0.525 and 0.843 respectively. Individual fruit weight also exhibited a positive and highly significant correlation with total fruit weight (0.765). The positive correlation between plant height at flowering and number of branches per plant is an indication that taller plants at flowering will result in higher number of branches. Higher number of branches will result in more leaves per plant. Plant height at flowering, plant height at maturity, number of branches, number of leaves at flowering, fruit width, fruit length, number of fruits and individual fruit weight recorded positive and significant correlation with total fruit weight. This implies that taller plants will be highly branched with high number of leaves will result into greater number of fruits and bigger number of fruits will invariably cumulate into higher yield per plant.

TABLE 3: Correlation Coefficient Between Fruit Yield and Its Related Characters in the Okra genotypes

	DTF	PHTF	DTM	PHTM	NBP	NLPF	FW	FL	NFP	NRF	IFWT	TFWT
DTF	-	0.109	1.000**	-0.425*	-0.320	0.040	-0.377	-0.325	-0.481*	-0.463*	0.228	-0.217
PHTF			-0.109	0.559**	0.379*	0.287	0.623**	0.56**	0.345	0.729**	0.226	0.657**
DTM				-0.425*	-0.320	0.040	-0.377	-0.325	-0.481*	-0.463*	0.228	-0.387*
PHTM					0.547**	0.667**	0.712**	0.622**	0.818**	0.590**	0.396*	0.772**
NBP						0.518**	0.705**	0.500*	0.569**	0.705**	0.490*	0.619**
NLPF							0.455*	0.318	0.492*	0.350	0.491*	0.505**
FW								0.931**	0.660**	0.845**	0.129	0.483*
FL									0.635**	0.728**	-0.015	0.384*
NFP										0.525**	0.331	0.843**
NRF											0.084	0.361
IFWT												0.765**
TFWT												

DTF= Days to flowering; PHTF= Plant height at flowering(cm); DTM= Days to maturity(days); PHTM=Plant height at maturity(cm); NBP= Number of branches per plant; NLPF= Number of leaves per plant; FW= Fruit width(cm); FL= Fruit length(cm); NFP= Number of fruits per plants; NRF= Number of ridges per fruit; TFWT= Total fruit weight (g); IFWT= Individual fruit weight (g).

The estimates of the phytochemical composition of the okra genotypes are presented in Table 4. The phytate composition ranged between 21.01 and 32.960mg/g. The highest phytate level is

recorded in G10 (32.960mg/g) followed by G2 (32.130mg/g) followed by G3 (29.250mg/g) whereas the lowest level of phytate is recorded in G9(21.010mg/g). As regards the tanin level, it ranged from 0.030 to 0.080mg/g being maximum in G16 and G24 (0.080mg/g) and minimum in G9 and G17(0.030mg/g). for the saponin content, the value ranged between 5.80 and 10.200mg/g being maximum in G7, G14 and G24 (10.20mg/g) whereas it is minimum in G6 (5.80mg/g). the oxalate content varied from 1.800 to 3.690%mg/g. the highest level of oxalate is recorded in G4 (3.690mg/g) followed by G9 (3.500mg/g) followed by (3.190mg/g) whereas the lowest level is recorded in G16 (1.800mg/g). As regards the phenol content, it varied between 0.100 and 0.460mg/g being maximum in G14 (0.460mg/g) and minimum in G7, G11 and G22 (0.100mg/g). as regards the flavonoids content, the level varied between 9.900 and 11.300mg/g being maximum in G10 (11.300mg/g) and minimum in G9 (9.900mg/g).

TABLE 4: PHYTOCHEMICAL COMPOSITION OF THE OKRA GENOTYPES

GENO. NO	PHYTATE (mg/g)	TANIN (mg/g)	SAPONIN (mg/g)	OXALATE (mg/g)	PHENOLS (mg/g)	FLAVONOIDS (mg/g)
1	28.840a	0.050ab	7.800ab	2.390b	0.310ab	11.000a
2	32.130a	0.070a	6.600bc	2.610ab	0.240b	11.200a
3	29.250a	0.040b	6.200bc	2.790ab	0.400a	10.100ab
4	28.420a	0.070a	6.150bc	3.690a	0.320ab	10.720a
5	27.130ab	0.041b	7.050ab	2.250b	0.150bc	9.900ab
6	27.600ab	0.070a	5.800bc	3.190a	0.220b	11.000a
7	28.840a	0.050ab	10.200a	2.790ab	0.100c	10.900a
9	21.010b	0.030bc	9.000a	3.500a	0.150bc	9.900ab
10	32.960a	0.070a	7.250ab	2.160b	0.230b	11.300a
11	28.420a	0.030bc	6.600b	3.110a	0.100c	10.900a
14	21.850b	0.070a	10.200a	2.880ab	0.460a	11.260a
16	27.190ab	0.080a	8.150ab	1.800bc	0.310ab	11.050a
17	28.420a	0.030bc	9.800a	2.390b	0.330ab	11.300a
18	28.840a	0.040b	8.200ab	2.210b	0.410a	10.010ab
19	28.010a	0.040b	7.900ab	2.610ab	0.140c	10.900a
20	28.840a	0.070a	8.100ab	2.610ab	0.170bc	10.700a
21	28.420a	0.050ab	7.650ab	2.70ab	0.140bc	11.000a
22	25.950ab	0.050ab	8.400ab	2.610ab	0.100c	11.200a
23	24.720ab	0.050ab	9.900a	2.480b	0.120c	11.100a
24	28.420a	0.080a	10.200a	2.610ab	0.140bc	10.300ab

G1=NGB00245; G2= NGB00297; G3= NGB00298; G4=NGB00304; G5= NGB00305; G6= NGB00322; G7=NGB00323; G9=NGB00338; G10=NGB00339; G11=NGB00342; G14=NGB00380; G16=NGB00416; G17=NGB00438; G18=NGB00466; G19=NGB00535; G20=NGB02212; G21=NGB02423; G22=NGB02428; G23=NGB02433; G24=NGB07726

DISCUSSIONS

The significant level of some of the characters among the okra genotypes is an indication that the okra genotypes were from diverse background. This findings corroborates the findings of Rambabu *et al.*, (2019). The phenotypic variance and phenotypic coefficient of variation were generally higher than the genotypic variance and genotypic coefficient of variation which imply that the expression of the characters are under the influence of the environmental factors. This finding is synonymous to the findings of Ranpise *et al.*, (2018). They reported higher phenotypic coefficient of variation than genotypic coefficient of variation for number of fruits, and total fruit weight. The moderately high and high heritability and high genetic advance recorded in number of branches, number of leaves at flowering, fruit length, number of fruits, number of ridges and individual fruit weight is similar to the findings of Melaku *et al.*, (2020) and Akotkar *et al.*, (2020). the positive and significant correlation between fruit yield and plant height at flowering, number of branches, fruit width and individual fruit weight in this study corroborates the findings of Akotkar *et al.*, (2020) which imply that these characters can be selected for in okra breeding program. The results of the phytochemical analysis revealed that the okra varieties were different from one another based on the composition. The variations in the phytochemical composition of the okra genotypes corroborates the findings of Hassan, and Mahfouz (2016).

CONCLUSION

It can be concluded from this study that the okra genotypes were from different backgrounds. It can also be concluded that the expression of the characters studied were under the influence of genetic factors coupled with environmental factors. The significant correlations observed between fruit yield and plant height at flowering, plant height at maturity, number of branches, number of leaves at flowering, fruit width, fruit length, number of fruits and individual fruit weight indicated that the selections and improvement of these characters will result in higher fruit yield. The results of phytochemical analysis recorded in this study is an indication that okra is a very rich source of anti-oxidant and anti inflammatory potential.

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