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SEM STUDIES OF SEED COAT IN SOME ASIATIC *VIGNA* SPECIES AND THEIR TAXONOMIC AND PHYLOGENETIC SIGNIFICANCE

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Seed morphology, hilum structure and cellular structure of seed coat of predominently cultivated and wild species of the genus Vigna *in India were studied using scanning electron microscopy (SEM). The important species include mung bean* (Vigna radiata *(L.) Wilczek), urid bean* (V. mungo *(L.) Hepper), rice bean* (V. umbellata *(Thunb.) Ohwi and Ohashi), moth bean* (V. aconitifolia *(Jacq.) Marechal) and Pillipasera* (V. trilobata *Ait.) and their wild occurring closely related putative progenator forms particularly* V. radiata *(L.) var. sublobata Verdc.,* V. mungo *(L.) var. silvestris Lukoki, Marechal and Otoul, wild forms of* V. umbellata *and* V. aconitifolia. *Seed coat cellular structure and hilium were characteristic to each species and appeared to be species-specific. Considerable morphological similarties were observed between cultivated plants of* V. radiata *with its related wild species* V. radiata *var. sublobata,* V. mungo *with that of* V. mungo *var. silvestris and* V. umbellata *with its wild form. In* V. aconitifolia, *cultivated and wild types differed drastically in their seed coat surface pattern. SEM seed coat structure was found to be useful in establishing taxonomic and phylogenetic relationship in these Indian species of* Vigna.

Seed coat morphology using scanning electron microscopy (SEM) have been used as taxonomic characters which may provide very useful and reliable evidence on phylogeny (Heywood, 1971; Brisson and Peterson 1976; Barthlott, 1981). The above investigators amply justified the utility of seed morphology and SEM seed coat surface structure in solving a variety of problems concerning systematics and evolution. Recent findings on scanning electron microscopy of seed coat surfaces showed this to be a consistant and important source of character states at the species level (Canne, 1980; Wofford, 1981). SEM studies of seed coat cellular morphology were used in establishing species relationship among *Vigna-mungo-radiata-sublobata* and *silvesteris* complex to provide evidences about the possible ancestors of the Indian domesticated species of *Vigna* (Chandel, 1980; Chandel *et al.*, 1984; Trivedi and Gupta, 1985, 1986). SEM seed coat morphology of *V. mungo, V. radiata* and their wild relatives were studied earlier as well (Sharma *et al.*, 1977, 1983). The wrong identification of *Vigna* species collected from Poona-Khandala and Mahablashwar regions of Western Ghats by the latter authors led to misinterpretation of SEM studies of seed coat patterns and further complicated the taxonomic and phylogenetic relationship in this complex. Trivedi and Gupta (1985, 1986) concluded that *V. mungo, V. sublobata* and *V. radiata* resemble each other in generally having reticulate seed coat pattern and it is therefore, difficult to separate them distinctly on this basis. Seed coat surface structure in *Vigna* possesses a strong heritable base (Rajendra *et al.*, 1979) in a cross combination involving *V. sinensis* with *V. sesquipedalis* where the single gene control for two types of macrosclereid traits was observed. The above findings support the genetic control of seed coat structure and thus this structure should be considered as a valuable heritable taxonomic character in the study of systematics and phylogenetic relationship. The present studies have been carried out to investigate the detailed SEM seed coat surface patterns of various cultivated species of *Vigna* and wild occurring related taxa collected from diverse natural habitats in the Indian Gene Centre. Taxonomic implications and relevance of SEM seed coat surface morphology in establishing phylogenetic relationship between cultivated and wild *Vigna* species have been elucidated.

MATERIALS AND METHODS

Specimens of several wild species of *Vigna* occurring in natural and disturbed habitats in India were collected from different ecological regions/microniches (Arora *et al.*, 1973; Arora, 1986; Chandel, 1984). Scanning electron microscopy of seed coat and hilum structure was undertaken involving different cultivated species and their close wild relatives (Table 1). The present investigations included cultivated species namely mung bean (*V. radiata* syn. *Phaseolus radiatus* (L.) Roxb.), urid bean (*V. mungo* syn. *Phaseolus mungo* L.), rice bean (*V. umbellata* syn. *Phaseolus calcaratus* Thunb.) and moth bean (*V. aconitifolia* syn. *P. aconitifolius* Jacq.), *V. trilobata* syn. *P. trilobus* Ait. The wild related taxa included in the present study are *V. radiata* var. sublobata, *V. mungo* var. silvestris, wild putative progenitor forms of rice bean (*V. umbellata*), and moth bean (*V. aconitifolia*).

Table 1. List of Vigna species/forms, their accession numbers and sources of collection.

S. No.	Name (Species)	Common name	Accession No.	Source
1.	*Vigna radiata* (L.) Wilczek	Mung bean (Cultivated)	Pusa Baisakhi	IARI, New Delhi.
2.	*V. radiata* (L.) var. sublobata (Roxb.) Verdc.	Wild progenator	PLX 274	Rishikesh, Utter Pradesh
3.	*V. mungo* (L.) Hepper	Urad bean (Cultivated)	NC55947	NBPGR, New Delhi.
4.	*V. mungo* (L.) var. silvestris Lukoki, Marechal and Otoul	Wild progenator	PLX 409	Khopli, Maharashtra.
5.	*V. mungo* (L.) var. silvestris Lukoki, Marechal and Otoul	Wild progenator	PLX 410	Khopli, Maharashtra.
6.	*V. umbellata* (Thunb.) Ohwi and Ohashi	Rice bean (Cultivated)	IC 18136	NBPGR, New Delhi.
7.	*V. umbellata* (Thunb.) Ohwi and Ohashi	Rice bean (Wild form)	IW 3176	Shimla Hills, Himachal Pradesh
8.	*V. aconitifolia* (Jacq.) Marechal	Moth bean (Cultivated)	PLMO 211	NBPGR, New Delhi
9.	*V. aconitifolia* (Jacq.) Marechal	Moth bean (Wild form)	C-2515	Rajendranagar, Andhra Pradesh
10.	*V. trilobata* Ait.	Pillipasera	IC 24830	NBPGR, New Delhi.

Seeds of these species were thoroughly cleaned and were dried at room temperature. Two or three well developed seeds of each species were mounted on brass stubs using silver paste with adhesive and were uniformly coated with a thin film of gold using ion sputter JEOL JFC-1100. The coated samples were examined at uniform tilt angle (45°) in scanning electron microscope model Jeol JSM-840A at an accelerating voltage of 20 kv and photographed. Surface structure of seed coat pattern was scanned uniformly around the hilum. The voucher specimens were deposited in the National Herbarium at NBPGR, New Delhi.

RESULTS AND DISCUSSION

Vigna radiata and *V. radiata* var. sublobata

The seed shapes, hilum structures and seed coat cell patterns of *V. radiata* and its putative progenitor *V. radiata* var. sublobata exhibited considerable resemblance (Fig. 1-4). *V. radiata* possessed oblong, elongated, rectangular or wedge shaped seeds while in *V. radiata* var. sublobata was flattish, linear in shape and not raised. Surface structure in mung bean showed very elongated cells (5 to 6 times more in length than width), mostly rectangular or triangular in shape, arranged in parallel rows (Fig. 2). Wax deposition on the seed coat was observed to be very thick. External surfaces of waxy mounds on cell boundaries were quite irregular. Cell contours were distinct with varying thickness. Wax deposition in cell sutures was in the form of linear rows of globules. Due to distinct cell boundaries, uniform reticulum was formed on seed surface.

Seed coat surface structure and cellular details of *V. radiata* var. sublobata (PLX 274) showed significant variations in cell size (Fig. 4). In general, the seed surface was covered with a reticulum formed by cells varying from rectangular to ovate in shape. Cells were found to be elongated and were arranged side by side in wavy rows. Cell sutures were impregnated with wax forming mounds which were regular in outlines.

Vigna mungo and *V. mungo* var. silvestris

In *V. mungo*, seed shape was globular with roundish ends (Fig. 5), while in various wild accessions of *V. mungo* var. silvestris, shape of the seed varied from rectangular (Fig. 7) to oblong (Fig. 9). Structure of hilum was very distinct in urid bean and morphologically resembled the hilum in wild forms of *V. mungo* var. silvestris, being concave and deeply furrowed (Fig. 5, 7, 9). The hilum rim was very much raised and arilate and the suture was clearly marked while in PLX 409 where the hilum was not much raised and the suture was somewhat elongated rather than ovate. The seed coat in *V. mungo* was composed of hexagonal cells arranged in irregular rows forming a network on the seed

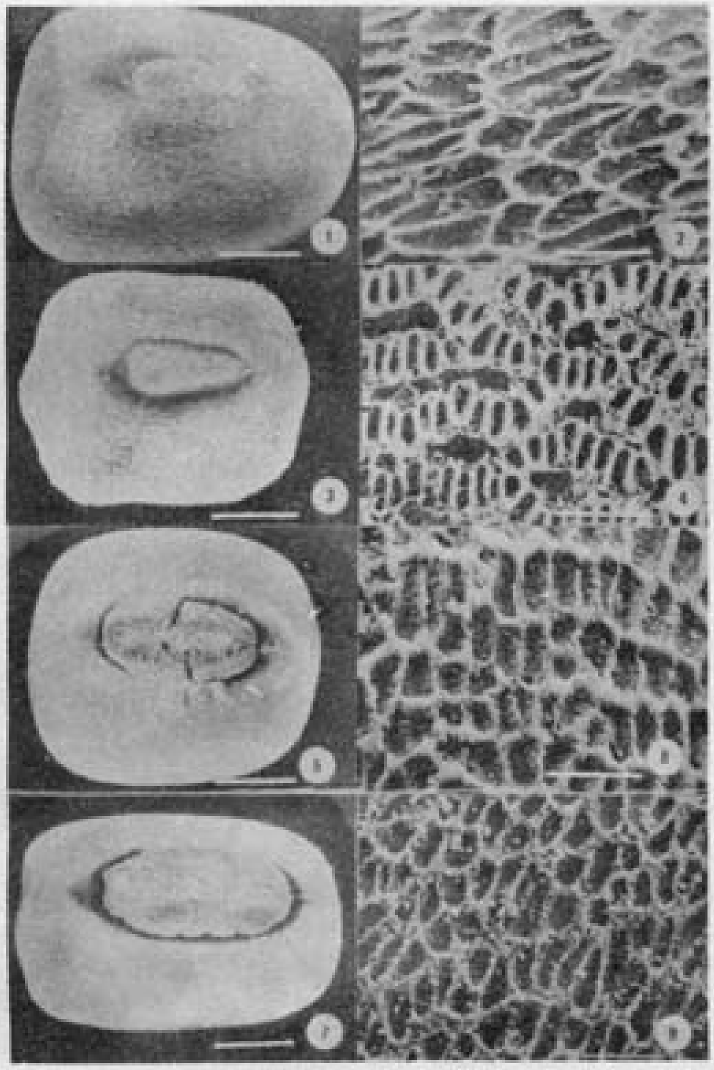

Fig. 1-8. SEM micrographs of seeds of *V. radiata*, *V. radiata* var. sublobata, *V. mungo* and *V. mungo* var. silvestris. Fig. 1. *V. radiata* wedge shape seed with linear hilum. Fig. 2. Seed coat pattern of *V. radiata*, rows of rectangular cells with wax deposition. Fig. 3. *V. radiata* var. sublobata (PLX 274) oblong seed with linear hilum. Fig. 4. Seed coat of PLX 274, showing rectangular cells arranged in wavy rows. Fig. 5. *V. mungo* globular seed with raised hilum. Fig. 6. Seed coat of *V. mungo* showing hexagonal cells arranged in network. Fig. 7. *V. mungo* var. silvestris (PLX 410) rectangular seed with hilum similar to *V. mungo*. Fig. 8. Seed coat pattern of PLX 410 showing almost hexagonal cells. (Fig. 1, 3, 5, 7-Scale Bar = 1 mm. Fig. 2, 4, 6, 8-Scale Bar = 100 μm).

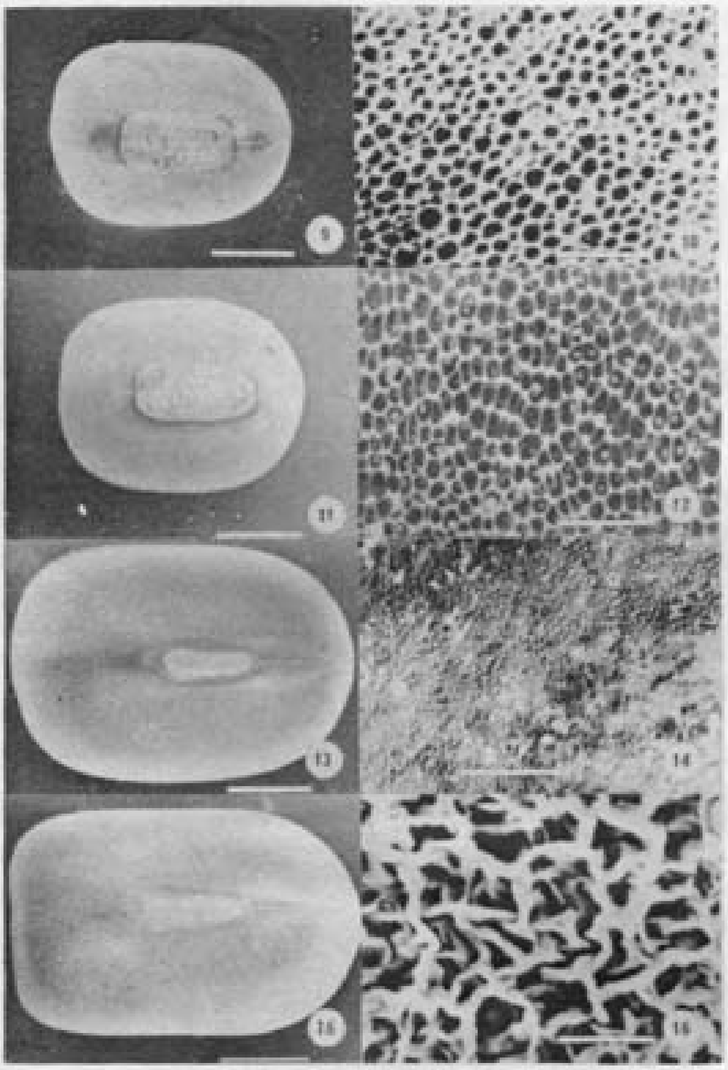

Fig. 9-16. SEM micrographs of seeds of *V. mungo* var. silvestris, *V. trilobata* and *V. aconitifolia*. Fig. 9. *V. mungo* var. silvestris (PLX 409), globular seed with semi-raised hilum. Fig. 10. Seed coat of PLX 409 with small hexagonal cells arranged in network. Fig. 11. *V. trilobata* globular seed with semi-raised hilum. Fig. 12. Seed coat of *V. trilobata* showing rectangular to hexagonal cells arranged in network. Fig. 13. *V. aconitifolia* cultivated, globular seed with lanceolate hilum. Fig. 14. Smooth seed coat of *V. aconitifolia* cultivated. Fig. 15. Wedge shaped seed of *V. aconitifolia* wild with hilum similar to cultivated. Fig. 16. Seed coat of *V. aconitifolia* wild showing heavy wax deposition. (Fig. 9, 11, 13, 15 -Scale Bar = 1 mm, Fig 10, 12, 14, 16-Scale Bar = 100 mm).

surface (Fig. 6). Heavy wax deposition on cell walls with unequal quantity was observed. Waxy mounds on cell boundaries exhibited irregular external surfaces. Wax deposition on cell surfaces and sutures was in the form of granules giving papillate appearance to the seed surface. Contours of epidermal cells were quite distinct with irregular thickness.

In the present study, two accessions (PLX 409 and PLX 410) of the wild putative progenitor *V. mungo* var. silvestris were studied for their seed coat pattern. The seed coat pattern of wild ancestral form *V. mungo* var. silvestris exhibited remarkably similar cell shape, size and configuration with *V. mungo*, but the degree of cell wall distinctness and wax deposition was variable (Fig 8, 10). Interestingly PLX 409 showed remarkably smaller (4-5 times) hexagonal cells typical of *V. mungo-silvestris* complex as compared to the other three wild types.

Vigna trilobata

In this semi-domesticated species, one accession (IC 24830) was studied, seeds were oblong to globular in shape with roundish ends. (Fig. 11). The hilum in this species was lanceolate in shape and not much raised. Seed coat surface structure showed the presence of hexagonal cells arranged in wavy rows (Fig 12). Deposition of wax was rather uniform and thin giving a smooth appearance to the surface. Sometimes unequal wax deposition was found along the cell sutures. Lateral cell walls possessed more wax deposition in comparison to transverse walls. Small papillae were observed on cell surface occasionally. Cell boundaries were distinct, rarely indistinct, forming a regular network of cells on seed surface (Fig. 12).

Vigna aconitifolia

One cultivated (PLMO 211) and one wild type (C2515) accession of *V. aconitifolia* were studied. Seed shape in cultivated forms was globular to elongated with roundish ends (Fig. 13) while in wild forms, it was rectangular to wedge shaped (Fig. 15). The hilum was found to be flattish, elongated, lanceolate and arilate in both the forms (Fig. 13, 15). Cultivated type (PLMO 211) exhibited very smooth, thin and uniform wax deposition on seed surface. Low magnification (250x) revealed the presence of very small, elongated and hexagonal epidermal cells arranged in a reticulate pattern (Fig. 14). In contrast to the cultivated strain, wild type showed thick and heavy deposition of wax on surface forming irregular reticulations (Fig. 16). These reticulations formed by the interweaving of finger like waxy tubercles of varied thickness. Due to such irregular wax deposition, cell boundaries were found to be indistinct with no clear cut suture and cell walls (Fig. 16).

Vigna umbellata

Wild form of *V. umbellata* (IW 3178) was studied in comparison to its cultivar (IC 18136). Seeds in both the forms were globular and elongated with roundish ends (Fig. 17, 19). The hilum in the cultivar was prominent, raised, rectangular in shape and set towards the lower end of the seed (Fig. 17). In the wild accession, the hilum was linear, not much raised and rectangular in shape (Fig. 19). The seed coat in both cultivated and wild forms, was very smooth due to uniform and thin wax deposition (Fig. 18, 20). Waxy pattern on seed coat was not observed in both the forms however, at higher magnification, densely packed epidermal cells with indistinct cell walls were found (Fig. 18, 20). Seed coat surface of wild type showed small irregular patches of wax at some sites.

The taxonomical nomenclature, origin and evolutionary relationships of the cultivated taxa with that of wild-occurring closely related species of the genus *Vigna* have remained complex and blurred.

SEM studies of the seed coat showed that in *V. radiata*, the seed coat possesses elongated cells, mostly rectangular or wedge shaped, arranged in parallel rows with their wall and forming reticulum superimposed with heavy wax deposition on cell boundaries. The wild form *V. radiata* var. sublobata possessed similar structure and configuration of cells and remarkable morphological similarities exist with *V. radiata*. *V. mungo* — another important cultivated species and its well authenticated ancestral form *V. mungo* var. silvestris exhibited similar seed coat cellular morphology and hilum structure. The two wild forms of *V. mungo* var. silvestris (PLX 410 and PLX 409) possessed hexagonal epidermal cells with heavy wax deposition resulting in varying degree of cell wall distinctness. These forms of *V. mungo* var. silvestris resembled its cultigen species *V. mungo* with exception of PLX 409 which possessed a hilum like *V. radiata* var. sublobata and seed coat structure like *V. mungo* var. silvestris.

Sharma *et al.* (1977, 1983) reported the presence of elongated hexagonal cells forming reticulum with varying degree of wax deposition in *V. sublobatus* (*V. radiata* var. sublobata). While the present and earlier studies on this complex showed such pattern in *V. mungo* and in its wild form *V. mungo* var. silvestris (Chandel *et al.*, 1984; Trivedi and Gupta, 1986). Further Sharma *et al.*, (1977, 1983) wrongly designated wild *Vigna* species collected from Pune, Khandala and Mahabaleshwar regions of Western Ghats as *V. sublobatus* (*V. radiata* var. sublobata). This is also contrary to other published reports (Arora *et al.*, 1973; Chandel, 1984; Chandel *et al.*, 1984 and Arora, 1986) that this region has predominent natural distribution of *V. mungo* var. silvestris but not of *V. radiata* var.

sublobata. It is relevent to mention here that wild form accession PLX 416, collected from Khandala region of Western Ghats by NBPGR (Arora *et al.*, 1973) was designated as holotype for *V. mungo* var. silvestris (Lukoki *et al.*, 1980). The present findings strongly support the view that *V. radiata* and *V. mungo* are two separate species and their wild ancestral forms are *V. radiata* var. sublobata and *V. mungo* var. silvestris, respectively (Chandel, 1984; Chandel *et al.*, 1984). Conclusions drawn by Jain and Babu (1982) that *V. calcarata* is the putative progenitor of *V. radiata* and *V. sublobata*, *V. mungo*, *V. calcarata* and *V. radiata* are closely related to one another, do not conform with the present studies.

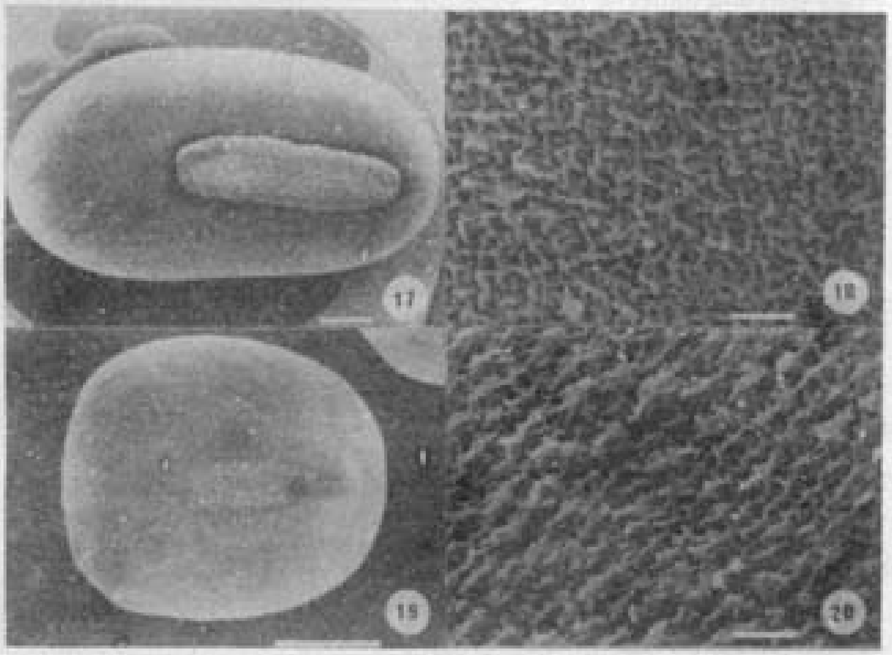

Fig. 17-20. SEM micrographs of seeds of *V. umbellata*. Fig. 17. *V. umbellata* cultivated, globular seed with raised hilum. Fig. 18. Smooth seed coat of *V. umbellata* cultivated, showing epidermal cells. Fig. 19. Globular seed of *V. umbellata* with hilum not much raised. Fig. 20. *V. umbellata* wild, smooth seed coat, epidermal cells with wax deposition at some sites. (Fig. 17, 19-Scale Bar = 1 mm, Fig. 18, 20-Scale Bar = 10 mm).

Intraspecific variations and overlapping of some seed coat surface characters recorded among the various forms of *V. radiata* var. sublobata and *V. mungo* var. silvestris may be influenced by interactions between genetic and environmental factors due to their occurrence in different ecological habitats and micronitches (Ojomo, 1972; Wyatt, 1984; Matthews and Levins, 1986) or sometimes due to their sympatric distribution (Chandel, 1981, 1984).

SEM seed coat surface structure and hilum morphology of *V. trilobata, V. aconitifolia* and *V. umbellata* suggested the distinctness of these species from each other and from *V. mungo* and *V. radiata*. It is important to mention here that seed coat surfce pattern of cultivated forms of *V. aconitifolia* drastically differed from its wild type. Thick waxy deposition present in wild form in this species might have become reduced in the course of evolution towards cultivated type where it is very thin and uniform. In *V. umbellata,* definite resemblance in SEM seed coat structure was observed in cultivated and wild types. Thus the present studies clearly indicate species-specific nature of SEM seed coat structure and hilum morphology in these five Asiatic *Vigna* species. SEM seed coat morphology also proved to be useful in establishing phylogenetic relationship between wild and cultivated types in *V. mungo, V. radiata* and *V. umbellata*.

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