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The Phylogenetic Relations and Biogeography of Three Indian and Two African Species of *Abrus* Adanson

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Abstract: Phylogenetic interrelationship between five species of *Abrus* Adanson was studied based on SDS-PAGE protein profile of their seeds. These included *Abrus precatorius* Linn. and *A. pulchellus* Thw, two circumtropical species from India and *A. canensis* Welw. ex Bak. and *A. fruticulosus* W. & A., two species from continental Africa. The fifth species was also *A. precatorius* but with white seeds. Pairing affinity values of this species was highest with *A. precatorius* having red black seeds but varied considerably in their protein profile. *A. precatorius* was also found to be more related to the other Indian species *A. pulchellus*. On the other hand *A. pulchellus* which has been placed as a separate species by Verdcourt but considered to be a subspecies of *A. fruticulosus* by Breteler, were found to be two separate species. *A. precatorius* and *A. fruticulosus* are the most distantly related species.

Key Words: Phylogenetic interrelationship, *Abrus*, SDS-PAGE, protein profile, seeds, Indian species and African species

Introduction

Abrus Adanson is a pantropical genus and is a member belonging to the tribe Vicieae in the sub-family Papilionaceae of the Leguminosae. As a genus it was first described by Adanson in 1763 who based it on *Glycine abrus* L. (Breteler, 1960). This genus is well distinguished by the presence of 9 connate stamens. It is often placed in its own tribe Abreae (Polhill, 1981). Morphologically, it has affinities with the Vicieae because of the presence of twining stems and paripinnate leaves ending in a bristle (Hutchinson, 1964). Based on the general appearance, it has affinities with Dalbergieae and Phaseoleae (Baillon, 1876) and also with the African genus *Millettia* because of its tendency to twine and its pseudoracemes (Pohill, 1981). Recent studies on chloroplast trm K/mat K sequences (Hu et al., 2000) and rbcL data (Doyle et al., 1997) which have been used successfully in phylogenetic studies at the generic level places *Abrus* as a sister group to the core Millettieae plus *Galactia.* According to Hu et al. (2000), *Abrus* shows affinity with core Millettieae members is having a pseudoraceme inflorescence, an absence of canavanine (except in the *Philonoptera* clade) and chromosome number of x=11.

There still prevails a controversy regarding the taxonomy of this genus as there are conflicting reports of the exact number of species in this genus. The first worldwide revision of this genus was done by Bretler (1960). He recorded four species in the genus viz. *Abrus precatorious* L., *A. canescens* Welw. ex Baker, *A. diversifoliolatus* Breteler nom. nov. and *A. fruticulosus* Wall. ex W. & A. *A. canescens* is confined to continental Africa, *A. diversifoliolatus* is found in Madagascar and the remaining two have a more or less circumtropical distribution and are found in Africa, America and Asia. It is quite amazing and unacceptable to see the number of variation which he classified under the name *A. fruticulosus* (Table 1). According to Breteler's revision later (Breteler, 1994), he felt that such variation is not well described and insufficiently illustrated which is a minus point to its acceptability. He felt that this group consisting of numerous species appears to be morphologically complex and it is debatable whether this confused areas of specimens needing taxonomic order, represents a pluriform species or

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a complex of perhaps closely allied species or just infraspecific taxa. Verdcourt (1970) however reported thirteen species (*A. canescens* Welv ex Baker, *A. diversifoliolatus* Breteler, *A. fruticulosus* Wall ex. W. & A., *A. grandiflorus* Vig., *A. parvifolius* (Vig) Verdc., *A. sambiranensis* Vig., *A. schimperi* Bak., *A. bottae* Defl., *A. mandagascariensis* Vig., *A. laevigatus* Mey., *A. pulchellus* Thw., *A. aureus* Vig. and *A. precatorius* L.). He further recorded three subspecies under *A. schimperi*, five under *A. pulchellus*, two subspecies under *A. aureus* and two subspecies under *A. precatorius* (Table 1). Unlike Breteler, Verdout described the variation in detail and hence proposed many subspecies to accommodate the specimens examined by him. However his description of morphological characters, often derived from the leaves, are sometimes not consistently, combined with geographical separation. Hutchinson and Dalziel (1958) reported three species of *Abrus*, *A. precatorious* L., *A. pulchellus* Wall. and *A. canescens* Welw. ex Baker in tropical West Africa. Labat (1991) reported a new species *A. longibracteatus* Labat from Laos and Vietnam, while Thulin (1994) published *A. baladensis* Thulin and *A. gawwenensis* from Somalia which was *species nova.* Thus a wide disagreement about the classification of a plant group can be very problematic for those working with this group of plants.

Table 1: Taxonomy of *Abrus* Adanson (Breteler, 1994)

Breteler (1960)		**Verdcourt (1970)**		
Species	**Distribution**	**Species**	**Subspecies**	**Distribution**
A. canescens Welw. ex. Bak.	Africa	*A. canescens*		Africa
A. diversifoliolatus Bret.	Africa (Madagascar)	*A. diversifoliolatus*		Africa
A. fruticulosus W. & A.	Africa, America, Asia	*A. fruticulosus* s.s		Asia (India)
		A. grandiflorus Vig.		Africa(Ma dagascar)
		A. parvifolius (Vig.) Verdc.		Africa (Madagascar)
		A. sambiranensis Vig.		Africa (Madagascar)
		A. schimperi Bak.		Africa
			ssp. *schimperi*	Africa (R.C.A., Sudan, Ethiopia, Uganda)
			ssp. *africanus* (Vatke) Verdc.	Africa (Kenya, Tanzania, Zambia, Zimbabwe)
			ssp. *oblongus* Verdc.	Africa (Malawi, Zimbabwe)
		A. bottae Defl.		Asia (Arabia)
		A. madagascariensis Vig.		Africa (Madagascar)
		A. laevigatus Mey.		Africa (S. Africa, Mozambique)
		A. pulchellus Thw.		Africa, America, Asia
			ssp. *pulchellus*	Asia (India, China, Malesia)
			ssp. *cantoniensis* (Hence) Verdc.	Asia (Thailand, China)
			ssp. *mollis* (Hance) Verdc.	Asia (Assam, Malay Peninsula, Java, Vietnam, Thailand, China, Philippines, Sarawak, Borneo, Papua)
			ssp. *suffruticosus* (Boutique) Verdc.	Africa (Sierra Leone, N. Nigeria, R.C.A. Zaire, Tanzania, Zambia, Angola)
			ssp. *tenuiflorus* (Benth.) Verdc.	America, Africa (from Senegal to Sudan, Angola and Mozambique)
A. precatorius Linm. s.l.	Circumtropical	*A. aureus* Vig.		Africa
			ssp. *aureus*	Africa (Madagascar)
			ssp. *littoralis* (Vig.) Verdc.	Africa (Madagascar)
		A. precatorius	ssp. *precatorius*	Asia
			ssp. *africanus* Verdc.	Africa (including Madagascar), Seychelles, Mauritius, Introduced in America & Australia

Seed protein studies have proved to be an excellent parameter to resolve the problems of identification of critical taxa and to understand their relationships and taxonomic status (Esen and Hilu, 1991; Khan, 1992; Sanchez-Yelano et. al., 1992). Seed protein patterns are highly stable and are unaffected by environmental conditions (Harborne and Turner, 1984). The present paper reports the study of phylogenetic interrelationship between five species of *Abrus* Adanson based on SDS-PAGE protein profile of their seeds. These included *Abrus precatorius* Linn. and *A. pulchellus* Thw, two circumtropical species from India and *A. canensis* Welw. ex Bak. and *A. fruticulosus* W. & A., two species from continental Africa. The fifth species was also *A. precatorius* but with white seeds.

Materials and Methods

Collection of seeds

Seeds of *Abrus precatorius* Linn. were collected from mature pods from plants growing in Santiniketan, West Bengal, India while the seeds of *A. pulchellus* Thw were collected from plants growing in Kanyakumari, Tamil Nadu, India. The seeds of *A. fruticulosus* W. & A. were collected from Usambara (North-East Tanzania), south-east Africa and Ivory Coast or Côte d'Ivoire (near Tiassate), west Africa while those of *A. canensis* Welw. ex Bak. was collected from Burundi, Bujumbura Province, south east Africa and Liberia, East of Ganta, road to Buchanan, west Africa. The seeds were sterilized in 10% (v/v) chlorox – 0.1% (v/v) Tween 20 for 5 min (Mondal et al., 1998). After rinsing in sterilized distilled water for 30 min, the seeds were immersed in sterilized distilled water overnight and used for protein extraction.

Protein extraction from seeds

For the extraction of protein from the seeds, a modification of Gifford's method (Gifford et al., 1982) was used. Protein was extracted from seed endosperm plus embryo in Tris-glycine buffer (0.01 M Tris; 0.08 M glycine), pH 8.2 [TGP buffer] containing 2% NaCl and clarified by centrifuging at 19,000 g for 20 min at 4°C. The supernatant containing the soluble proteins was pooled off while the pellet containing the insoluble storage proteins was suspended in the same TGP buffer and an equal volume of 62 mM Tris-HCl (pH 6.8) buffer with 3.05% (w/v) SDS and 10.7% (w/v) glycerol and boiled for 8 min (Jenson and Lixue, 1991). After centrifugation, the supernatant was used for SDS-PAGE.

Gel electrophoresis

Protein electrophoresis by SDS-PAGE was carried out according to the method of Laemmli (1970) with samples containing 80 µg of protein each. The samples were boiled for 3 min with equal amount of sample buffer (0.6 M Tris HCl (pH 6.8), 1% SDS, 10% sucrose, 0.5% mercaptoethanol, 0.01% bromophenol blue] at 100°C and applied to each well of a mini-gel (8X7 cm gel). The acrylamide concentration in the gel was 15% and electrophoresis was performed with electrode running buffer, pH 8.4 (0.05 M Tris, 0.192 M Glycine, 0.1% SDS). A constant current of 50 mA was supplied until bromophenol blue entered the separating gel and then the current was increased to 60 mA until the dye moved to the bottom of the gel. After electrophoresis the gel was stained with 0.1% Coomassie Brilliant Blue R250 and destained with methanol: acetic acid: water (4:1:5) mixture.

Numerical analysis

Pairing affinity or similarity index was calculated by the method described by Sokal and Sneath (1963) and Romero Lopes *et al.,* (1979). Based on the results of electrophoretic analysis, the degree of pairing affinity (PA) was calculated by the following formula:

$$P.A. = \frac{\text{Bands common to species A and B}}{\text{Total bands in A and B}} \times 100$$

Dendogram expressing the average linkage was computed using the cluster method of Unweighted Pair Group Method with averages (UPGMA relationship) [Sneath and Sokal, 1974]. NTSYSpc.2.2 software was used to compute the dendogram.

Results and Discussion

Abrus is a genus where the characters of the inflorescence and pod proved to be more useful in distinguishing between species as flowers of the species do not show any character leading to specific segregation. The morphological appearances of the four species are presented in Table 2. The results showed that they are four distinct species of *Abrus.* However the fifth species (*A. precatorius* but with white seeds) did not show any morphological variation from *A. precatorius* with scarlet seeds having a black spot around the hilum.

Table 2: Comparative account of the 4 species of *Abrus*

	***A. precatorius* Linn.**	***A. pulchellus* Thw.**	***A. canensis* Welw. ex Bak.**	***A. fruticulosus* W. & A.**
Plant	Winding or trailing woody vine or climber, stem slender, with slender herbaceous branches, much branched with glabrescent mostly greenish yellow young branches, sparsely white strigose	Lianas, climbing, stem slender, sparsely yellow strigose or villous	Winding climber with woody pubescent branches, finally glabrescent	A climber or a diffuse creeping fastigiated or straggling shrub or undershrub, generally 3-5 m in length, young branches pubescent, finally glabrescent
Leaves	Leaves 8-16 jugate, petiole 5-15mm long	Leaves paripinnate, alternate, petiole short,	Leaves 10-14 jugate, petiole short	Leaves 5-20 jugate
Leaflets	Leaflets 6-25mm long, 3-10mm wide, ovate, obovate or oblong, top obtuse or acuminate, base rounded or subcordate, upper surface glabrous or pubescent, lower surface densely or sparsely appressed-pubescent	Leaflets 6-10-paired, opposite, 6-20mm long, 3-12mm wide, suboblong, oblong, or obovate-oblong, , top truncate, base rounded or subcordate, membranous, upper surface sparsely white strigose or densely white villous, lower surface glabrous or pilose	Leaflets 6-20mm long, 2-7mm wide, rectangular, white or grey pubescent on both sides	Leaflets 3-46mm long, 2-15mm wide, varying in shape and pubescent, ovate, obovate or oblong, top acute, rounded, obtuse or truncate-emarginate, base cordate, rounded, or cuneate, often unequal-sided, upper surface pubescent, glabrescent, lower surface densely or sparsely appressed-pubescent
Inflorescence	Inflorescence rigid, thick, strongly falcate, bracts and bracteoles 0.5-1mm long, flowers crowded, subsessile, calyx 3-4mm long, pubescent, corolla 3-4 times as long as the calyx, pale purple to yellowish	Inflorescence racemes axillary, flowers dense; calyx campanulate, 4-toothed, white strigose or densely gray villous, 3-5mm long, corolla 4-9 mm long pink, purple, or purple-red	Inflorescence terminal; flowers subsessile, usually in separated fascicles, bracts and bracteoles as long as calyx,3-6mm long, corolla 10-13mm long, purple	Inflorescence terminal, lateral or axillary; flowers in groups, bracts and bracteoles half as long as calyx, corolla 3-6 times as long as calyx, pale purple to yellowish
Pods	Pods roughly rectangular, bulgy, 2-4 cm long, 1-1.5 cm wide, densely warty, tomentose, beak hook shaped, reflexed, 3-7 seeded	Pods oblong, 3-6.5 cm long, 0.8-1.5 cm wide, densely white hairy, dehiscent, 4-12 seeded	Pods broad, linear, nearly straight, 3-6cm long, 8-12mm wide, beak hook shaped, recurved, pubescent, 6-9 seeded	Pods oblong, 2-10 cm long, 0.5-1.5cm wide, flattened, broadly rounded or cuneate at base, rounded at apex, pubescent or glabrescent, 4-12 seeded
Seeds	Seeds ovoid, 5-7mm long, 4-5mm broad, scarlet, with a black spot around the hilum, glossy	Seeds elliptic or ovoid, compressed, 3-6mm long, 2-5 mm broad, black-brown or black, glossy to slightly glossy	Seeds ovate, 4-6mm long, 3-4mm broad, light brown black, glossy	Seeds oval, laterally compressed, 3-6mm long, 2-5mm broad, brown black, mostly glossy

Table 3: Pairing affinity values (%) of the seed protein of *Abrus* sp. based on electrophoretic patterns

	A. canensis	***A.pulchellus***	***A. fruticulosus***	***A. precatorius***	***A. precatorius* (white seeds)**
A. canensis	100				
A. pulchellus	52.9	100			
A. fruticulosus	53.3	58.8	100		
A. precatorius	43.8	42.1	38.9	100	
A. precatorius (white seeds)	60.0	68.8	47.1	78.6	100

Study of the phylogenetic interrelationship between the five species of *Abrus* based on SDS-PAGE protein profile of their seeds and numerical analysis of the data obtained (pairing affinity values) shows that the two Indian species *A. precatorius* and *A. pulchellus* are more related (Table 3) having the affinity of 68.8%. However the two species of *A. precatorius* which did not differ morphologically however showed a variation in the protein profile and instead of showing 100% affinity showed a pairing affinity of 78.6%. According to Breteler (1960), the seeds of *A. precatorius* sometimes appear to be entirely black or white. He explained that this is accidental and is not correlated with any morphological character. This seems to be contradictory to the results of the present study. On the other hand *A. pulchellus* which has been placed as a separate species by

Verdcourt but considered to be a subspecies of *A. fruticulosus* by Breteler, were found to be two separate species showing 58.8% affinity. Thus the taxonomy of *A. fruticulosus* which has long remained debated and has been reduced as a synonym of *A. pulchellus* and *A. mollis* and others by Breteler (1960) needs to be treated separately now as proposed by Verdcourt (1970). *A. fruticulosus* has also been reported from India but as sparse in some parts of its range in India particularly coastal forest in Tamil Nadu. It is believed to have suffered a population decline over the last 10 years here, however, this is not thought to be greater than 30%, and there are possibly more than 10,000 mature individuals (Sanjappa pers comm., 2011). The other species *A. canensis* was more related to *A. fruticulosus* (53.3%) followed by *A. pulchellus* (52.9%) and *A. precatorius* (43.8%). *A. precatorius* and *A. fruticulosus* are the most distantly related species with only 38.9% affinity.

Conclusion

The results of the present investigation highlights the usefulness of seed proteins as an excellent parameter to resolve the problems of identification of critical taxa and shows the demerit of over dependence on morphological and especially morphometric studies for taxonomic elucidation of species. *A. precatorius* with white seeds and *A. precatorius* with scarlet seeds having a black spot around the hilum, which did not show any morphological variation proved to be chemotaxonomically different and needs to be treated separately. *A. precatorius* with white seeds may be placed as a subspecies under *A. precatorius.* This however needs further investigations and may be proved through molecular studies particularly DNA. *A. precatorius* and *A. pulchellus* are more related while *A. precatorius* and *A. fruticulosus* are the most distantly related species. *A. pulchellus* which has been placed as a subspecies of *A. fruticulosus* by Breteler needs to be treated as a separate species and this is in confirmation with the placement of the species by Verdcourt. *A. canensis* is more related to *A. fruticulosus* followed by *A. pulchellus* and *A. precatorius.*

a)

b)

Fig. 1: a) A twig of *Abrus precatorius* with pods b) The seeds of *A. precatorius.*

a)

b)

Fig. 2: a) Pod of *Abrus precatorius* with white seeds b) The seeds of *A. precatorius* (white seeds).

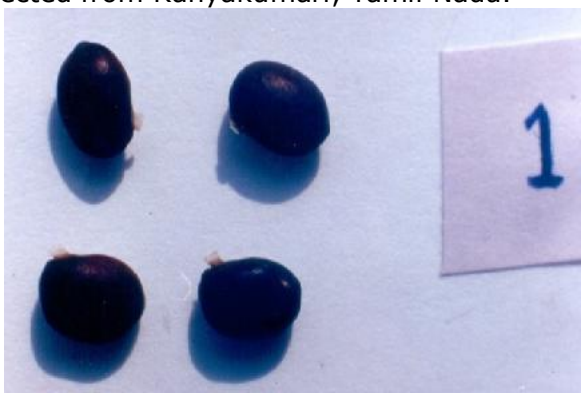
a)

b)

Fig. 3: a) A flowering twig of *Abrus pulchellus* b) The seeds of *A. pulchellus.*

Fig. 4: A flowering twig of *Abrus fruticulosus* collected from Kanyakumari, Tamil Nadu.

a)



b)

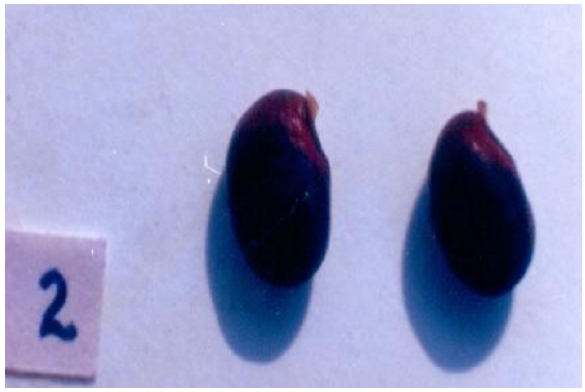
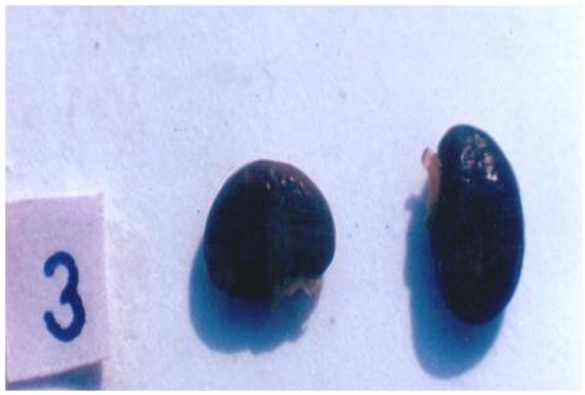


Fig. 5: The seeds of *A. fruticulosus* collected from a) Usambara (North-East Tanzania), south-east Africa and b) Ivory Coast or Côte d'Ivoire (near Tiassate), West Africa.

a)



b)

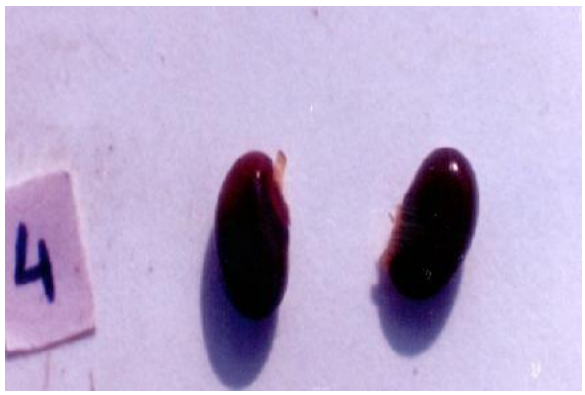


Fig. 6: The seeds of *A. canensis* collected from a) Burundi, Bujumbura Province, south east Africa and b) Liberia, East of Ganta, road to Buchanan, West Africa.

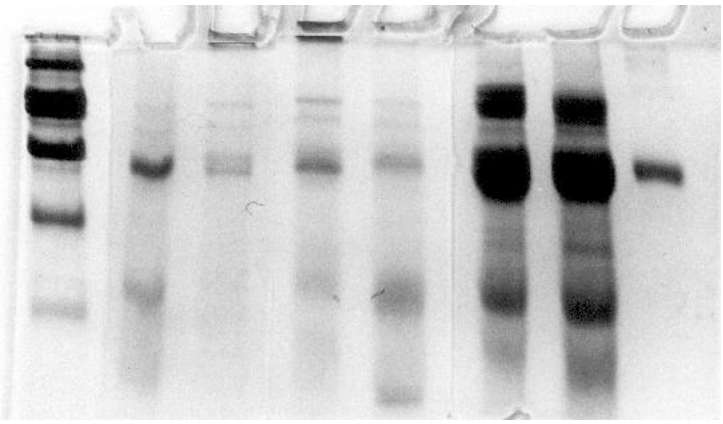


Fig. 7: SDS-PAGE protein profile of the seeds of *Abrus* a) Marker proteins, b) *A. canensis,* c&d) *A. pulchellus,* e) *A. fruticulosus,* f) *A. precatorius,* g) *A. precatorius* (white seeds), h) 66kDa protein (BSA).

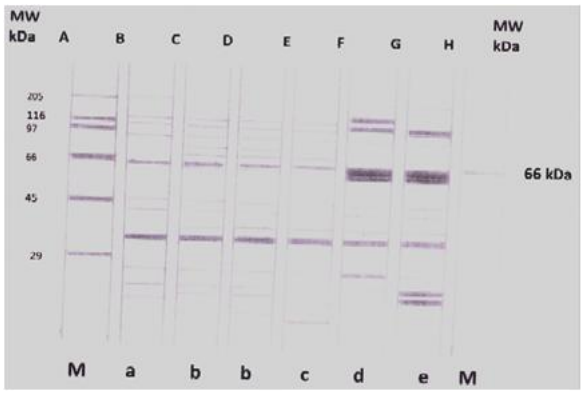


Fig. 8: Diagramatic representation of the SDS-PAGE protein profile of the seeds of *Abrus.*

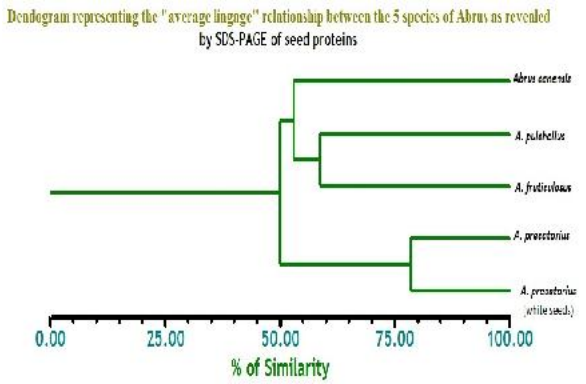


Fig. 9: Dendogram representing the "average linkage" relationship between the 5 species of *Abrus* as revealed by SDS-PAGE of seed protein.

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