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STABILITY OF FOLIAR EPIDERMAL ATTRIBUTES IN SESAME (*Sesamum indicum* L.)

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ABSTRACT

The response of foliar epidermis to different nutrient sources was investigated at the Research Garden of the Biological Sciences Department in Kogi State University, Anyigba. Seeds of sesame (*Sesamum indicum* L.) were obtained from Ilorin, Kwara State, Nigeria. Top soil from the research site was separately mixed with N.P.K fertilizer, chicken dung and cow dung while the one without fertilizer application serves as the control. The data obtained from adaxial and abaxial leaf surfaces were subjected to Analysis of Variance (ANOVA) while means with significant difference were separated using Duncan Multiple Range Test (DMRT). Two attributes on the adaxial surface and three traits on the abaxial surface did not show statistical significant variation in their response to nutrient sources which implies that they are stable; their expressions are under strong genetic control and will play a significant role in sesame taxonomy.

Keywords: Foliar, epidermis, N.P.K fertilizer, chicken dung, cow dung.

1. Introduction

Sesame belongs to the division Spermatophyta, subdivision Angiospermae, class Dicotyledoneae, order Tubiflorae, family Pedaliaceae and genus *Sesamum* (Falusi and Salako 2001). Langham and Wiemers (2002) reported that a wide range of soils are suitable for sesame cultivation but Mahajan *et al*., (2007) specifically reported that sesame performs best on fertile, well drained soils such as silt loamy soils. *Sesamum* species has its production requirements for optimum yield such as appropriate time of planting, mode of planting, fertilizer application, planting spacing, weed management, harvesting and post-harvest practices (Ram Materials Research and Development Council, 2004).

The external factors that influence sesame productivity include climatic factors such as temperature, rainfall, day length and soil type (Adebisi *et al.*, 2005). Variations in climatic and edaphic conditions according to Muhamman and Gungula (2008), affect sesame yields and performance but the major constraints identified to militate against optimum production of sesame in most countries are instability in yield, lack of wider adaptability, drought, non - synchronous maturity, poor stand establishment, poor of response to fertilizer application, profuse branching, lack of seed retention, low harvest index and susceptibility to insect pests and pathogens (Mahajan *et al*., 2007). Alege *et al*., (2011) opined that all these factors should be taken into account when planning conservation strategies for sesame breeding programs. Alege *et al*., (2009) reported that some morphological characteristics in okra responded significantly to different nutrient sources. Likewise, the study of Alege *et al*., (2013) on the response of morphological, proximate and mineral attributes in sesame to different nutrient sources revealed that some of these traits are affected by the fertility conditions of the soil.

Epidermal features have become widely studied from three main perspectives which include ontogenetic, phylogenetic and taxonomic perspectives. Adegbite (1995) stated that stomata structures are under strong and highly integrated genetic control and their modification could reflect evolutionary trend. Mbagwu *et al*., (2007) concluded that leaf possesses anatomical attributes that are of potential taxonomical significance even at the genus and species level. Erkara and Kogunco (2007) stressed the need to shed more light on evolutionary and stability of some attributes in plants.

Sesame varieties selected from local landraces according to Mahajan *et al.*, (2007) are generally adapted only to the environments from which they were derived. In contrary, Ercan *et al.*, (2002) reported that numerous modern varieties and ecotypes of sesame adapt to various ecological conditions. However, the cultivation of these modern varieties is limited due to insufficient genetic information on them. Alege *et al.*, (2009) reported that the wide diversity in a sizeable number of sesame characteristics no doubt poses a problem to proper identification of the plant's taxa. Alege *et al.*, (2011) addressed this identification problem by indicating the vegetative and pod characteristics of sesame plants that are species specific for the purpose of their easy and quick identification.Therefore, it is necessary to study the stability of leaf epidermal features in sesame to different nutrient sources since published information on this aspect is very scarce. The objective of this study is therefore to investigate the effect of nutrient sources on the stability of leaf epidermal attributes of sesame with a view to the give genetic and taxonomic implications of our findings on the studied plants.

2. Materials and Methods

2.1 Soil Analysis

Samples of the top soil (about 0-15 cm deep) were taken from the Research Garden of Biological Sciences Department, Kogi State University, Anyigba (research location) to determine the nutrient status of the soil. The soil

sample was air-dried, ground, sieved through 5mm mesh, enveloped and labelled for analysis using analytical methods of IITA (1982). Soil sample was investigated to determine the physico-chemical parameters such as water holding capability, soil pH and organic matter, total Nitrogen (N), Potassium (K), Phosphorus (P), Magnesium (Mg), Calcium (Ca), percentage sand, clay and silt.

2.2 Seed Source and Planting

Seeds of black seeded sesame were obtained from Oja-oba in Ilorin, Kwara state, Nigeria in 2008.The seeds were planted in 2008 and 2009 at the research garden of the Biological sciences Department of Kogi State University, Anyigba, Kogi State, Nigeria. This was done to adapt the plant to the environment where the research will take place (Anyigba) and to eliminate variations induced by environmental differences. The research location is between latitude $8^0$43´ and $9^0$5´ South of the equator and between $6^0$6´ and $7^0$45´ West of the Meridian. Seeds of sesame were broadcast on a raised bed and watered until seedlings were fully established. Top soils from the research location were collected and mixed thoroughly. The soil was divided into four (4) portions and 10kg of each portion was mixed separately with 500g each of NPK fertilizer, poultry dung and cow dung respectively, the fourth portion was without treatment representing the control. Each treatment was replicated five (5) times and three (3) seedlings were transplanted to each bag which was later thinned to two seedlings per bag. The bags were labelled appropriately.

A - bags containing N.P.K fertilizer (inorganic fertilizer)
B - bags containing poultry dung (organic fertilizer)
C - bags containing cow dung (organic fertilizer)
D - bags without any treatment (control)

Complete Randomized Design (CRD) was adopted for the set up

2.3 Leaf Epidermal Studies

Fresh leaves were collected from plants grown from each of the four nutrient sources. Each leaf was painted with finger nail polish on both the adaxial and abaxial surfaces and allowed to dry. After drying, short clear cellophane tape was firmly pressed over the dried nail polish on the surfaces according to the method of Mbagwu *et al.,* (2007). Epidermal strips were taken from the median portion of matured leaves, stained in alcoholic safranin and mounted in 50% glycerine jelly for microscopic examination. Epidermal strips from both the adaxial and abaxial surfaces were prepared and mounted separately. Photographs of good preparations were taken at a magnification of X400 objective with photomicrograph. The length and width of epidermal cells and stomata apparatus were measured with micrometer eyepiece graticule. The number of stomata and epidermal cells were observed and recorded. Ten peelings were mounted for each leaf surface, while observations and measurements were made from 30 microscope fields of focus at × 40 objectives.

2.4 Data Analysis

Data obtained from each leaf epidermal attributes on both the abaxial and adaxial surfaces were subjected to Analysis of Variance (ANOVA) and means with significant difference separated using Duncan Multiple Range Test (DMRT).

The Stomata Index (SI) and Guard Cell Area (GCA) were estimated for the leaf surfaces using the following formulae as described by Wilkinson, (1979).

Guard cell area = Length of stomata x Breadth of stomata x K

Where K = Franc's constant = 0.78524

$$\text{Stomata Index (SI)} = \frac{S}{(S + E)} \times 100$$

Where S = Number of stomata per unit area

E= Number of epidermal cells in the same unit area

3. Results

Table 1: Physical and Chemical Characteristics of the Soil Used for Growing *Sesamum indicum* plants.

SOIL PROPERTIES	VALUE
Field capacity (mm)	111.08
Permanent wilting (mm)	104.31
Availability of water (mm)	6.77
Sandy	27.78
Silt %	4.17
Clay %	68.05
pH %	6.50
Organic matter %	17.90
Nitrogen (N) %	66.67
Phosphorus (p) %	2.909
Sodium (Na) %	12.00
Potassium (K) %	4.30
Calcium (Ca) %	91
Magnesium (Mg) %	15.10

The physico-chemical conditions of the soil in table 1 showed that the soil is rich in clay content (68.05%), slightly acidic (PH of 6.5), rich in organic matter (66.67%) and also rich in Nitrogen (17.90%).

Table 2: Some Foliar Epidermal Attributes for the Adaxial and Abaxial surfaces for the Different Nutrient Sources.

Nutrient Sources	Surface	Shape of Epidermal Cells	Epidermal Wall Pattern	Stomata Type	Density of Unicellular Trichomes	Density of Multicellular Trichomes	Leave Conditions
NPK	Adaxial	P,I	S,C	Tetracytic	+	+ + +	Hypoamphistomatic
	Abaxial	I	U	Tetracytic	+	+	
Chicken dung	Adaxial	P,I	S,C	Tetracytic	+	+ +	Hypoamphistomatic
	Abaxial	I	U	Tetracytic	+	+	
Cow dung	Adaxial	P,I	S,C	Tetracytic	+	+ + + + +	Hypoamphistomatic
	Abaxial	I	U	Tetracytic	+	+	
Control	Adaxial	P,I	S,C	Tetracytic	+	+ + + +	Hypoamphistomatic
	Abaxial	I	U	Tetracytic	+	+	

Key:

P-Polygonal	+- very low density
I-Irregular	+ +- low density
S-Straight	+ + +- moderate density
C-Curve	+ + + +- high density
U- Undulating.	+ + + + +- very high desity

The shapes of epidermal cells on the adaxial surface were polygonal and irregular while only irregular shaped epidermal cells occurred on the abaxial surfaces (table 2 and plates 1a – h). The epidermal wall pattern was straight and curve on the adaxial surface while the abaxial surface consisted of only undulating wall pattern. Both the abaxial and adaxial surfaces had tetracytic stomata types and very low densities of unicellular trichome but the density of multicellular trichomes vary across the different nutrient sources with cow dung producing the highest density of multicellular trichomes while the least density on the adaxial was recorded for chicken dung. It could be observed from table 2 and plates 1a - h that the leaves across the different nutrient sources are hypo - amphistomatic (i.e having more stomata on the abaxial than the adaxial surfaces).

Table 3: Means of measurements for the adaxial leave surfaces across the different nutrient sources.

Nutrient sources	Epidermal cell number	Stomata Number	Length of Epidermal cell(*u*m)	Width of Epidermal cell(*u*m)	Length of Stomata (*u*m)	Width of Stomata (*u*m)	Guard Cell Area ($u m^2$)	Stomata Index (%)
NPK	112.40^{b}	14.67^{b}	3.56	1.52	1.40^{b}	0.91^{b}	1.00	11.54
Chicken dung	79.00^{c}	10.70^{c}	3.33	1.59	2.78^{a}	1.75^{a}	3.82	11.93
Cow dung	122.47^{a}	22.33^{a}	2.94	1.57	1.38^{b}	0.84^{b}	1.16	15.42
Control	106.73^{b}	13.07^{bc}	3.03	1.68	1.49^{b}	0.84^{b}	0.98	10.91
LSD (0.05)	4.52	2.11	NS	NS	0.23	0.22	-	-

❖ Means with the same letter are not significantly different

From the result obtained in table 3, only the length and width of the epidermal cell on the adaxial leaf surface did not show significant variations across the different nutrient source while number of epidermal cell, number of stomata, length and width of stomata all showed significant differences across the different nutrient sources. A significantly higher number of epidermal cell and stomata of 122.47and 22.33 respectively were obtained from plant grown with cow dung while leaves from plant grown with chicken dung showed significantly higher length and breadth of stomata of 2.78*u*m and 1.75*u*m respectively.

The highest Guard Cell Area was observed in leaves obtained from chicken dung use as nutrient source ($3.82 u m^2$) while the least Guard Cell Area was recorded for leaves obtained from control pot ($0.98 u m^2$). The highest Stomata Index was obtained from leaves obtained from cow dung (15.42%) but leaves from plants gotten from the control pot had the least Stomata Index of 10.91%.

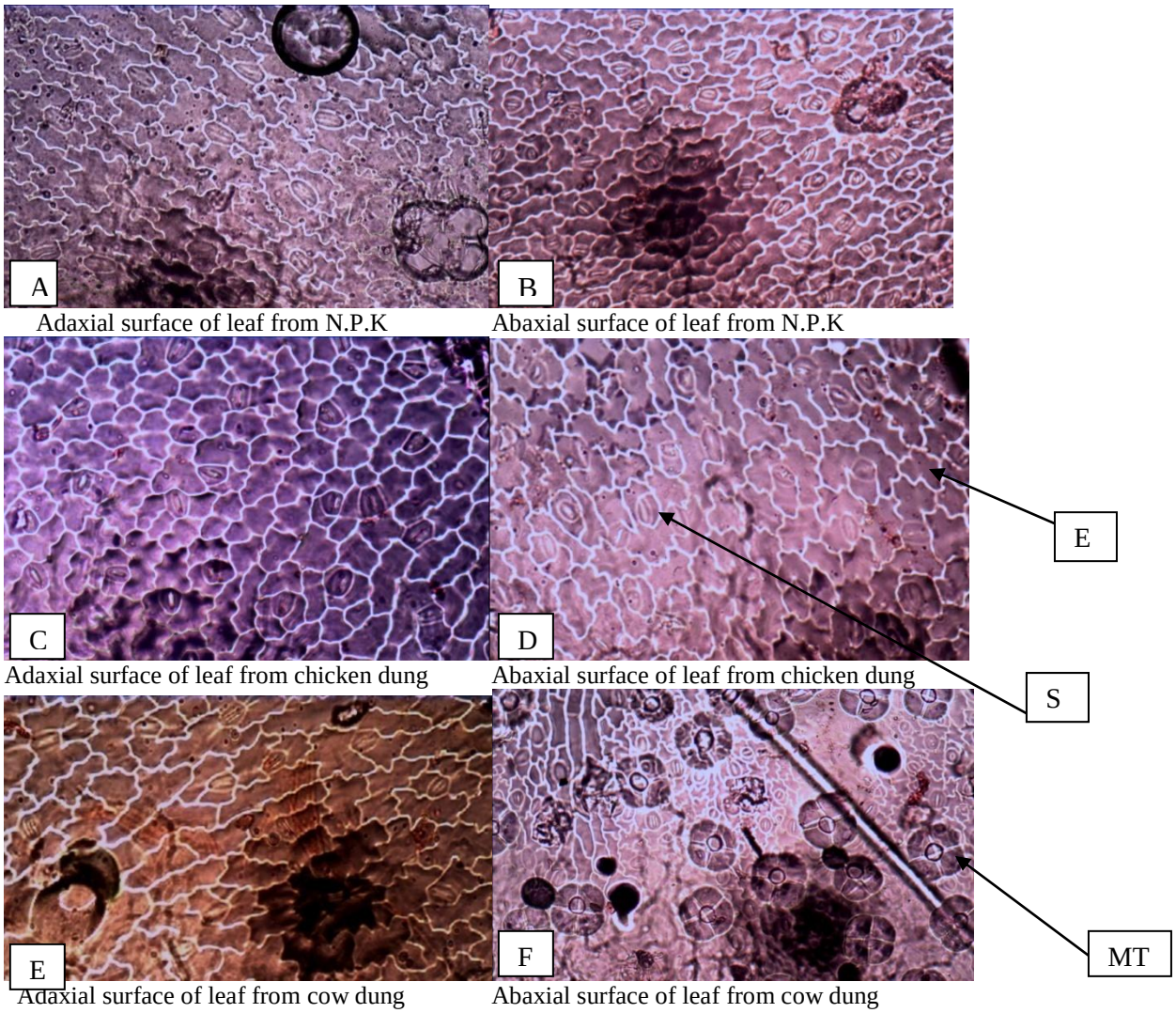
Table 4: Means of measurements for the abaxial leave surfaces across the different nutrient sources.

Nutrient sources	Epidermal cell number	Stomata Number	Length of Epidermal cell (*u*m)	Width of Epidermal cell (*u*m)	Length of Stomata (*u*m)	Width of Stomata (*u*m)	Guard Cell Area (um^2)	Stomata Index (%)
NPK	169.26	31.83[b]	2.91	0.42	1.03[b]	0.63[b]	0.51	15.83
Chicken dung	160.20	31.93[b]	2.51	0.39	1.05[b]	0.56[c]	0.46	16.62
Cow dung	160.87	33.20[b]	2.99	0.42	1.35[a]	0.78[a]	0.83	17.11
Control	169.07	39.63[a]	5.65	0.64	1.37[a]	0.69[b]	0.74	18.99
LSD (0.05)	NS	2.53	NS	NS	0.31	0.07	-	-

❖ Means with the same letter are not significantly different.

Table 4 showed that on the abaxial leaf surface the number, length and width of the epidermal cell did not show significant variations across the different nutrient source while number of stomata, length and width of stomata all showed significant differences across the different nutrient sources. A significantly higher number of stomata of 39.63 were obtained from plant grown without fertilizer (control) while the least was recorded for plants grown with N.P.K fertilizer (31.83). Leaves obtained from plant grown in control pot showed higher length of stomata (1.37*u*m) while the shortest stomata were recorded for plants grown with N.P.K fertilizer (1.03*u*m). The widest stomata were recorded for plant grown with cow dung (0.78*u*m) but plant grown with chicken dung produced the thinnest stomata (0.56*u*m).

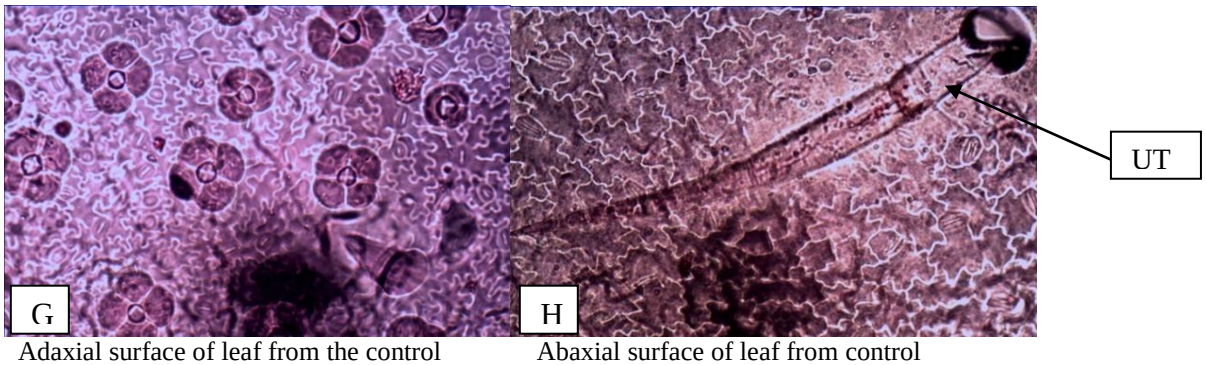
The highest Guard Cell Area was observed in leaves obtained from cow dung use as nutrient source (0.83um^2) while the least Guard Cell Area was recorded for leaves obtained from chicken dung used as nutrient source (0.46 um^2). In the contrary, the highest Stomata Index was obtained from leaves obtained from plants grown without the addition of fertilizer i.e control pot (18.99%) while leaves from N.P.K fertilizer had the least Stomata Index of 15.83%.



Adaxial surface of leaf from N.P.K Abaxial surface of leaf from N.P.K

Adaxial surface of leaf from chicken dung Abaxial surface of leaf from chicken dung

Adaxial surface of leaf from cow dung Abaxial surface of leaf from cow dung



Adaxial surface of leaf from the control Abaxial surface of leaf from control

Plates A- H: Photomicrographs of the adaxial and abaxial leaf surfaces From Different Nutrient Sources.

Key:

E-Epidermal cell UT – Unicellular Trichome

S-Stoma MT – Multicellular Trichome

4. Discussion

Essiett *et al.,* (2012) reported that taxonomists lately realized the importance of microscopic features of the leaf epidermis and recently taxonomic monographs are now considered incomplete without them.The physico-chemical conditions of the soil in table 1 showed that the soil is rich in clay content (68.05%), slightly acidic (PH of 6.5), rich in organic matter (66.67%) and Nitrogen (17.90%). This soil condition is within the range recommended by Langham and Wiemer (2002) for optimum sesame production.

This present study revealed the presence of polygonal and irregular shapes epidermal cells on the adaxial surface, but only irregular shaped epidermal cell occurred on the abaxial surfaces. Straight and curve shape epidermal wall patterns were found on the adaxial surface while only undulating wall patterns were recorded on the abaxial surface. Also, tetracytic stomata type and hypo - amphistomatic leaf conditions were common to both the abaxial and adaxial surfaces (plates 1a – h and table 2). This is an indication that all these attributes are stable, under strong genetic effect and are traits typical to the studied plant species. Therefore, they are all good taxonomic indicators in sesame plant. This finding corroborates the report of Abdulraman and Oladele (2003) that *Sesamum indicum* possess hypo-amphistomatic leave and tetracytic stomata type is the most abundant stomata type.

The density of unicellular trichome on both leaf surfaces and the density of multicellular trichomes on the abaxial surface did not vary across the different nutrient sources (table 2). This indicates that the traits are stable and can be used for the delimitation of sesame, but the density of multicellular trichome on the adaxial surface is environment dependent and is a very poor taxonomic indicator for the delimitation of the studied plants, it is therefore not under strong genetic influence because its expression greatly depend on the fertility status of the soil. Essiett *et al.,* (2012) reported that trichome density can be reasonably employed for the delimition of plant species. Also Ogundipe and Pereira-sheteolu (2006) reported that the presence and types of trichomes are useful diagnostic features in the pedialiaceae family.

Only the length and width of the epidermal cell on the adaxial leaf surface did not show significant variations across the different nutrient source while number of epidermal cell, number of stomata, length and width of stomata all showed significant differences in their expressions across the different nutrient sources (table 3). This is an indication that the length and width of epidermal cell on the adaxial surface are under strong genetic control and will be useful in the taxonomy of the plant since the soil fertility condition did not influence their expressions in the studied plants.

Higher Guard Cell Area and Stomata Index were observed in leaves grown with either organic or inorganic nutrient sources compared with leaves from control pot on the adaxial surface (Table 3), which indicates that the application of fertilizer especially chicken dung increased the size of the stomata and the guard cell.

The number, length and width of the epidermal cell on the abaxial leaf surface did not show significant variations across the different nutrient source while number of stomata, length and width of stomata all showed statistical significant differences across the different nutrient sources. This is an indication that the epidermal cell attributes on the abaxial surface are stable in sesame and would be a better tool for sesame taxonomy than stomata traits because all the stomata characteristics considered in this study vary with the application of organic and inorganic fertilizers. This agrees with the report of Chen *et al*., (2009) that stomata density may depend on the environmental factor.

Contrary to the observation on the adaxial surface, the Guard Cell Area and Stomata Index on the abaxial surfaces reduce slightly with the application of organic and inorganic fertilizer. This indicates that the application of fertilizer especially N.P.K fertilizer and chicken dung reduced the size of stomata and guard cell. This may have adaptive implication for water conservation in the studied plant since the abaxial surface is responsible for loss of water through the stomata (transpiration). The reduction in stomata size in the study of Rai and Mishra (2013) was attributed to adaptive response of the studied plants to avoid entry of harmful constituents of exhaust.

By comparing the epidermal attributes on the adaxial (table 3) and abaxial (table 4) for the studied plants, only 2 attributes were stable across the different nutrient sources on the adaxial surface while 3 foliar epidermal attributes were stable on the abaxial surfaces which implies that the epidermal characteristics on the abaxial surface will be more valuable in the systematics of sesame than those of the adaxial surface. This report contradicts the findings in the previous work of Adegite (1995) on *Aspilia* species that the epidermal characteristics of the adaxial surface in a better

tool for the delimitation of the genus *Aspilia* than the abaxial surface. The findings in this study showed that the expressions of foliar epidermal attributes in sesame plant to some extent depend on fertility status of the soil.

5. Conclusion

In this study generally, the epidermal cell characteristics are stable traits and therefore proofed to be a better tool for the delimitation of sesame than stomata traits because the former is under strong genetic control and not environment dependent while the later varies with the fertility status of the soil. Also for better taxonomic result, more attention should be paid to leaf epidermal characteristics on the abaxial surface than those of the adaxial surface in sesame (*Sesamum indicum* L).

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