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SIZE VARIATION AND SURFACE STRUCTURE OF PODS AND SEEDS OF *BAUHINIA RACEMOSA* LAMK.

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Abstract

Size variation and surface structure of pods and seeds of *Bauhinia racemosa* are described. The immature and developing fruits of *B. racemosa* are twisted strap like dark green, and photosynthesizing. The mature fruits mostly twisted, oblong and compressed chocolate brown, turgid, and apex tapered. Pod surface has ridges and grooves. The mature pods of *B. racemosa* averaged to 15.79 ± 0.273 cm (4.60 – 25.70 cm) in length and 2.02 ± 0.016 cm (1.20-2.8 cm) in breadth. An average pod weighed 9.19 ± 0.23g (1.56 – 18.25g; CV: 36.64%) and yielded on an average 10.27 ± 0.35 seeds. The surface of the pods had non-glandular unicellular conical trichomes (80-150 µm), denser in the depressed areas. The inner diameter of trichome stub was 7.98 µm and outer diameter of 17.1 µm. The lumen of broken trichomes was often filled with occluded materials. In *B. racemosa*, stomata were present only on the outer epidermis of the pericarp but not on the inner epidermal lining of the seed chamber. Pod wall in *B. racemosa* appeared to have some air spaces. The epidermal lining of the seed chamber appeared to be quite smooth and provided with so many openings of the micro-tunnels running in the pod wall. Single seed weight based on 1170 seeds weighed individually averaged to 93.20 ± 0.74mg (25.0 – 194.0 mg, CV: 27.14%) i.e. varying by 7.76-folds. The seeds were foveolate (having pits on the surface). The results are discussed in view of the possible role of green pod wall and seeds in pod development and gaseous exchange across pod wall.

Introduction

Genus *Bauhinia* L. includes c 300 species (Yakovlev, 1991). According to Larsen (1975), however, 600 species of pantropical distribution are included in the genus *Bauhinia* L. Eight species of Bauhinia L. are reported from Pakistan namely *B. corymbosa, B. linnaei, B. purpurea, B. retusa, B. racemosa, B. tomentosa, B. vahlii* and *B. variegata* (Ali, 1973). Ponomarenko and Pavlova (2003) have divided *Bauhinia* species studied by them (13 in number) into two groups on the basis of seed structure. Group I species (*B. aculeata, B. purpurea* and *B. variegata*) have seeds that are laterally flattened, disk shaped, slightly convex on one side and concave on the other. The colour is brown and the surface is matte and wrinkled. Endosperm is absent, and no aleurone layer is present. Stomata are present on the seed surface, which always remain open. The group II species (*B. acuminata, B. cumanensis, B. diphylla, B. galpinii, B. hookerii, B. mononadra, B. racemosa, B. rufescens, B. tomentosa* and *B. violacea*) have seeds with elliptic contour and are less flattened laterally. The colour is black and dark brown. The surface is lustrous and smooth. There is no stomatal apparatus and surface cuticle shows no distinct sculptural pattern and looks coarsely pitted. Endosperm is thick and aleurone layer is present. Group I seeds are heavier than group II seeds. However, spermoderm of group II seeds is thicker than that in seeds of group I species. Also, seeds of group I species have no hypodermis whereas group II seeds have hypodermis.

Rugenstein and Lersten (1981) examined 45 species of *Bauhinia* (evenly distributed in old and new World tropics) and found stomata on mature seeds of eight species viz. *B. acuminata, B. glauca, B. grandiflora, B. microstachya, B. purpurea, B. retusa, B. vahlii* and *B. variegata*. Four of these species viz., *B. purpurea, B. retusa, B. vahlii* and *B. variegata*, are Pakistani Bauhinias showing the presence of stomata on the seeds. The occurrence of stomata on seeds is an unusual character which is considered to be of taxonomic value (Rugenstein and Lersten (1981). Schleiden's (1839) report on *Canna maculata* was the first observation on the occurrence of stomata on seed coats (Werker, 1997 (bears reference of Schleiden (1839); Johri, 2012). At present c. 30 families are known to bear stomata on seeds (Johri, 2012). The presence of stomata on outer seed integument is rare (Netolitzky, 1926; Werker, 1997; Paiva *et al.*, 2006). Stomata on seed coat are although poorly developed when present, they play important role in seed imbibition and these permanently open stomata also take part in gas exchange during seed development (*Paiva et al.*, 2006). According to the hypothesis of Jernstedt and Clark (1979) stomata in *Eschscholzia* take part in gas exchange when developing seeds photosynthesize within fruit. Gaseous exchange through pod wall has been demonstrated in some studies of legumes (Sheoran and Singh, 1987; Flinn *et al.*, 1977; Setia *et al.*, 1987).

In present paper, some pods and seeds characteristics of *B. racemosa*, a legume of great economic importance and distributed in Sindh and Punjab, are studied, particularly for variation in their mass and for their surface structure under Scanning Electron Micrography. It is a semi-deciduous tree, reaching 10m or so. It grows in sunshine. Elephants feed on this tree in India. Seedling morphology of *B. racemosa* has been described by Khan *et al.* (2015). They have reported paracytic, anisocytic, anisotricytic, anomocytic, staurocytic types of stomata in the leaves and cotyledons of this species. In *B. racemosa*, the developing pods and seeds are green. We hypothesized that there should be some alternate way other than stomatal apparatus for gaseous exchange from photosynthesizing seeds in this species.

Materials and Methods

Green immature and ripe pods from a mature tree (c 10m high with DBH around 50 cm, canopy area 56 m^2 and basal area around 27 dm^2) of *B. racemosa* Lamk. (Burmese Silk Orchid, Jhinjera, Kosundra or Sonpatta tree), growing in the campus of University of Karachi, were collected. The mature fruits were air-dried for few months in shade. The seeds were recovered from them and stored in a brown glass bottle. The number of seeds recovered from each pod was recorded. A sizeable number of seeds were weighed individually on electrical balance to follows seed weight variation. The green pods were studied for pod and developing seed sizes by opening them longitudinally by ventral and dorsal sutures. For examination of surface structure of pods and seeds, sections of air-dried pericarp and seeds were mounted on brass stubs and then coated with a layer of 300 nm (A^o) gold in a sputter-coater (JFC-1500). SEMs were made at 15 and 30 kV with JEOL JSM – 6380 LV Scanning Electron Microscope at various magnifications. The images were saved digitally on computer.

Results and Discussion

The immature and developing fruits of *B. racemosa* are twisted strap like dark green, and photosynthesizing (Fig. 1A). In literature, there are many reports of green photosynthetic characteristics of legume pods (Crookston *et al.*, 1974; Flinn *et al.*, 1977; Sambo *et al.*, 1977; Atkins and Flinn, 1978; Sheoran *et al.*, 1987; Ma *et al.*, 2001; Futbank *et al.*, 2006; Wang *et al.*, 2016). The mature ripe fruits were mostly twisted, oblong, compressed chocolate brown, hard, tough and swollen and apex tapered. Pod surface had ridges and grooves (Fig. 1B). The mature pods of *B. racemosa* averaged to 15.79 ± 0.273 cm (4.60 – 25.70 cm) in length and 2.02 ± 0.016 cm (1.20-2.8 cm) in breadth (Table 1). Pod length, was more variable character (CV: 25.37%) than pod breadth (CV: 11.42%). An average pod weighed 9.19 ± 0.23g (1.56 – 18.25g; CV: 36.64%) and yielded on an average 10.27 ± 0.35 seeds (CV: 46.09%).

The surface of the pods bears non-glandular unicellular conical trichomes (80-150 μm), denser in the depressed areas (Fig. 2 and 3). The trichomes are often broken due to random events and their stubs may be seen distributed over the pericarp (Fig. 4 A, C and E). The inner diameter of trichome stub was 7.98 μm and outer diameter of 17.1 μm. The lumen of broken trichomes may often be filled with occluded materials presumably lipids (Fig. 5 A and B).

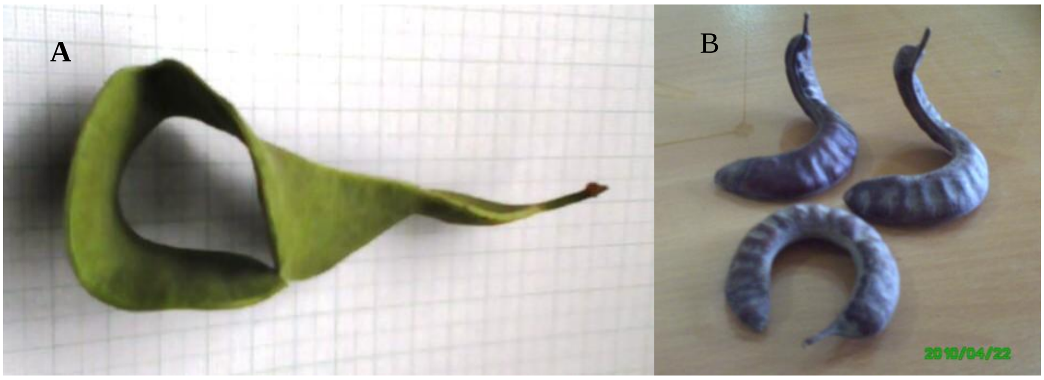

Fig. 1. The pods of *Bauhinia racemosa* L collected from Karachi University campus, Karachi. A, Young green strap like thin pod and B, Mature fruits are chocolate brown shiny flat and generally twisted in one plane but often in two or more planes also. Note the ridges and grooves. The ridges correspond with the location of seed inside. The depressed areas are relatively rougher than the raised areas which are smoother.

In *B. racemosa*, stomata were present only on the outer epidermis of the pericarp (Fig. 4 D and F) but not on the inner epidermal lining of the seed chamber. Pod wall in *B. racemosa* appeared to have some air spaces (Fig. 6). The epidermal lining of the seed chamber appeared to quite smooth, devoid of any stomata but provided with so many openings of the micro-tunnels running in the pod wall (Fig. 7 A and B).

The dark brown healthy seeds of *B. racemosa* are variously shaped and broadly classifiable into two categories (Fig. 8). The seeds of upper row are more or less oval to somewhat elliptic in shape and those of the lower row are circular or near circular in shape. Such seeds were comparatively lesser in number. Single seed weight (SSW) based on observation on 1170 seeds averaged to 93.20 ± 0.74 mg (25.0 – 194.0 mg, CV: 27.14%)

i.e. varying by 7.76-folds (Table 1). The smaller seeds (25-75 mg) were 24.4%, Medium seeds (76-125mg) were around 65.1% and large seeds category was merely 10.5%. The modal class (76-100mg) seeds were 35.7% of the total seeds. The sorted medium to large seeds measured 7.37 ± 0.094mm in length, 5.27 ± 0.072mm in breadth and 3.27 ± 0.075mm in thickness (Table 1). Yücadaĝ and Gültokin (2011) have reported 1000-seeds weight of *B. racemosa* to be 130.0 ± 10.0g i.e. on average 130 mg per seed. Ponomarenko and Pavlova (2003) have reported *B. rac*emosa normal healthy seed to weigh 144.3 mg. Our seed weight estimate of *B. racemosa* is comparatively lower than that reported above which may probably be attributed to seed lot composition of unsorted seeds with substantial number of smaller seeds (24.4%) or the geographical variation of the environment of the mother plants. The seeds of *B. racemosa* are reported to be rich in calcium and iron and glutelins fraction of proteins. Seeds contained essential amino acids - lysine, tyrosine, and phenylalanine and anti-nutritional factors of free phenols, tannins, L-DOPA, and haemagglutinating and trypsin inhibitor activities were not particularly high (Rajaram and Janardhanan (1991). Since *B racemosa* seed endosperm consists of Galactomannans (Shcherbukhin and Anulov, 1999), *B. racemosa* can serve as a source of the valuable substances for various industries (Ponomarenko and Pavlova, 2001).

Table 1. Location and dispersion parameters of some pods and seeds characteristics.

Parameter	PL (cm)	PB (cm)	PW (g)	NSP	SSW (mg)	SL * (mm)	SB* (mm)	ST* (mm)
N	215	215	215	180	1170	30	30	30
Mean	15.785	2.0244	9.185	10.267	93.20	7.367	5.267	3.268
SE	0.2731	0.0158	0.2295	0.3527	0.740	0.0942	0.0719	0.07453
Median	15.10	2.00	8.870	10.0	93.90	7.00	5.00	3.29
CV (%)	25.37	11.42	36.64	46.09	27.14	7.33	7.47	12.49
g1	0.199	-0.187	0.216	0.083	-0.009	-0.514	0.508	-0.544
Sg1	0.166	0.166	0.166	0.181	0.072	0.427	0.427	0.427
g2	-0.297	1.063	-0.542	-0.046	-0.062	0.7.31	-0.382	2.739
Sg2	0.330	0.330	0.330	0.360	0.143	0.833	0.833	0.833
Min	4.60	1.20	1.16	0.0	25.0	6.0	4.5	2.17
Max	25.70	2.80	18.25	23.0	194.0	8.0	6.0	4.28
KS-z	1.037	2.282	0.793	0.915	1.143	1.724	1.557	1.047
p	0.233	0.001	0.555	0.372	0.147	0.005	0.016	0.223

PL, pod length; PB, pod breadth; PW, Pod weight; NSP, Number of seeds per pod, SSW, Single seed weight (mg), SL, seed length (mm), SB, seed breadth (mm) and ST, seed thickness (mm). *, sorted medium to large seeds.

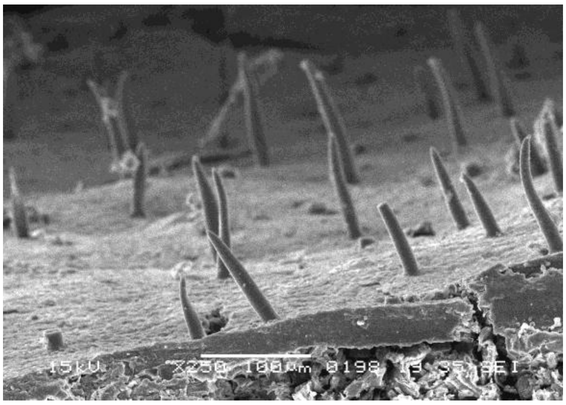

Fig. 2. SEM of depressed part of pod surface showing conical hairs (80-150 µm).

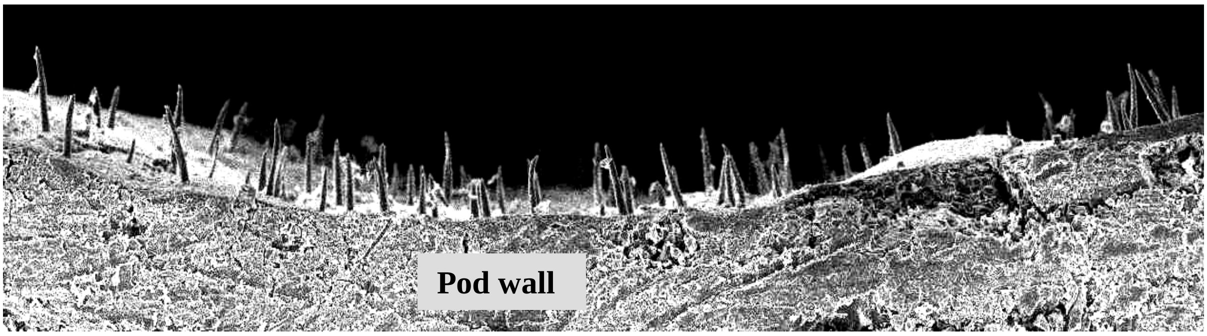

Fig. 3. Denser crop of trichomes in the depressed part of the pod surface.

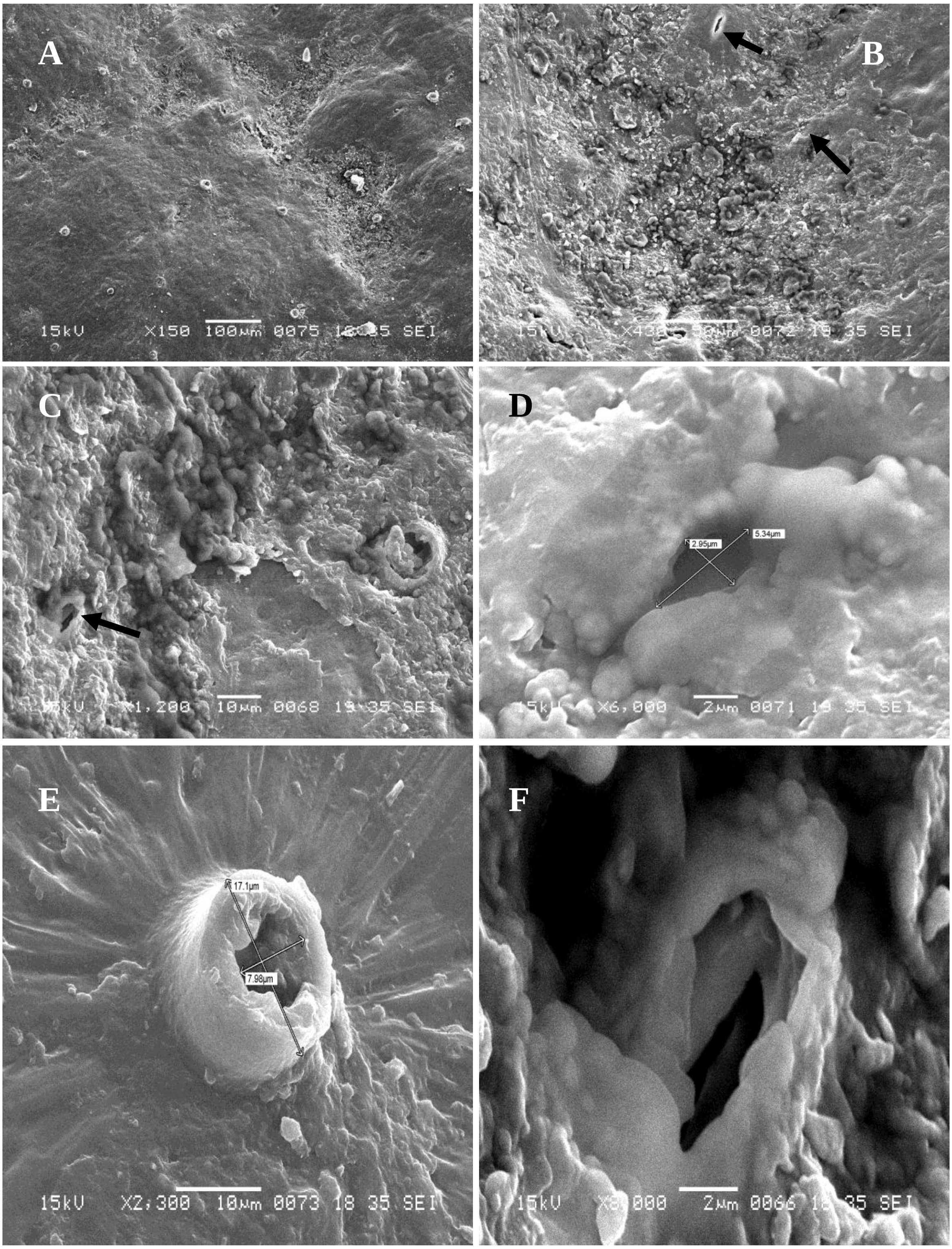

Fig. 4. SEMs of the dry pod surface of *Bauhinia racemosa*. Surface showing broken hair (A). There are several clogged stomata, only few open ones as indicated by the arrow in relatively rougher region of the dry pod (B and C). The stomatal pore admeasures 5.34 x 2.96 µm in size (6,600 X) (D). The basal remains of a trichome (E) in the depressed area of the pod (x 2,300) – inner diameter, 7.98µm and outer diameter, 17.1 µm. The stomata on outer wall of pericarp showing inner and outer rims of the aperture (F) and deposition of occluded material around (Magnification 8000X).

There were no stomata on the seed surface. In stead, seeds were foveolate with large number of small pits more or less regularly-equidistantly distributed on the seed surface (Fig. 9) and visible at very high magnification. The pits were very small, 3.4 x 2.7 μm in size and reaching inside the spermoderm. According to Ponomarenko and Pavlova (2003) stomatal character on seeds is an ancestral character. The absence of stomata on *B. racemosa* seeds indicated that it should be relatively more advanced species among Bauhinias.

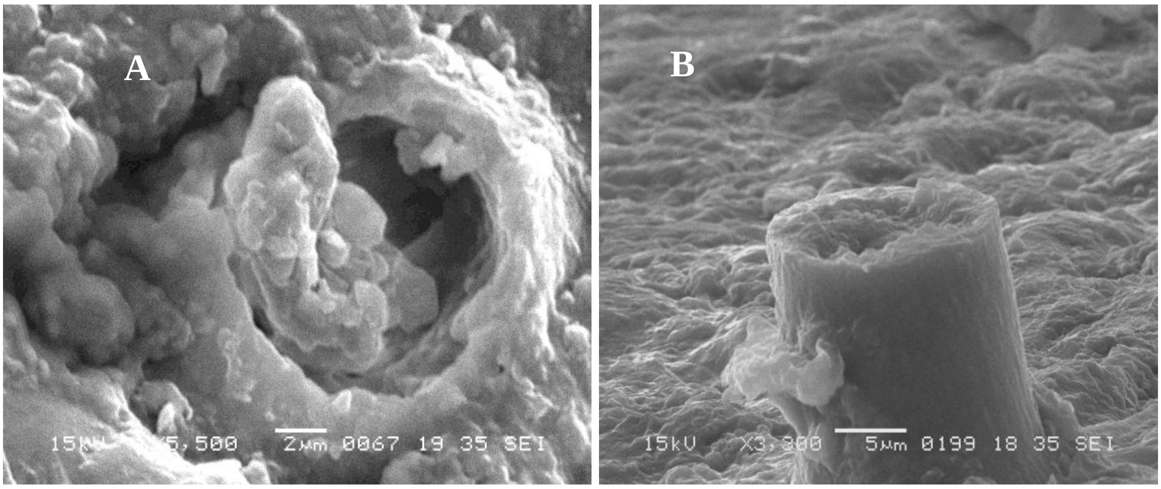

Fig. 5. A close up of a broken trichome (A, lumen showing occluded material (magnification 5,500 X) and (B, almost completely clogged lumen of the trichome with Occluded material, presumably lipid (3000 x).

The development of pod in *B. racemosa* appears to be a biphasic phenomenon. After fertilization, in the first phase pod (pod wall) grows rapidly and pod shows a strap like structure - twisted, thin and green and reaching the proportion of ripe pod but seed / ovule still small green structure around 1-2 mm in size (much lesser than the mature seed). When seed chambers are appropriately large in size, growth of seeds again takes place and they reach to their normal proportions. The developing seeds being green and photosynthesizing appear to contribute photosynthates to their development to an extent but major supply of nutrients appears to be available from pod wall, which as matures gradually, changes its colour from green to dark brown. In mature pods, however, still thick mesocarp persists. According to Sheoran and Singh (1987) pod wall (Mesocarp) being vascularized and chlorophyllous tissue fixes CO_2 in light during the early phase of seed development in *Cicer arietinum*. It contributes about 20% to seed dry matter and rest mass comes from foliar photosynthates or that contributed by seeds themselves. Like *B. racemosa*, inner wall of the pod wall of *C. arietinum* had no stomata (Sheoran and Singh, 1987). The inner layers of pea pods have been shown to be most active site for refixation of respired CO_2 (Flinn *et al*., 1977). Sheoran and Singh (1987) have shown that chickpea pod wall refixed $^{14}CO_2$ when fed through internal cavity more efficiently (90%) than seeds (10%). The mesocarp cells are metabolically more active as indicated by high amounts of starch, proteins and nucleic acid etc. present in the Mesocarp (Setia *et al*., 1987). Bennett *et al.* (2011) have shown that being photosynthetically active siliqua wall in Brassicaceae contributes assimilates and nutrients to fuel seed growth and it can, therefore, regulate the seed growth and maturation.

Setia et al. (1987) have reported pod wall to exhibit precocious growth in several legumes when compared with seeds. It acts as a major sink for reserves located in the pod wall which are broken down in mobilizable components by hydrolytic enzymes and translocated into seeds which are major sink during the late phase of pod development. According to Setia *et al.* (1987), pod wall has multiple functions – 1) contribute to photosynthetic pool and adds significantly to its reserve budget, 2) the inner wall layers fix CO_2 released in the pod cavity by respiring seeds and minimize CO_2 loss to atmosphere, 3) it acts as temporary sink for assimilates from leaves and other nutrients which are later translocated to seeds and 4) pod wall protects seed from environmental extremes, pests and pathogens.

It is to say that pod development in *B. racemosa* appears to be in two phases. First, it takes place in the pod wall till inflation phase and then in seeds during their later phase in development. The chlorophyll content in the leguminous pod wall is reported to be highest in very young pods, decreases gradually with development and becomes the minimum at maturity when the seeds attain maximum fresh weight (Atkins *et al.*, 1997; Setia and Malik, 1985). Throughout pod development, entire pod wall plays a very crucial role (Futbank *et al.*, 2006; Wang *et al.*, 2016).

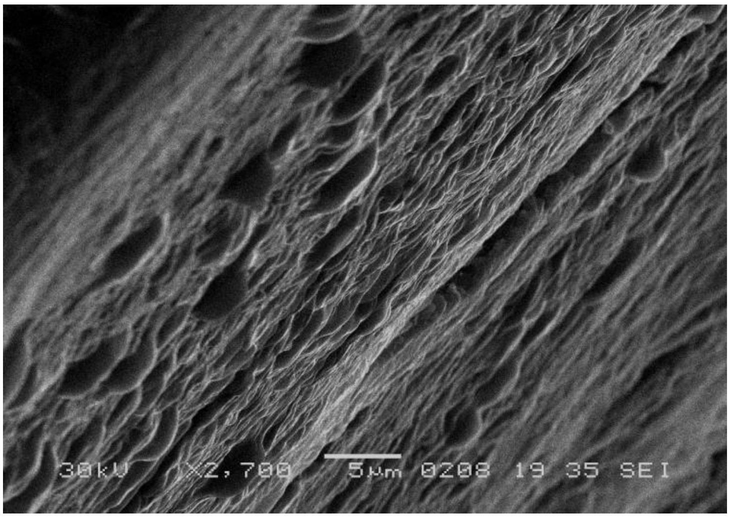

Fig. 6. Pod wall in LS – showing air spaces.

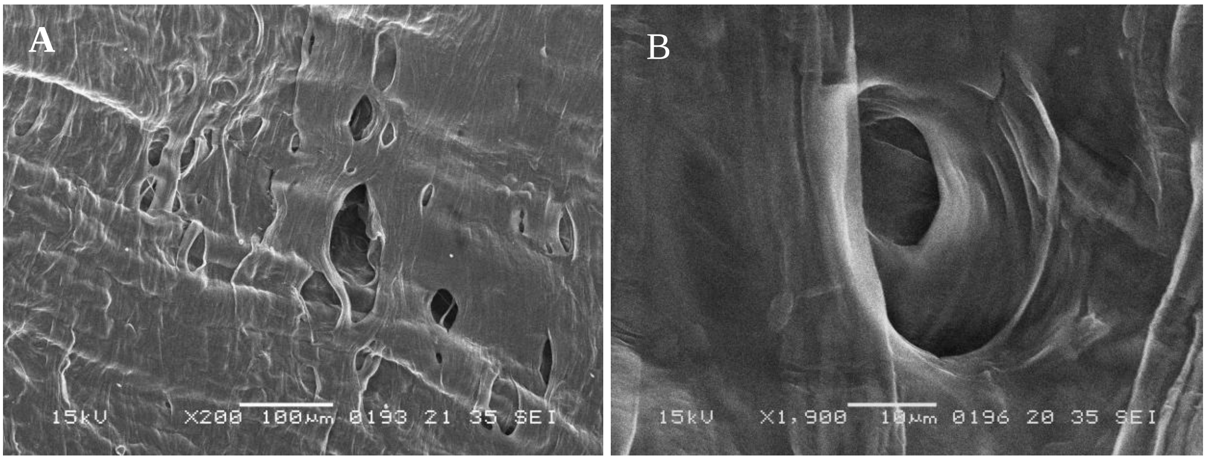

Fig. 7. A) SEMs of lining of seed chamber showing openings of several tunnels – presumably for gaseous exchange (200 X); B) One of the tunnel enlarged showing spatial passage of a tunnel (1900 X).

Fig. 8. Seeds of *Bauhinia racemosa*.

It is observed that there are no stomata on the inner epidermal lining of pod wall and seed integument in *B. racemosa*. It is, however, certain that being green the seeds photosynthesize during their development. Pod wall is also green and photosynthesize during pod development. It may be hypothesized that refixation of CO_2 as demonstrated in *Cicer arietinum* (Sheoran and Singh, 1987; Wang *et al.*, 2016) could also hold well in *B. racemosa*. There may be gaseous exchange between pod wall and the atmosphere around pod. The air spaces in the mesocarp of *B. racemosa* and tunnels ramifying in the mesocarp could provide gaseous passage between seed chamber and pod wall and stomata on the outer epidermis could participate in gaseous exchange between pod wall and the exterior environment as stomata on outer wall of pericarp should be functional (Ponomarenko and Pavlova, 2003). The pits on the seed surface may presumably facilitate gaseous exchange between developing seeds and the seed chamber. It is obvious that a great deal of research is needed to examine this hypothesis.

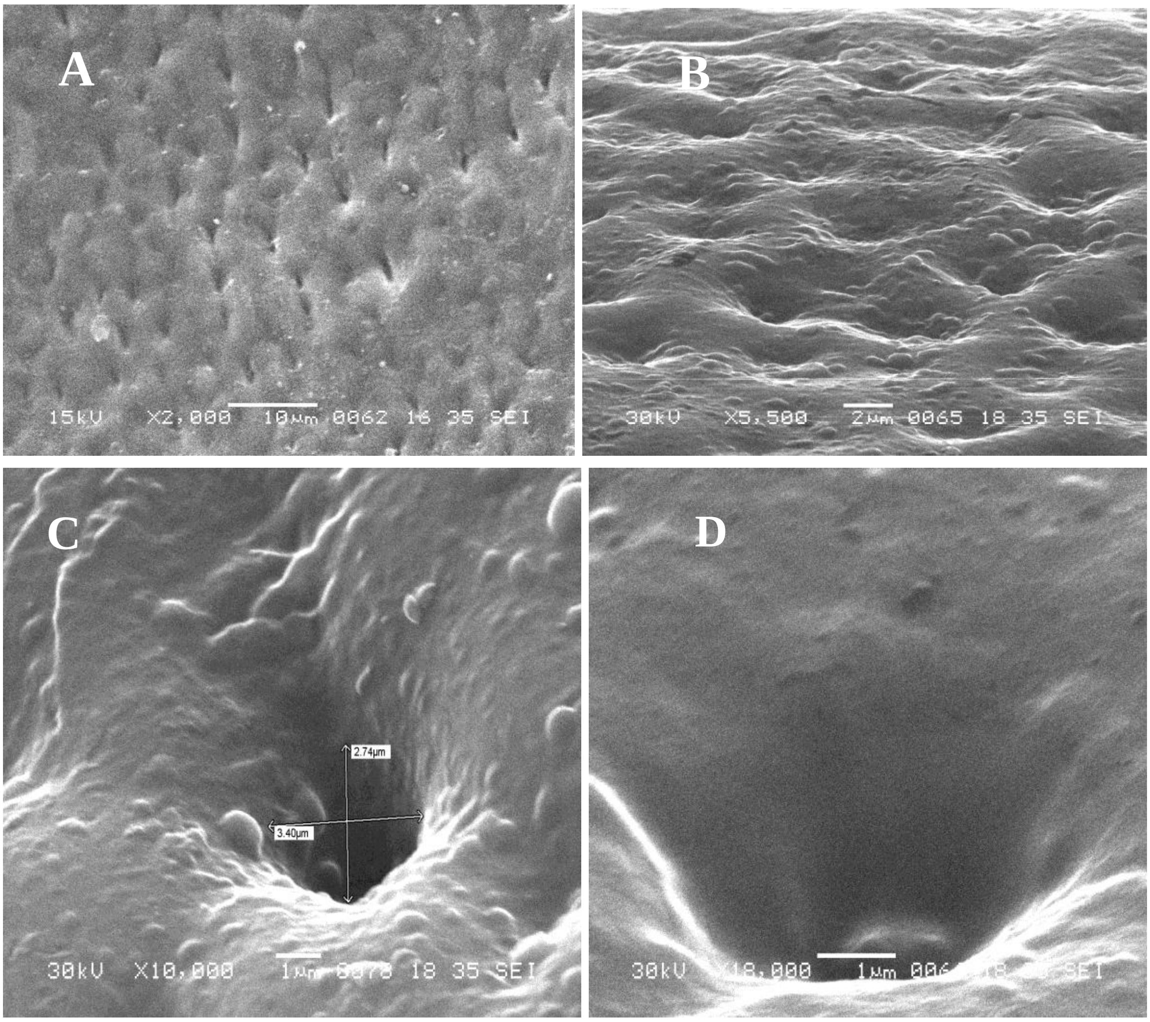

Fig. 9. SEM of seed surface of *Bauhinia racemosa*. A) Magnification 2,000 X; B) 5500 X; C) 10,000 X and D) 18,000 X. The pit size 2.74 x 3.40 µm. The droplet like structures is presumably the occluded material.

References

Ali, S.I. (1973). *Caesalpiniaceae.* Flora of West Pakistan. Fascicle # 54.

Atkins, C.A. and Flinn, M.A. (1978). Carbon dioxide fixation in the carbon economy of developing seeds of *Lupinus albus* (L.). *Plant Physiol.* 62: 486-490.

Atkins, C.A., Kuo, J. and Pate, J.S. (1977). Photosynthetic pod wall of pea (*Pisum sativum* L.). Distribution of carbon dioxide fixing enzymes in relation to pod structure. *Pl. Physiol*. 60: 779-786.

Bennett, E.J., Roberts, J.A. and Wagstaff, C. (2011). The role of the pod in seed development: strategies for manipulating yield. *New Phytol.* (4): 838-853.

Crookston, R.K., O'Toole, J. and Ozbun, J.L. (1974). Characterization of the bean pod as a photosynthetic organ. *Crop Sci.* 14: 708-712.

Flinn, A.M., Atkins C.A. and Pate, J.S. (1977). Significance of photosynthetic and respiratory exchanges in carbon economy of the developing pea fruit. *Pl. Physiol*. 60: 412-418.

Futbank, R.T., White, R., Palta, J.A. and Turner, N.C. (2006). Internal cycling of respiratory CO_2 in pods of chickpea (*Cicer arietinum*): the role of pod wall, seed coat and embryo. *J. Exp. Bot.* 55(403): 1687-1696.

Jernstedt, J.A. and Clark, C. (1979). Stomata on the fruits and seeds of *Eschscholzia* (Papaveraceae). *Am. J. Bot.* 66: 586-890.

Johri, B.M. (2012). *Embryology of Angiosperms.* Springer Science and Business Media. 834 Pp.

Khan, D., M.J. Zaki and M. Anis (2015). Seedling characteristics of Jhinjera (*Bauhinia racemosa* Lamk.). *Int. J. Biol. Biotech.* 12(1): 143-154.

Larsen, S.S. (1975). Pollen morphology of Thai species of *Bauhinia* (Caesalpiniaceae). *Grana* 14: 114-131.

Ma, Q., Behboudian, M.H., and Turner, L.C. (2001). Gas exchange by pods and subtending leaves and internal cycling of CO_2 by pods of chickpea (*Cicer arietinum* L.) subjected to water deficit. *J. Exp. Bot.* 52: 123-131.

Netolitzky, F. (1926). Anatomie der Angiospermen – Samen. In: K. Linsbauer (Ed.) Handbuch der Pfanyenantomie. Band X, Teil 2.

Paiva, A.S., Lemos-filho, J.P. and Oliveira, D.M.T. (2006). Imbibition of *Sweitenia macrophylla* (Meliaceae) seeds: the role of stomata. *Ann. Bot.* 98(1): 213-217.

Ponomarenko, S.F. and Pavlova, M.E. (2001). Seeds of genus *Bauhinia* L. (Fabaceae Lindl.) as a source for Galactomannan prosuction. IV. Mezhdunarodnyl simpozium: novye I netraditsionuye rasteniya I perspecktivy ikh ispolzovaniya (VI Int. Symp.: *New Alternative Plants and Outlooks of Their Use*”). Moscow – Pushchino. Vol 3. Pp. 572-574.

Ponomarenko, S.F. and Pavlova, M.E. (2003). Seed structure in the genus *Bauhinia* L. (Fam. Fabaceae Lindl.) in the context of taxonomy. *Biol. Bullet.* 30(4): 361-369.

Rajaram, N. and Janardham, K. (1991). Chemical composition and nutritional potential of the tribal pulses, *Bauhinia purpurea, B. racemosa* and *B. vahlii. J. Sci. of Food & Agriculture* 55 (3): 423-431.

Rugenstein, S.R. and Larsten N.R. (1981). Stomata on seeds and fruits of *Bauhinia* (Leguminosae: Caesalpiniaceae). *Am. J. Bot.* 68 (6): 873-876.

Sambo, E.Y., Moorby, J. and Milthorpe, F.L. (1977). Photosynthesis and respiration of developing soybean pods. *Aust. J. Pt. Physiol.* 4:713-721.

Setia, N. and Malik, C.P. (1985). Photosynthetic characterization of chickpea pod. In: Gohil, R.N. (Ed.): *Recent Trends in Botanical Research* (pp. 117-127). Scientific Publishers, Jodhpur, India.

Setia, R.C., Setia, N. and Malik, C.P. (1987). The pod wall structure and function in relation to seed development in some legumes. *Phyton* (Austria) 27(2): 205-220.

Shcherbukhin, V.D. and Anulov, O.A. (1999). Galactomannans of seeds of legumes (A Review). *Prikl. Biokhim. Microbiol.* 35(3): 257-274.

Sheoran, I.S. and Singh, R. (1987). Photosynthetic contribution of pod wall in seed development of chickpea (*Cicer arietinum* L.). *Proc. Indian Natn. Sci. Acad*. B53, No. 5 & 6: 531-534.

Sheoran, I.S., Singal, H.R. and Singh, R. (1987). Photosynthetic characteristics of chickpea (*Cicer arietinum* L.) pod wall during seed development. *Ind. J. Exp. Biol*. 25: 843-847.

Wang, H., Hou, L., Wang, M. and Mao, P. (2016). Contribution of the pod wall to seed grain filling in alfalfa. *Sci. Reports*. www.nature.com/scientific 6: 26586. DOI: 10.1038srep26586.

Werker, E. (1997). *Seed Anatomy*. Gebrüder Borntraeger, Berlin, Stuttgart. 424 Pp.

Yakovlev, G.P. (1991). Bobovyezemnogo Shara *(Legumes of the World),* Leningrad: Nauka.

Yücadaĝ, C. and Gültokin, H.C. (2011). The effect of sowing time on germination of twenty two leguminous seeds. *Afr. J. Agric. Res*. 6(16): 3809-3816.