Taxonomical Studies of Some Acacia Seeds Growing in Saudi Arabia

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Abstract: The aim of this work is to study the morphological and anatomical characters of seeds of eleven species and subspecies of genus Acacia which were collected from the western and southwestern region in Saudi Arabia. Morphological studies of seeds show many different characters related to the seed shape and size, central aerole, where size difference between small seeds in *A. raddiana*, *A. tortilis* and large seeds in *A. gerrardii*, *A. ehrenbergiana.* Studying the characters of the central aerole shows that they are undistinguished in species *A. ehrenbergiana* and distinguished in all other investigated species. Also open or closed central aerole with divergent straight equal or unequal arms, and area inside the aerole and the ratio between the central aerole area and seed surface area, where consider as very diagnostic characters for seed morphology. The character related to the level of the central aerole was used as specified characters Scanning electron microscope of seeds surface show many different ornamentation such as, regulous: reticulate tuberculate, reticulate rugose, Jagged with grooves, granulate colliculate, rough, crimpled foveolate, granulose, striated papillosed, micro granulate, reticulate foveolate. Anatomical studies of seed coat, of the eleven investigated species indicate the presence of different characters such as different lengths of Malpighian cells and structure of the light line. Two keys conclude the results, one is based on the morphological characters of seed, and the second is based on the anatomical characters of the seed coat**.**
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Key words: Acacia seeds, central aerole, ornamentation seed surface, Malpighian cell

1- Introduction

Acacia is a genus of shrubs and trees belonging to the subfamily Mimosoideae of the family Fabaceae, first described in Africa by the Swedish botanist Carl Linnaeus in 1773. (Reference 2008).

Many non-Australian species tend to be thorny, whereas the majority of Australian acacias are not. They are pod-bearing, with sap and leave typically bearing large amounts of tannins and condensed tannins that historically in many species found use as pharmaceuticals and preservatives(Simmons, 1987) . The genus Acacia previously contained roughly 1300 species, about 960 of them native to Australia, with the remainder spread around the tropical to warm-temperate regions of both hemispheres, including Europe, Africa, southern Asia, and the Americas (Orchard and Maslin 2003). However, in 2005 the genus was divided into five separate genera , *Acacia*, *Vachellia*, *Senegalia*, *Acaciella* and *Mariosousa* (Kodela and Wilson 2006), most of the traditional acacias of Africa are now treated in the genera *Vachellia* and *Senegalia* while some of the American species are placed in *Acaciella* and *Mariosousa*. The majority of species still treated in the genus *Acacia* are confined to Australia. (Seigler and Ebinger 2005).

Trees and shrubs are important in preserving the ecosystem of any region, particularly in areas where the vegetation is rich (Wasson 2003). The number of tree species in Saudi Arabia is only 97, which is about 4% of the total floristic elements. Out of these, more than 80% are present in the southwestern and western regions, including Taif region. Among the tree genera, *Acacia* contains the highest number of species (16 species) Acacias, in general, are the most dominant tree species in Saudi Arabia and elsewhere in the Arabian Peninsula.

Acacia seeds have been morphologically studied by a few authors (Brenan 1970), (Vassal 1972), (Al-Kinany 1981), (Mahmoud *etal.* 1981), (Behawi and Mohamed 1982) and (Singh 1982) .Number per pods and arrangement on the placentaion were discussed by (Andrews 1956) , (White 1962), Tackholm (1974) and (Chaudhary 1983). (Gunn1984) and (Martin and Williams 1973) describe the shape and color of the seeds rather oval to elliptic, black to greenish. AL-Gohary (2007) study surface sculpture of 11Egyptian Acacia species and construct an indented key. Paula Venier (2012) studied the seed coat structure through histochemical analysis in five Neotropical Acacia species from xerophytic forests of central Argentina. The present study on Acacia seeds in Saudi Arabia show at least 10 morphological characters and 5 anatomical characters in the 11 Acacia species under investigation

2- Material and Methods:

2-1- Sample collection:

Fresh materials of 10 Acacia species growing

wild in western region and west south region were collected during different seasons 2004-2005. Attention was paid for studying specimens who were collected from localities representing the geographical range of each species (Table 1). One herbarium specimens were studied where the fresh materials was not available during this seasons. The collected materials were identified according to Migahid (1978) Batanouny (1981), Collenette (1985), Boulos (2000) and Chaudhary (2001). Samples of the identified materials were kept at Botany Department Faculty of Science King Abdul-Aziz University (Girls section).

Table (1) Collected specimens of Acacia species in Saudi Arabia

Species	*Locality*
Acacia abyssinica	El Hadda road
A. ehrenbergiana	Old Jeddah – makkah road
A. etbaica	Makkah road –wadi elsal
A. etbaica ssp.uncinata	El Hadda road
A. gerrardii	El Baha – Belgrashi road
A. gerrardii var. najdensis	El Madina Road (Gebel elfaqra)
A. hamulosa	Herbarium sheet
A. mellifera	Jeddah – El Madina road
A. nubica	Hadda el sham region
A. raddiana	Assfane road
A. tortilis	Assfane road

2-2- Morphological studies

Acacia seeds were collected from mature pods, seeds were studied from both surface noted shape, size, central aerole and the position of funicle ,length of arms, area inside the aerole and the ratio between the central aerole area and seed surface area.

Examination takes place by Nikon light microscope and photographed by Nikon camera 4500. Measurements given are the mean of 10 seeds.

2-3- Anatomical studies

Anatomical sections were made from seeds soaked in a mixture of equal parts water, Glycerol, and ethyl alcohol for 30 hours, then sectioned with a razor blade. Sections were stained in saffranin and light green dehydrated in alcohol-xylol series, cleared in clove oil and mounted in Canada balsam, photographed by Nikon Microscope. Measurements given are the mean of 10 seeds.

2-4- Scanning electron microscope

For scanning electron microscopic, seeds were dehydrated in an acetone series, critical point dried using carbon dioxide and, together with dry seeds, were mounted directly on stubs using double-side adhesive tape, and sputter-coated with gold. Observations were made in a Philips LX-20 Auto scan SEM. Terminology of seed-coat surface sculpturing basically follows Stearn (1992) and Font Quer (1993).

3- Results

In table (2&3) the 11 investigated *Acacia* species were arranged according to systematic treatment Chaudhary (2001) vertically while the morphological and anatomical characters of seeds were arranged horizontal.

Table (2): Seed morphological characters of the 11 Acacia species growing in Saudi Arabia

Species	**Size L x W/ mm**	**Shape**	**Color**	**Texture**	**Funicle**	**Central aerole**			
						Shape	Length of arms	Size	Color
A. abyssinica (Fig.1 A&B)	4x6	Elliptic compressed with pointed apex	Reddish brown	Reticulate tuberculate	subterminal twisted long thick	closed equidistance concave	-	1/3 of seed area	Dark
A.ehrebergiana (Fig.2 A&B)	4x6	Elliptic compressed with pointed apex	Dark brown	Reticulate rugose	subterminal twisted long thick	not distinguish	-	-	-
A. etabica (Fig.3 A&B)	5x7	Elongated compressed with pointed apex	Light brown	Jagged with grooves	lateral non twisted short thick	open equidistance flat	equal arms	1/2 of seed area	Dark
A.etabica ssp. uncinata	6x10	Elongated compressed	dark green	granulate colliculate	subterminal twisted	open equidistance	equal arms	1/2 of	Dark

(Fig.4 A&B)		with pointed apex	shinning		long thick	convex		seed area	
A.gerrardii (Fig.5 A&B)	4x7	Elliptic compressed with round apex	Yellowish green	Jagged rough	subterminal twisted long medium thick	open equidistance convex	not equal arms	1/2 of seed area	Dark
A.gerrardii ssp. najdensis (Fig.6 A&B)	5x6	Rhombic compressed	Dark green	crimpled foveolate	subterminal no twisted short thick	open equidistance	equal arms	3/4 of seed area	Light
A.hamulosa (Fig.7 A&B)	7x8	circular compressed with apiculate apex	Yellowish brown shinning	granulose	subterminal twisted long thin deciduous	open V-shape	not equal arms	1/4 of seed area	Dark
A.mellifera (Fig.8 A&B)	7x8	Quadrate compressed with pointed apex	Greenish brown shinning	striated papillosed	subterminal twisted long thick	open cress-shape concave	not equal arms	1/4 of seed area	Light
A. nubica (Fig.9 A&B)	4x5	Ovate with round apex	Light brown	microgranulate	subterminal twisted long thin	open concave	equal arms	1/3 of seed area	Dark
A.raddiana (Fig.10 A&B)	2x4	Ovate with pointed apex	Dark brown	reticulate tuberculate	subterminal twisted long thin	open flat	not equal arms	1/2 of seed area	Dark
A.tortilis (Fig.11 A&B)	3x4	Ovate compressed with pointed apex	Reddish brown	reticulate foveolate	subterminal twisted long thin	open equidistance flat	not equal arms	1/2 of seed area	Dark

Table (3): Seed anatomical characters of 11 Acacia species growing in Saudi Arabia

Species	**Outer integuments**			**Inner integument**	**Endosperm**	
	Cuticle	Malbegian cell	Light line		Cell type	Reserve food
A. abyssinica (Fig.1C&D)	Thin	Bone shape with conical end 58 µm length	In the above 1/3 portion	Number of rows of lignified parenchyma cells	Thin wall parenchyma cells	color substance
A.ehrebergiana (Fig.2 C&D)	Thick	Bone shape with straight end 35 µm length	In the middle portion	One row of thin parenchyma cells	Thick wall parenchyma cells	crystals and color substance
A. etabica (Fig.3 C&D)	Thick	Bone shape with straight end 56µm length	In the middle portion	Number of rows of thin parenchyma cells	Medium thick wall parenchyma cells	color substance
A.etabica ssp. uncinata (Fig.4 C&D)	Thin	Bone shape with conical end 61 µm length	In the above 1/3 portion	Number of rows of thin parenchyma cells	Thin wall parenchyma cells	-
A.gerrardii (Fig.5 C&D)	Thick	Bone shape with straight end 84µm length	In the middle portion	One row of thick parenchyma cells	Thick wall parenchyma cells	color substance

A.gerrardii ssp. najdensis (Fig.6 C&D)	Thin	Bone shape with conical end 83 µm length	In the above 1/3 portion	Number of rows of lignified compressed parenchyma cells	Thick wall parenchyma cells	oil substance
A.hamulosa (Fig.7 C&D)	Thick	Bone shape with conical end40 µm length	In the above 1/3 portion	One row of thin parenchyma cells	Thick wall parenchyma cells	color substance
A.mellifera (Fig.8 C&D)	Very thick	Bone shape with conical end55 µm length	not distinguish	One row of thin parenchyma cells	Thick wall compressed parenchyma cells	color substance
A. nubica Fig.9 C&D) (	Thick	Bone shape with conical end57 µm length	In the middle portion	Number of rows of lignified parenchyma cells	Thick wall parenchyma cells	color substance
A.raddiana (Fig.10 C&D)	Thin	Bone shape with conical end30 µm length	In the above 1/3 portion	One row of thick parenchyma cells	Thick wall compressed parenchyma cells	color substance
A.tortilis (Fig.11 C&D)	Thick	Bone shape with conical end83 µm length	In the middle portion	Number of rows of thin compressed parenchyma cells	Thin wall parenchyma cells	color substance

4- Discussion

Our study was concentrated first on the description of the seeds for the 9 species and 2 subspecies of *Acacia* under investigation. About 7 characters have been recorded, viz; seed shape and size, areole features including open or closed with divergent, straight equal or unequal arms, area inside the areole ratio between control areole area and seed surface area, also surface texture, Hassen (1989) employed some of this characters in his study. All these characters help to distinguish between the 11 species and subspecies of *Acacia* plant under investigation.

A. ehrenbergiana is specialized by its undistinguished central aerole between all the often investigated species. Also A. abyssinica is specialized by its distinguish closed central aerole , while A. raddiana, A. mellifera, A. hamulosa, A. gerrardii were distinguish by the open central aerole and unequal arms, as well as. The central aerole occupies half the seed surface area in A. gerrardii and less than half in left other species. Central aerole characters can be described by Esau (1977) as a major character to differentiate between leguminous seeds. In case of open central aerole with equal arms, is specialized for the species and subspecies A. etbaica, A. etbaica ssp.uncinata, A. gerrardii var. najdensis, A. tortilis, A. nubica.

In this study we recognize the level of the central aerole, as a specific character. Species of A. abyssinica and A. mellifera can be distinguish by their concave central aerole, while in A. hamulosa and A. etbaica the central aerole is flat, and in A. raddiana, A. nubica, A. gerrardii var. najdensis the central aerole is convex.

Scanning electron microscope investigation of the seed surface shows some variation between the species; different types of seed surface ornamentation are recognized A. abyssinica is reticulate tuberculate, A. ehrenbergiana is reticulate rugose, A. etbaica is jagged with grooves, A. etbaica ssp.uncinata is granulate colliculate, A. gerrardii is jagged rought, A. gerrardii var.najdensis is crimpled foveolate, A. hamulosa is granulose, A. mellifera is striated papillosed, A. nubica is microgranulate, A.raddiana is Reticulate foveolate, , A. tortilis is reticulate tuberculate. Our results were agree with which used dry mature seeds of 26 species of 4 Leguminous genera, to standardize a procedure for identifying the seeds through SEM on the seed surface and seed sections

Anatomical structure of the seed coat of the investigated species shows many different characters that can be used in the separation of studied species according to Esau (1977) and Werker (1997).

The coat of the mature seed may be described as hard, cartilaginous, leathery, papery features determined by the structure of its cells.

In all investigated Acacia species, seed coat was so hard except A. hamulosa which contains a mucilaginous layer. Sections of testa in all investigated seeds species show the differentiation in protective layer (cuticle and sclerenchyma). Malpighian cells as mechanical layers and also responsible for its impermeability, light line which is a refractive zone across the upper half or in the middle of each cell running continuously along the

Malpighian layer. Inner integument layer with different cell shape and the inner most endospermic layer. Based on those characters, A. mellifera is specified by the undistinguished light line, while it is well distinguish in upper one third Malbighian layer in species A. hamulosa, A. abyssinica, A. tortilis, and subspecies A. gerrardii var.najdensis, A. etbaica ssp.uncinata; and in half Malpighian cell A. gerrardii, A. ehrenbergiana, A. nubica, A. raddiana, A. etbaica.

Length of malbighian cell show a great variety, short cells between 30-60µm in A. ehrenbergiana, A. nubica, A. etbaica A. hamulosa, A. abyssinica, A. tortilis or long cells between 61-80 µm in A. gerrardii, A. raddiana and subspecies A. gerrardii var.najdensis, A. etbaica ssp.uncinata.

The inner integument of all investigated species in addition to the endosperm is considered as a reserve tissue. Reserve materials, a nutritive tissue, sometimes proteins, starch, and lipid accumulate in the inner integument layer for germinating embryo. In species of A. nubica , A. abyssinica, A. gerrardii and subspecies A. gerrardii var. najdensis the inner integument formed of several lignified rows with reserve colored materials. In A. raddiana, A. etbaica and subspecies A. etbaica ssp.uncinata formed of thin wall cells in several rows A. gerrardii, A. raddiana were specified by one row, while, A. hamulosa, A. ehrenbergiana, A. mellifera, were distinguish by one row with thin wall integument cell. Crystals are quite common in seed coats in some species apparently and in protection against predators. They may be located in various layers, sometimes in specialized idioblasts. A. ehrenbergiana shows number of solitary crystals in the inner integument cell.

5- Artificial keys

5-1- Morphological key

I. Undistinguished central aerole..... ..... ***A. ehrenbergiana***

II. Distinguish central aerole

A. Distinguish closed central aerole……..... ***A. abyssinica***

B. Distinguish open central aerole

i. Open central aerole with equal arms

✸ The central aerole less than half the seed surface area…….. ***A. nubica***

✸ The central aerole equal or more than half the seed surface area

+ Flat central aerole

a)Seed surface jagged with grooves**…..... .....*A. etbaica***

b)Seed surface Reticulate foveolate …. **……..*A. raddiana***

+ Concave or convex central aerole

a)Seed surface granulate colliculate**……*A. etbaica ssp. uncinata***

b)Seed surface is crimpled foveolat**….. ……..*A. gerradii var. najdensis***

ii. Open central aerole and unequal arms

✸ The central aerole less than half the seed surface area and cress shape

+Seed surface granulose**..... *A. hamulosa***

+Seed surface striated papillosed. ……**. *A. mellifera***

✸ The central aerole equal or more than half the seed surface area

+Seed surface jagged rough**..... *A. gerrardii***

+Seed surface reticulate tuberculate……..…..……..… ***A. tortilis***

5-2- Anatomical key

I. Non distinguish Light line …... ***A. mellifera***

II. Distinguish Light line

A. light line passes across the middle of the Malpighian layer

i. short Malpighian cells between 30-60µm

✸ The inner integument formed of one row of thin cell… .***A. ehrenbergiana***

✸ The inner integument formed of several rows of cells

+ Thin wall parenchyma cells…… **….*A. etbaica***

+ Thick wall parenchyma cells……..…***A. nubica***

ii. Long Malpighian cells between 61-85

✸ The inner integument formed of one row of lignified cells ……..***A. gerrardii***

✸ The inner integument formed of several rows of thin cell**…….. *A. tortilis***

B. light line pass in the upper one third of the Malpighian layer

i. Short Malpighian cells between 30-60µm

✸ The inner integument formed of one row of thin cells….....***A. hamulosa***

✸ The inner integument formed of lignified cells

+ One raw of lignified parenchyma cells…….……..***A. raddiana***

+ Several rows of lignified parenchyma cell.....***A. abyssinica***

ii. Long Malpighian cells between 61-85

* The inner integument formed of several rows of thin cell ……***A. etbaica ssp. uncinata***

* The inner integument formed of several rows of lignified cells ..... ***A. gerradii var. najdensis***

Fig. 1 ***Acacia abyssinica*** A&B SEM seed and seed sculpture **,**C&D T.S. in seed coat 20x&40x

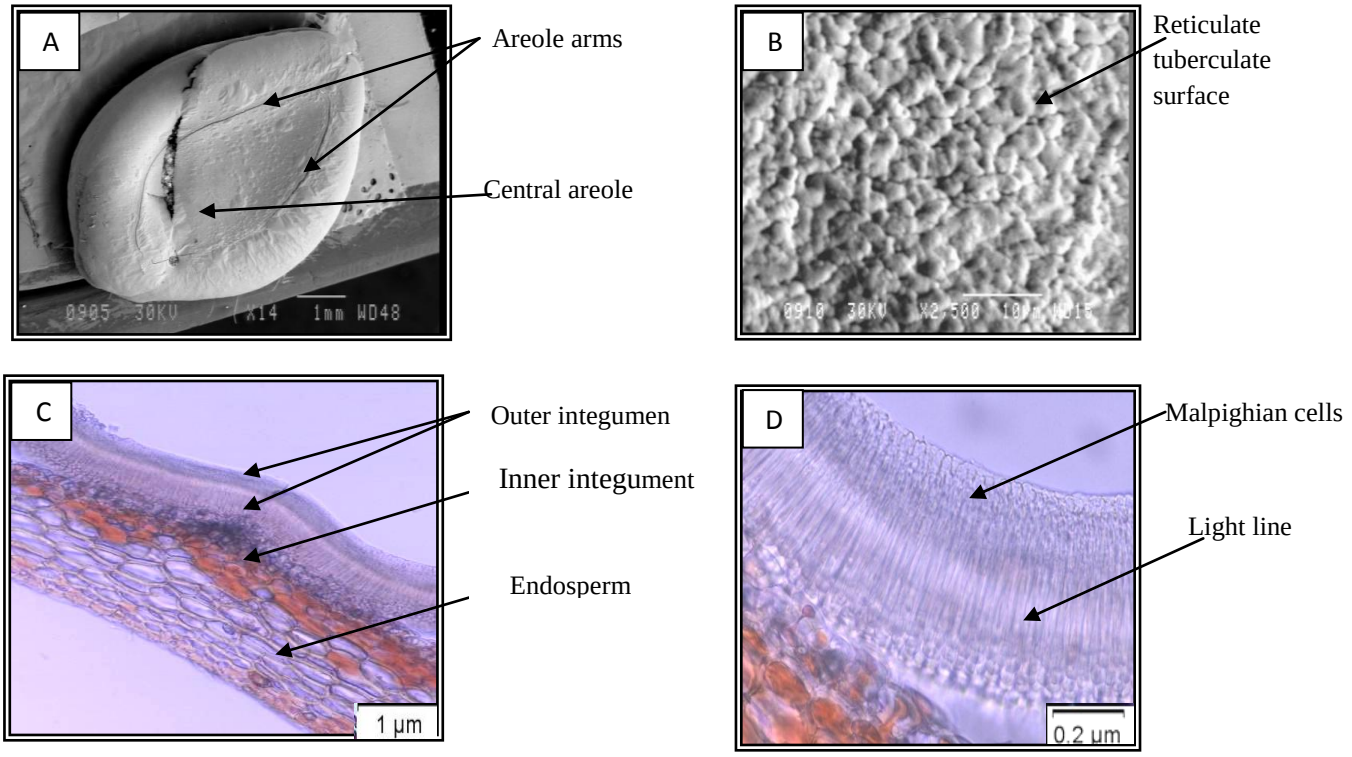


Fig 2 Acacia ehrenbergiana A&B SEM seed and seed sculpture **,**C&D T.S. in seed coat 20x&40x

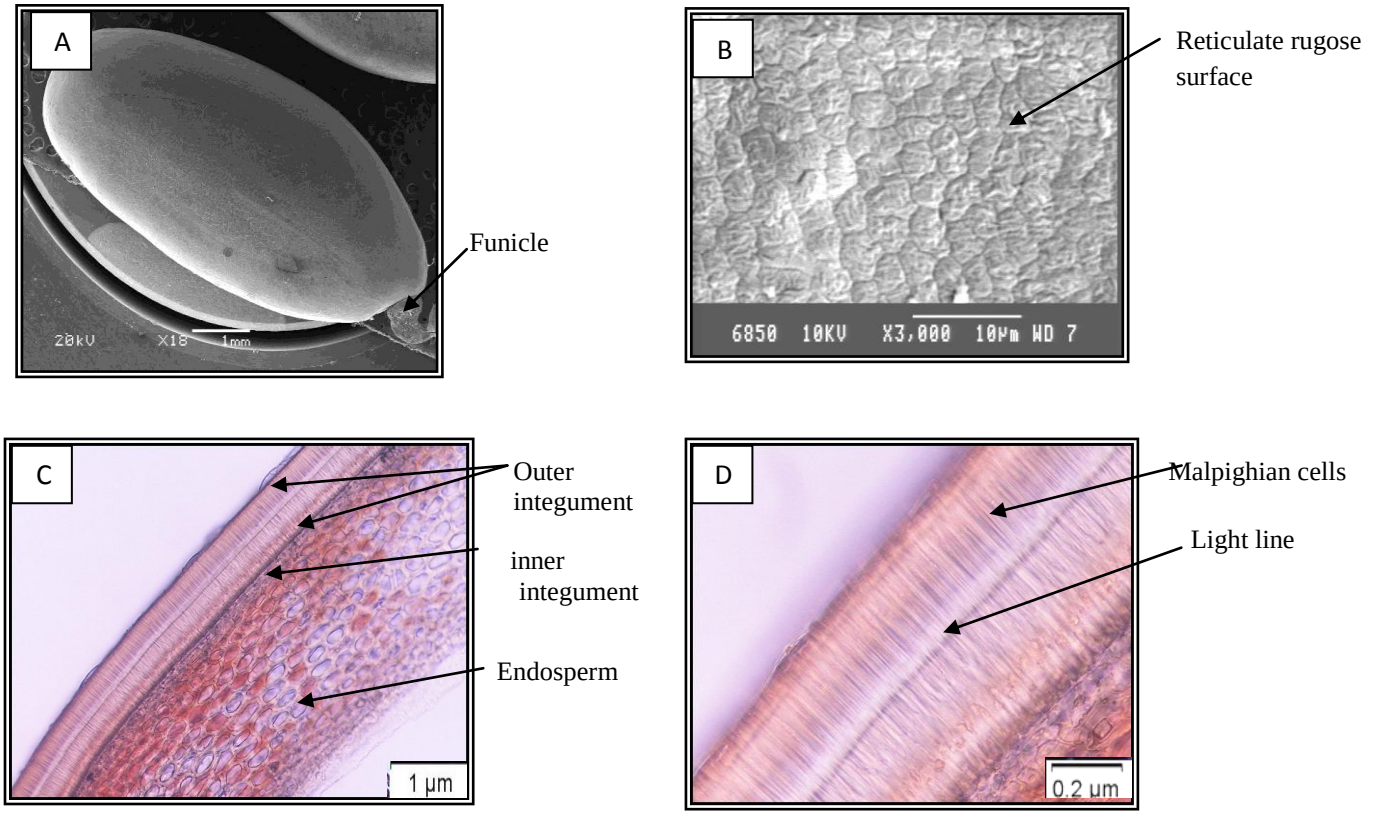


Fig 3 Acacia etbaica A&B SEM seed and seed sculpture **,**C&D T.S. in seed coat 20x&40x

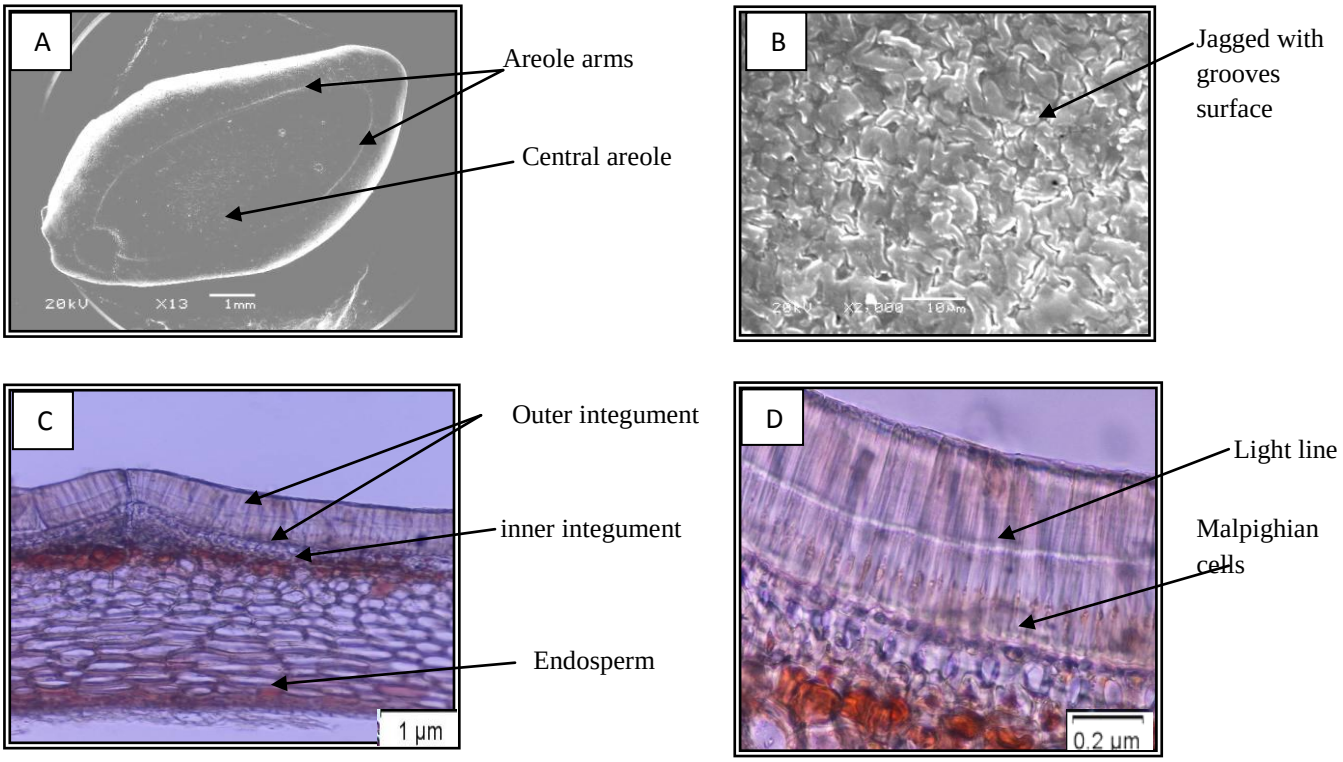


Fig.4 A. etbaica ssp. uncinata A&B SEM seed and seed sculpture **,**C&D T.S. in seed coat 20x&40x

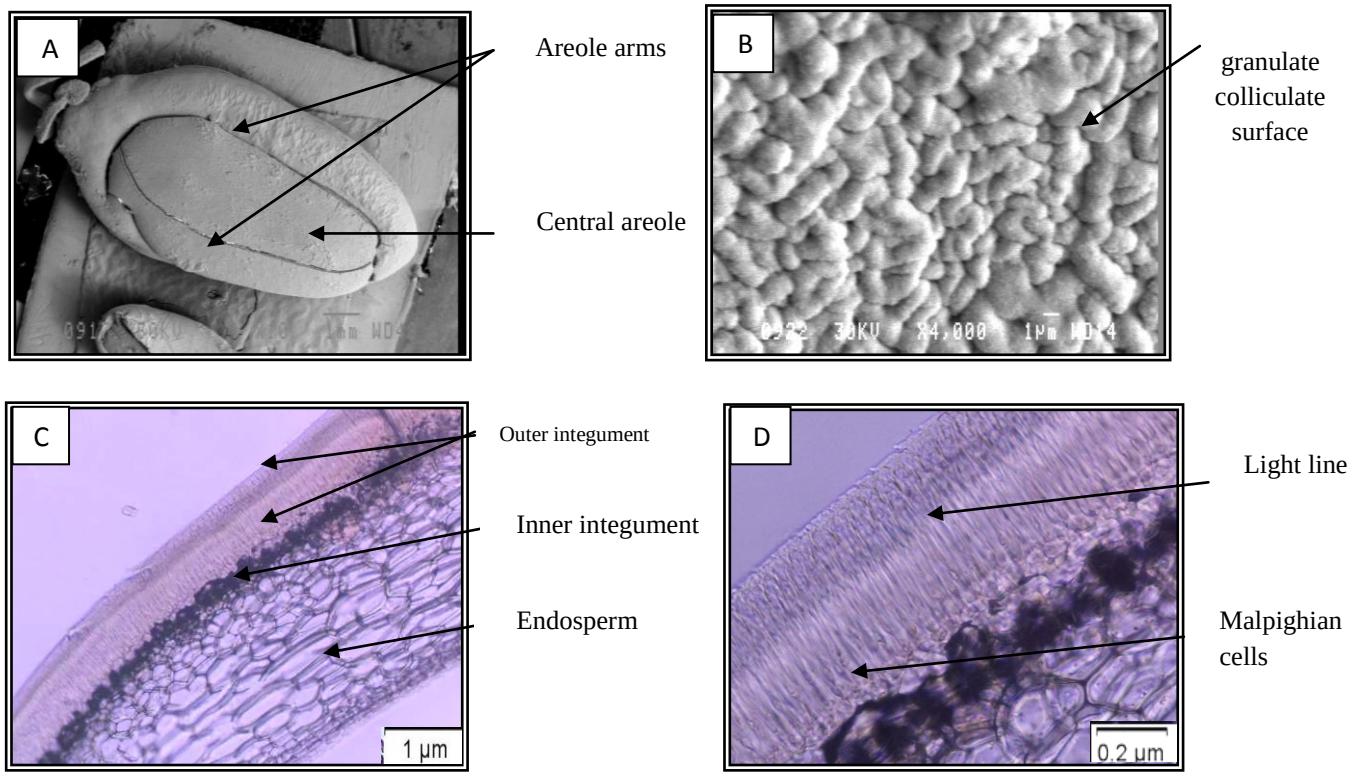


Fig 5 Acacia gerrardii A&B SEM seed and seed sculpture **,**C&D T.S. in seed coat 20x&40x

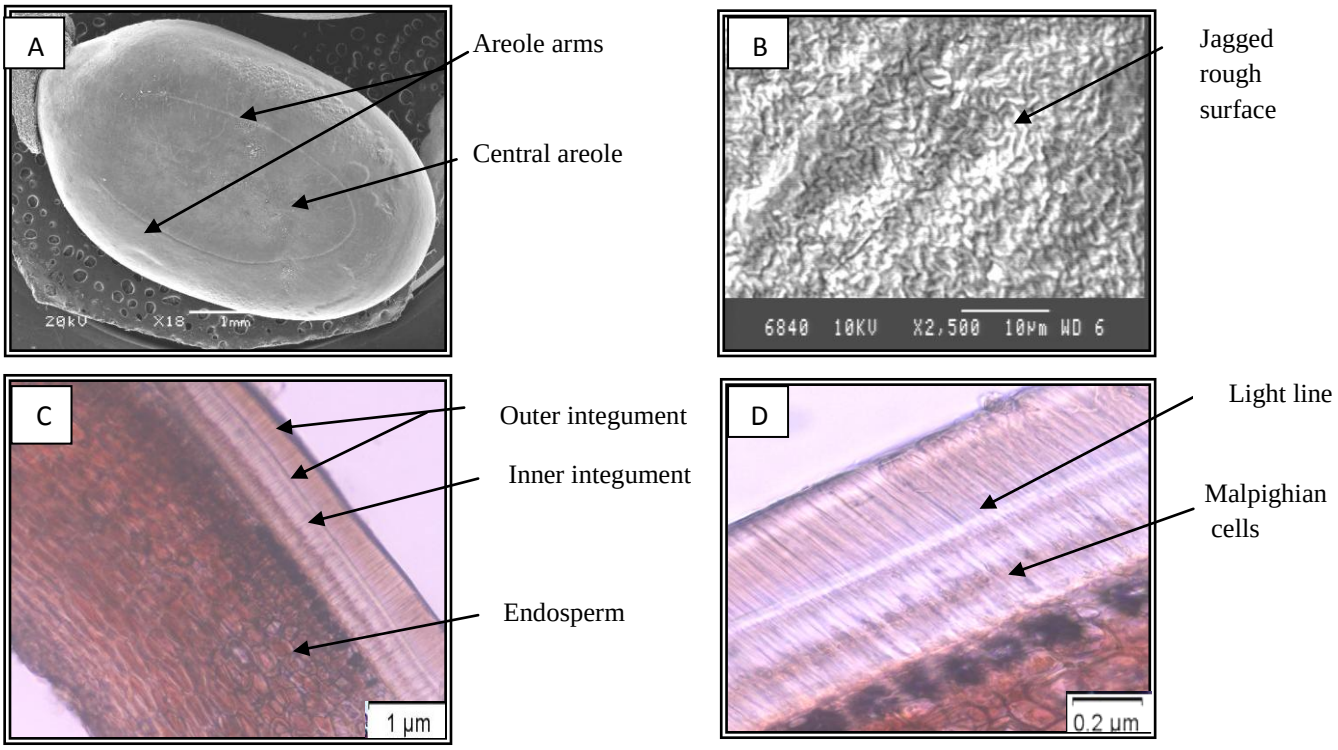


Fig 6 Acacia gerrardii var najdensis A&B SEM seed and seed sculpture **,**C&D T.S. in seed coat 20x&40x

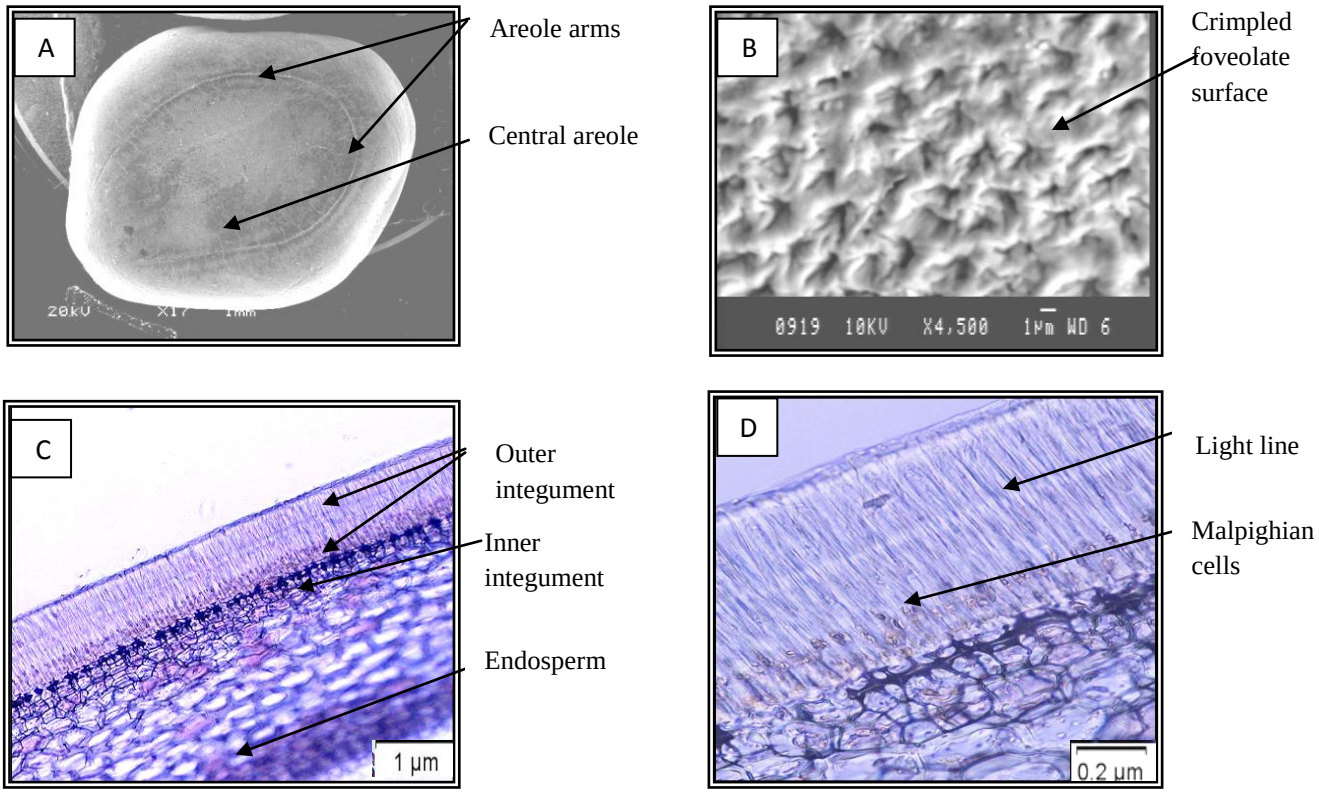


Fig.7 Acacia hamulosa A&B SEM seed and seed sculpture **,**C&D T.S. in seed coat 20x&40x

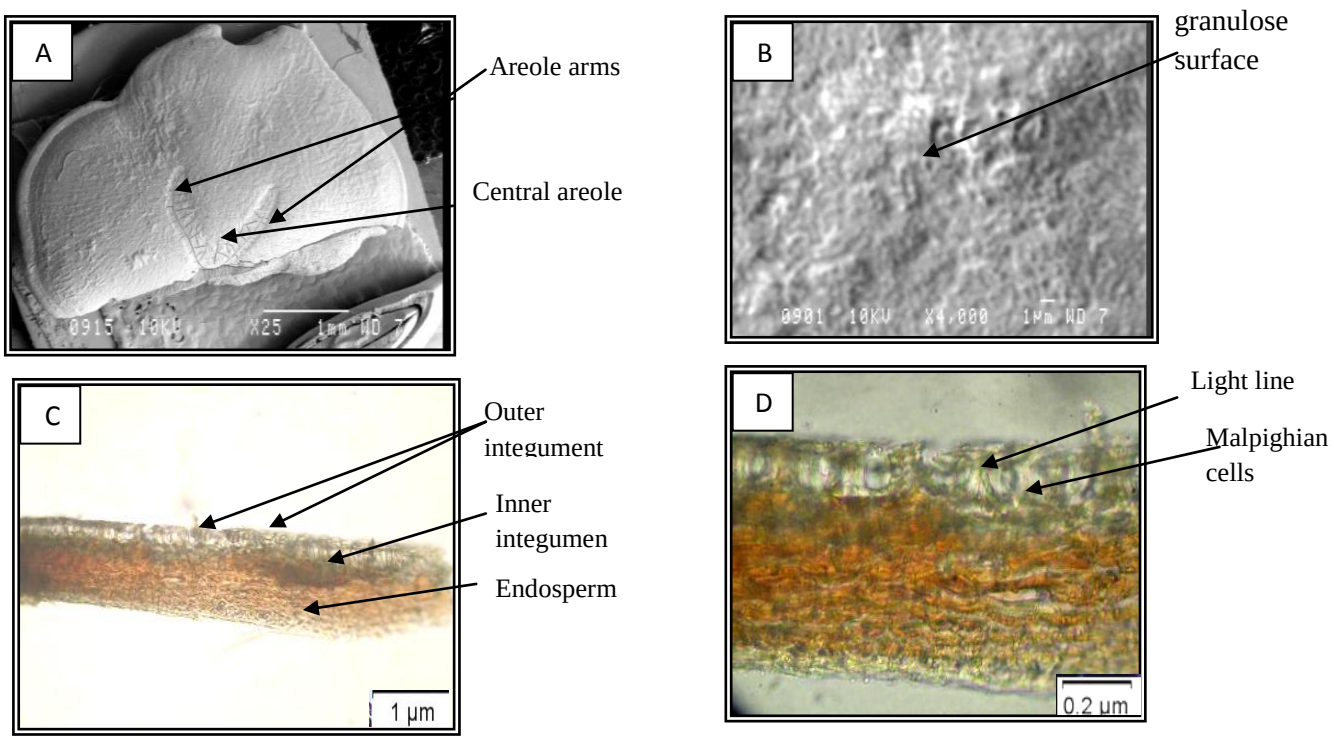


Fig.8 Acacia mellifera A&B SEM seed and seed sculpture **,**C&D T.S. in seed coat 20x&40x

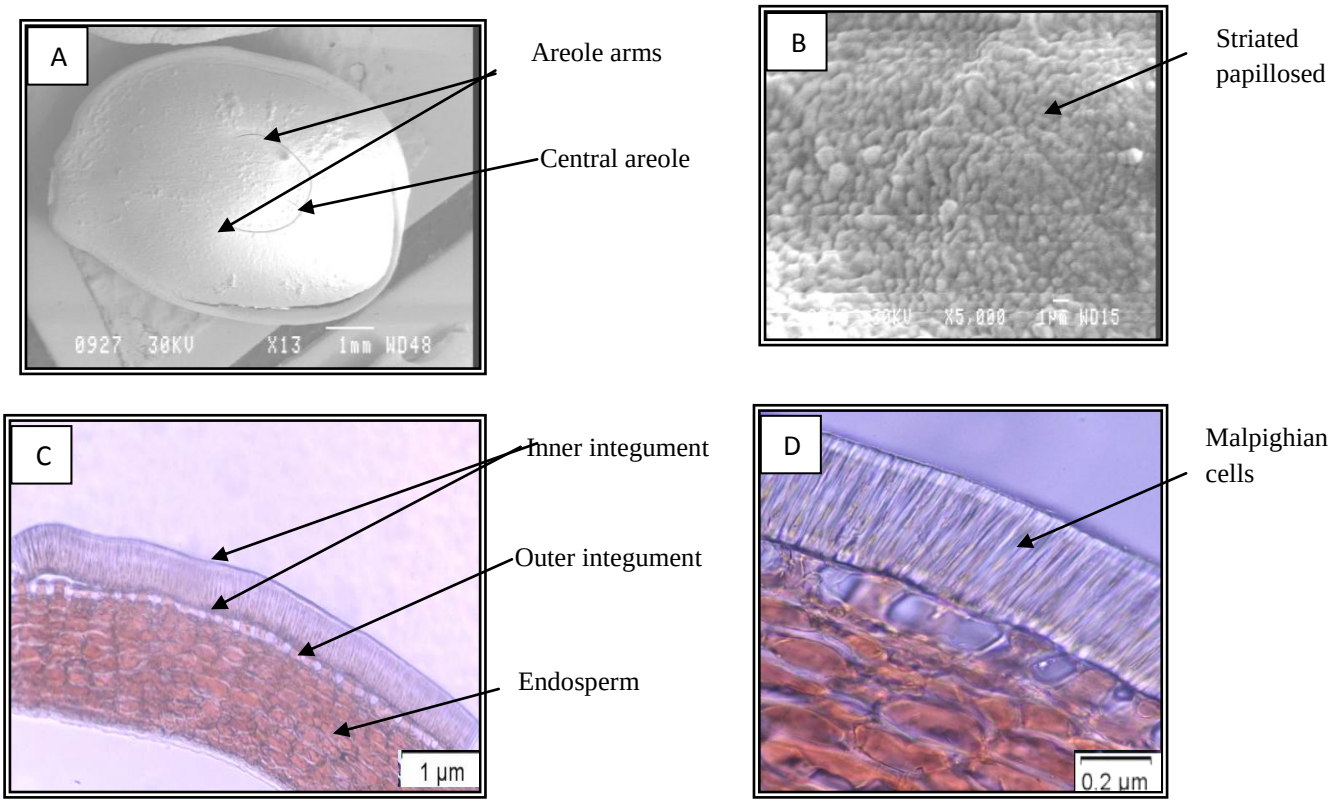


Fig 9 Acacia nubica A&B SEM seed and seed sculpture **,**C&D T.S. in seed coat 20x&40x

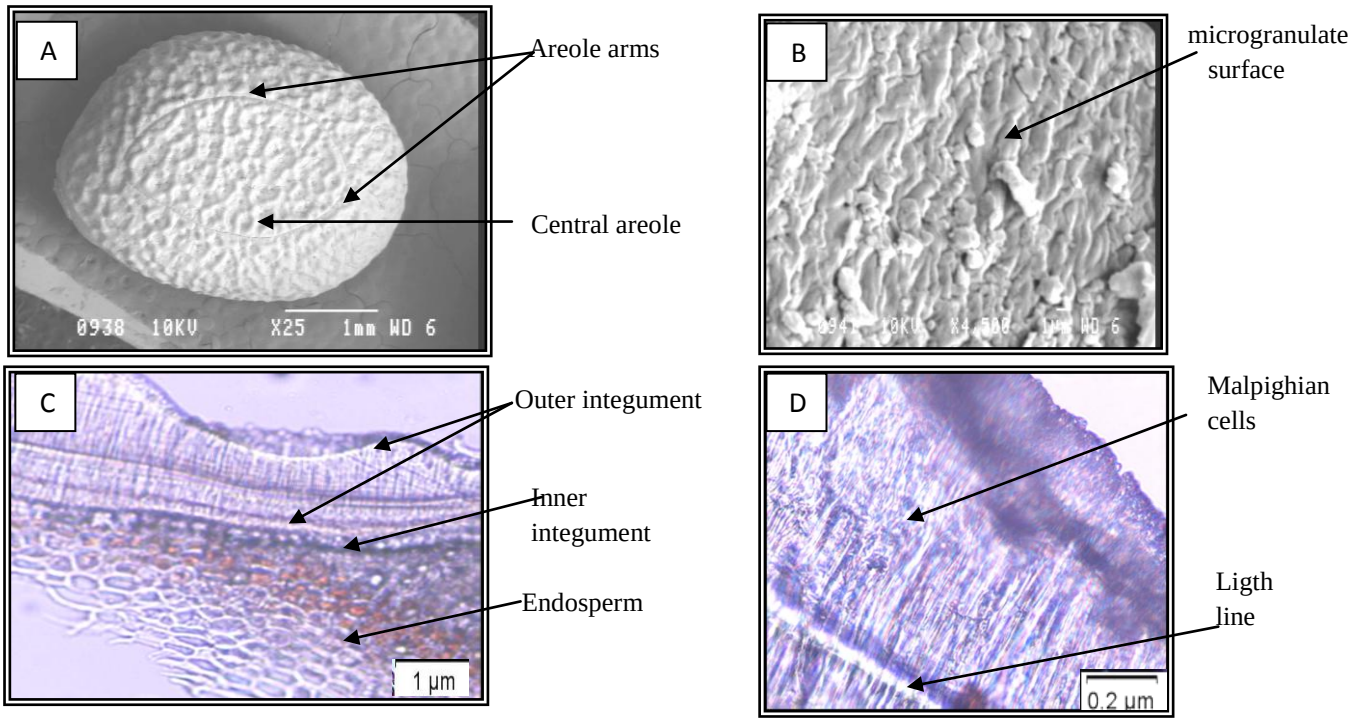


Fig.10 Acacia raddiana A&B SEM seed and seed sculpture **,**C&D T.S. in seed coat 20x&40x

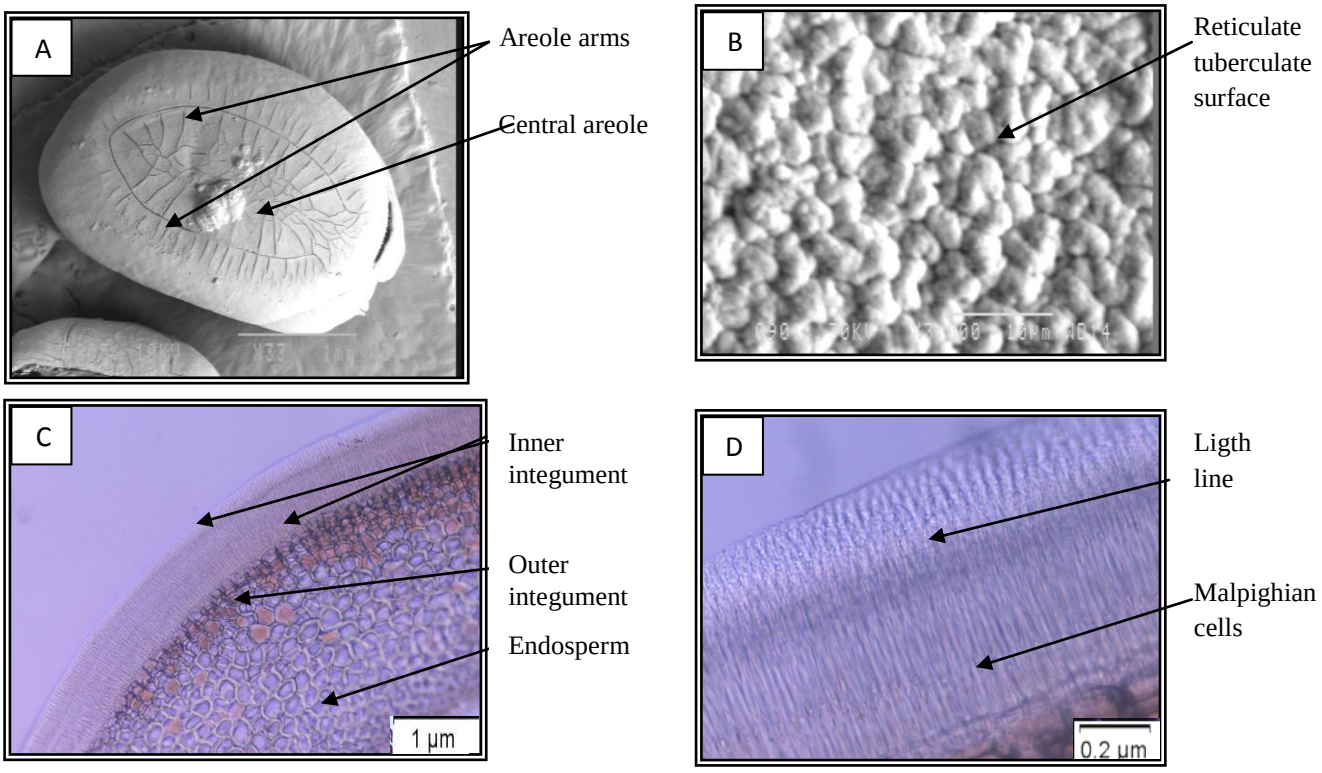
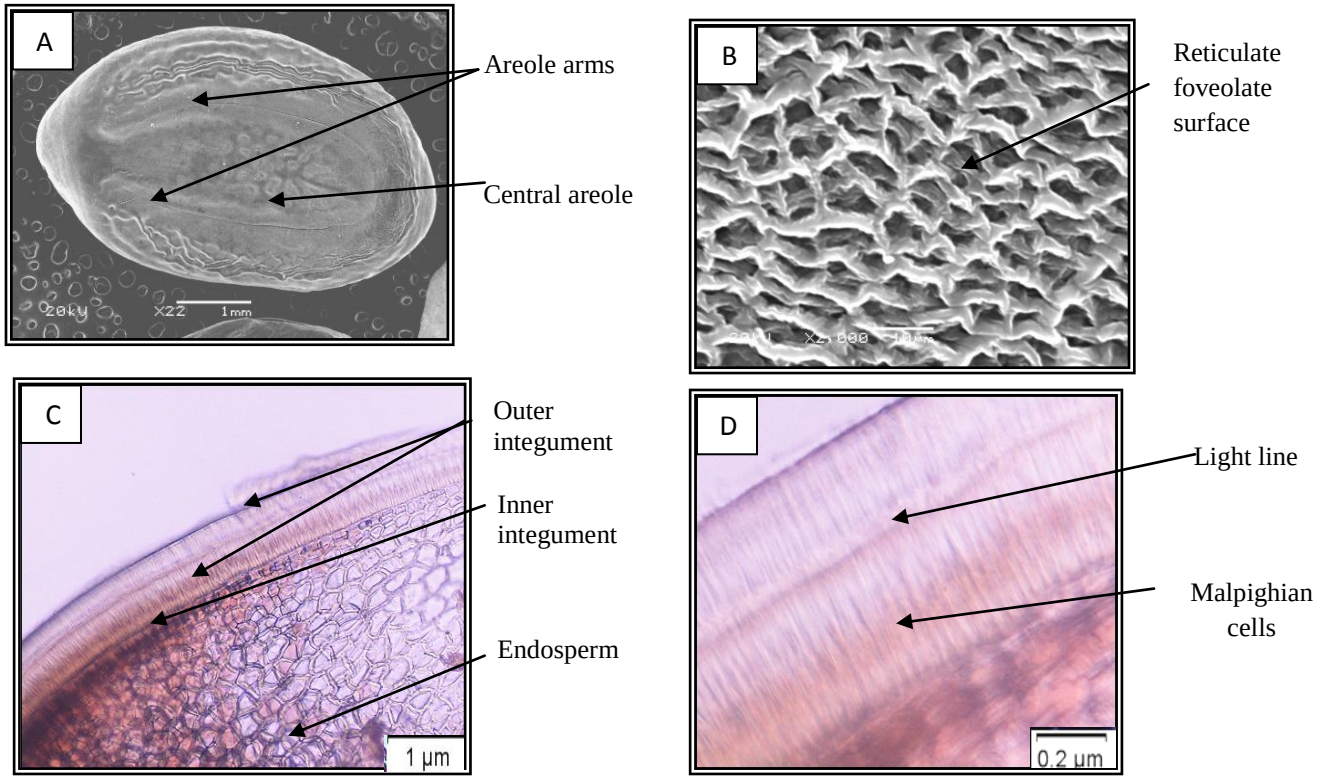


Fig.11 Acacia tortilis A&B SEM seed and seed sculpture **,**C&D T.S. in seed coat 20x&40x



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