Bangladesh J. Bot. **47**(2): 211-218, 2018 (June)

MORPHOLOGICAL AND ANATOMICAL FEATURES OF SEEDS OF SOME TAXA OF COSTACEAE

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Keywords: Costaceae, Exomorphic, Micromorphology, Aril, Seed anatomy, Microsite

Abstract

Exomorphic and anatomical characters of seeds of five species of Costaceae were examined using light and scanning electron microscopy. Seed anatomy was investigated to observe seed structural diversity as a taxonomic aid for the identification of taxa and to explain the adaptive characters for seed dispersal syndromes. Morphometric and micromorphological traits of seeds revealed considerable variation in size, shape and surface pattern. Among all the studied taxa, the taxonomic placement of *Tapeinochilos ananassae* was uncertain. But the micromorphological characters such as striate round dotted seed surface with tuberculate aril surface and areolate aril structure separate the *Tapeinochilos ananassae* from other taxa. Micromorphological and anatomical characters support the taxonomical delimitation of five species within the family Costaceae and are significant to analyze the aril structure in detail with implications on seed dispersal syndrome.

Introduction

Costaceae, the most recognizable crown group of the order Zingiberales (Specht *et al*. 2001) with species diversity is centered in South and Central America, Africa and Southeast Asia. Within Costaceae, the placement of *Tapeinochilos* with respect to other genera has been a source of debate in the taxonomic literature (Kress *et al*. 2001). Ants and birds are recognized as seed dispersers in Costaceae (Schemske 1983). There are no reports of seed dispersal in *Hellenia speciosa* so far. Moreover, detailed morphological and anatomical studies of seeds are limited in Costaceae.

Investigation on the micromorphological and anatomical characters of seeds of *Costus dubius* (Afzel.) K. Schum., *Costus laevis* Ruiz & Pav, *Costus woodsonii* Mass, *Hellenia speciosa* (J. Koenig *ex* Smith) S. Dutta (= *Cheilocostus speciosus* (J. Koenig) C.D. Specht) and *Tapeinochilos ananassae* (Hassk.) K. Schum. was made as a support for the taxonomic identity of five examined species within Costaceae and to evaluate the diversity of aril structure in detail with regard to seed dispersal syndrome.

Materials and Methods

The seeds of species were collected from natural populations of Athirapilly forest sites (10°18′31″N, 76°28′22″E) and Calicut University Botanical Garden (11°25′45″N, 75°45′50″E). Morphology and morphometry of seeds (n = 125) of five species were studied by stereo-microscopic illustrations and photomicrographic images using LEICA M80 stereomicroscope. Micromorphological parameters such as surface pattern, anticlinal cells, and periclinal cells were studied by scanning electron microscopy. For scanning electron microscopy, freshly collected seeds were dehydrated in alcohol series and mounted on a stub which sputters coated with gold

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for 80 sec. Scanning electron micrograph of seeds was examined using JEOL JSM-6390LA Analytical Scanning Electron Microscope.

For anatomical study, freshly collected seeds were hand sectioned using a commercial double edge razor blade. The sections were stained with safranin and toluidine blue for 5 - 10 min. Excess stain was removed by washing the stained sections in water. Finally the sections were mounted in microscopic slide with glycerine using a cover slip. Observations of anatomical preparations were done by Zeiss Axiolab A1 Phase Contrast Microscope. Seed dispersal syndrome was noticed in Konnakuzhy forest sites and areas of Calicut University Botanical Garden. Voucher specimens were deposited at Calicut University Herbarium (CALI).

The terminologies for seed morphology were based on the descriptions used by Barthlott (1981) and Koul *et al*. (2000). Anatomical terminologies provided by Werker (1977) were followed for seed anatomy. Statistical tests (mean ± standard error) on seed size, aril size and cell size of exotesta, mesotesta, endotesta, perisperm and endosperm were applied to assess the variation and accuracy of observations using IBM SPSS vs 20 software (USA).

Results and Discussion

Seeds of *Costus dubius* were glossy black, ellipsoid, 2.89 ± 0.07 × 1.70 ± 0.25 mm long with exserted orbicular operculum with punctate surface and circular hilum. Surface was striate with linear discontinuous anticlinal walls. Aril was white, lacerate wide, 7.30 ± 0.24 × 1.90 ± 0.10 mm long with verrucate surface (Fig. 1A-D). Seed anatomy revealed single-layered exotesta and mesotesta with rectangular cells. Endotesta with small rounded scattered sclerenchymatous cells were present. Perisperm was single-layered with elongated irregular shaped cells. Endosperm was multi-layered with broad irregular shaped cells. Aril cells were linear and elongated (Fig. 2A-F, Table 1).

Table 1. A comparison of morphometric characters of seeds.

Taxa	Aril cell size (µm)	Exotesta cell size (µm)	Mesotesta cell size (µm)	Endotesta cell size (µm)	Perisperm cell size (µm)	Endosperm cell size (µm)
C. dubius	1623.22±161.37 × 55.00±1.44	65.29±4.05 × 32.42±2.12	46.93±2.81 × 51.72±2.92	2.19±0.15 × 2.02±0.14	73.84±4.19 × 30.56±2.12	41.76±3.36 × 13.76±1.09
C. laevis	521.50±69.60 × 40.50±3.68	34.02±1.58 × 19.53±0.35(OE) 26.49±2.95 × 15.76±0.94(ME) 24.72±1.84 × 16.21±0.81(IE)	43.21±1.81 × 38.80±0.70	−	56.01±2.90 × 26.84±1.35	56.94±5.46 × 37.60±4.04
C. woodsonii	238.50±20.90 × 50.75±3.49	37.61±1.46 × 28.06±1.83	31.02±0.36 × 25.62±2.01	1.72±0.18 × 1.29±0.11	78.51±3.70 × 20.07±1.79	60.80±4.53 × 29.12±2.55
H. speciosa	57.10±15.55 × 25.30±5.50	25.97±1.27 × 23.93±0.71(OE) 09.94±1.47 × 22.44±0.43(IE)	49.85±3.53 × 38.45±4.66	7.42±1.13 × 5.10±0.62	76.03±10.51 × 29.07±2.39	69.26±5.21 × 43.38±3.70
T. ananassae	39.76±5.44 × 15.86±0.97	19.83±1.17 × 13.52±0.47	42.79±0.93 × 26.21±1.56	3.11±0.24 × 3.03±0.26	69.72±5.53 × 24.49±1.45	64.94±5.19 × 30.69±2.74

OE = Outer Exotesta; ME = Middle Exotesta; IE = Inner Exotesta.

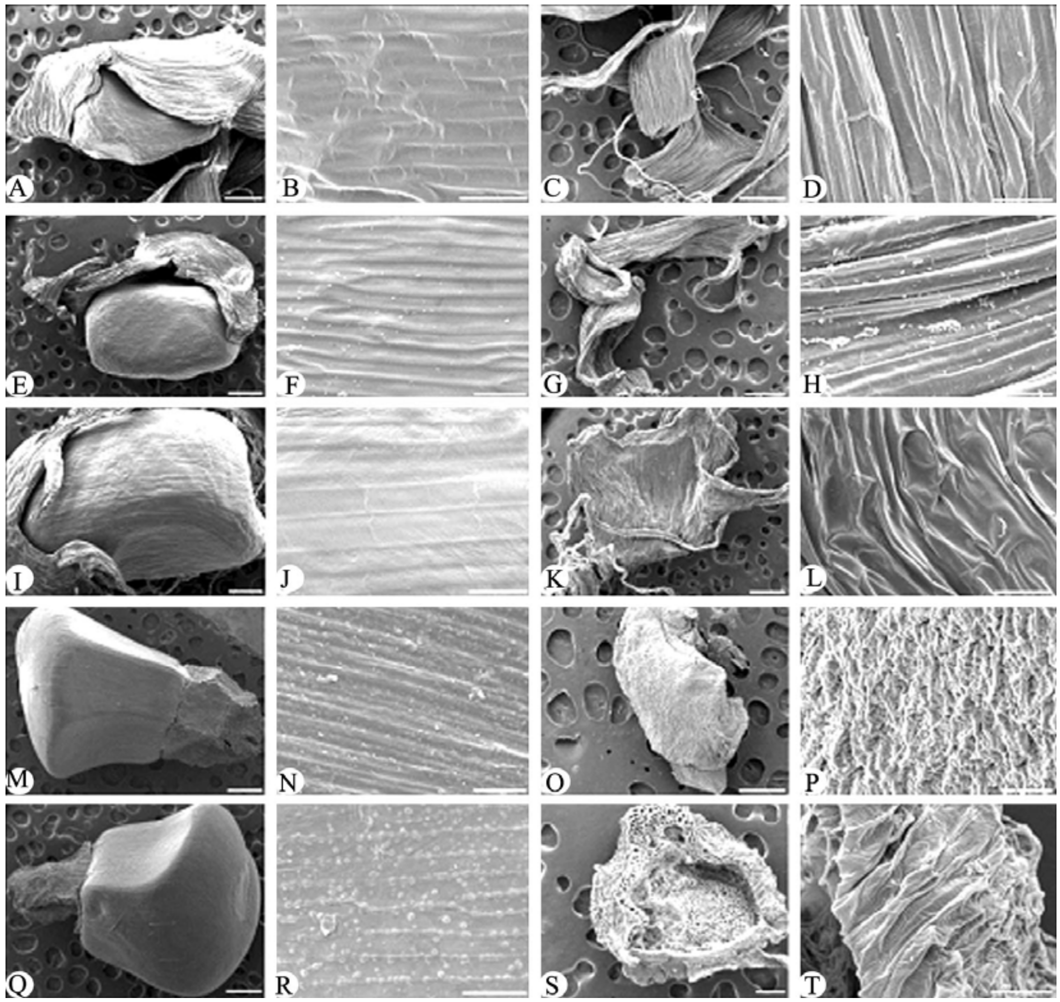

Fig. 1. SEM analysis of seed micromorphology of Costaceae. A - D: *C. dubius*, E - H: *C. laevis*, I - L: *C. woodsonii*, M - P: *H. speciosa* and Q - T: *T. ananassae*. Seed entire (A, E, I, M, Q). Seed surface pattern (B, F, J, N, R). Aril entire (C, G, K, O, S). Aril surface pattern (D, H, L, P, T). Scale bars: A, E, I, M, Q, C, G, K, O = 500 µm. S = 200 µm. L = 100 µm. B, D, F, H, J, N, P, R, T = 50 µm.

Costus laevis showed cuboid glossy black seeds, 2.70 ± 0.07 × 1.80 ± 0.02 mm long with exserted orbicular operculum with punctate surface and circular hilum. Surface was striate with linear continuous septate anticlinal wall. Aril was white, lacerate wide, 4.50 ± 0.29 × 1.60 ± 0.90 mm long with verrucate surface. (Fig. 1E-H). Seed anatomy showed multi-layered exotesta with outer exotesta of elongated rectangular cells, middle exotesta and inner exotesta with small irregular shaped cells. Mesotesta was single-layered with rectangular cell. Endotesta was absent. Perisperm was single-layered with elongated irregular shaped cells. Endosperm was multi-layered with broad irregular shaped cells. Aril cells were linear, septate and elongated (Fig. 2G-L, Table 1).

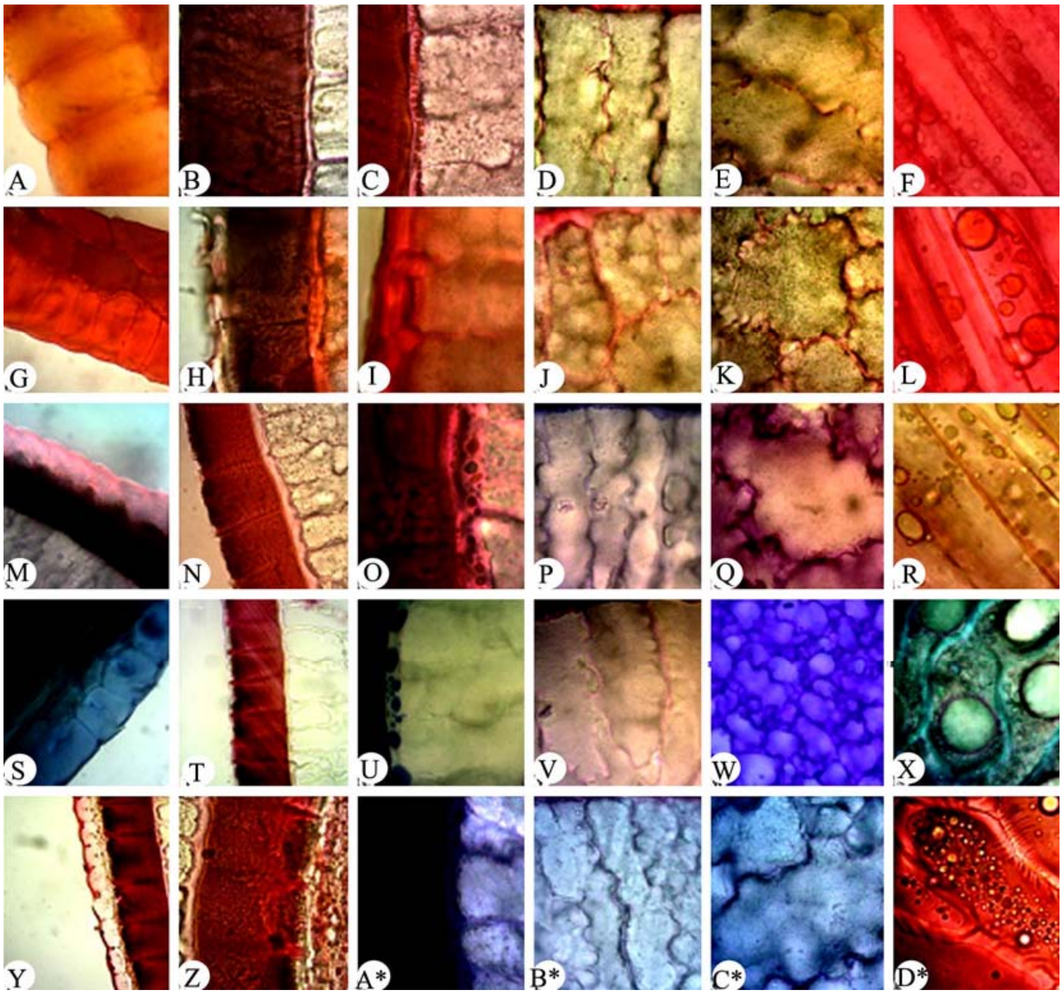

Fig. 2. Seed anatomy of Costaceae. A - F: *C. dubius*, G - L: *C. laevis*, M - R: *C. woodsonii*, S - X: *H. speciosa* and Y - D*: *T. ananassae*. Exotesta (A, G, M, S, Y). Mesotesta (B, H, N, T, Z). Endotesta (C, I, O, U, A*). Perisperm cell (D, J, P, V, B*). Endosperm cell (E, K, Q, W, C*). Aril cell (F, L, R, X, D*). Scale bars: M, L = 200 µm. W, F = 50 µm. R, T, Y, G = 20 µm. A, B, C, J, K, N, O, S, V, X, Z, B*, C*, D* = 10 µm. D, E, H, I, P, Q, U, A* = 5 µm.

Seeds of *C. woodsonii* were glossy black, broadly oblong, 4.15 ± 0.07 × 2.50 ± 0.10 mm long with inserted orbicular operculum with punctate surface and circular hilum. Surface was striate with linear continuous septate anticlinal wall. Aril was white, lacerate veil, 6.10 ± 0.34 × 2.90 ± 0.10 mm long with rugose surface (Fig. 1I-L). Seed anatomy revealed double-layered exotesta with outer rectangular cells and inner irregular shaped cells. Mesotesta was single-layered with rectangular cell. Endotesta was sclerenchymatous with small round cells. Perisperm was single-layered with elongated irregular shaped cells. Endosperm was multi-layered with broad irregular shaped cells. Aril cells were linear, septate and elongated (Fig. 2M-R, Table 1). *Hellenia speciosa* exhibited glossy black angular seeds, 3.55 ± 0.20 × 2.90 ± 0.10 mm long with prominent inserted orbicular operculum with punctate surface and circular hilum. Surface was striate with linear anticlinal wall. Aril was white, cushion-type, 6.10 ± 0.34 × 2.90 ± 0.10 mm long with tuberculate surface (Fig. 1M-P). Seed anatomy exhibited double-layered exotesta with outer exotesta of broad rectangular cells and inner exotesta with small irregular shaped cells. Mesotesta was single-layered with rectangular cells. Endotesta with small round to elliptic sclerenchymatous cells were present. Perisperm was single-layered with broad to elongated irregular shaped cells. Endosperm was multi-layered with broad irregular shaped cells. Aril cells were areolate with prominent nucleus (Fig. 2S-X, Table 1).

In *T. ananassae*, seeds were glossy black, angular, 2.60 ± 0.15 × 2.30 ± 0.11 mm long with flat operculum with punctuate surface and ovular hilum. Surface was striate and round dotted with continuous septate anticlinal wall. Aril was white, cushion-type, 1.80 ± 0.14 × 1.65 ± 0.15 mm long with tuberculate surface (1Q-T). Seed anatomy explained single-layered exotesta with broad rectangular cells and single-layered mesotesta consists of rectangular cell. Endotesta with small round sclerenchymatous cells were present. Perisperm was single-layered with elongated irregular shaped cells. Endosperm was multi-layered with broad cells. Aril cells were areolate (Fig. 2Y-D*, Table 1).

Comparative micromorphological studies have always been regarded as crucial to plant systematics which pursuit to explain plant diversity, phylogeny and evolution. Surface microstructure is especially diverse in reproductive parts such as petals, pollen, seeds, stigmas and other secretory surfaces (Endress *et al*. 2000) and often adapted for specialized pollination syndrome and seed dispersal mode. In the present study, the aril and seed coat showed much variation among species both morphologically and anatomically. Presence of oil drops in aril cells of all studied species is assumed to be an evolved mechanism for seed dispersal by ants.

Functionally seed dispersal is recognized as a fundamental process for building and sustaining species diversity and spatial patterns in plant communities (Carlo *et al*. 2005). More specifically seed dispersal by ants has evolved as the repeated evolution of plant-animal mutualisms and provides the seed with protection from seed predators, a safe place for seed survival during unfavourable periods and a microsite that is rich in nutrients (Lengyel *et al*. 2010).

Among all studied taxa, except *Tapeinochilos ananassae* different ant species were meant for seed dispersal. In *C. dubius*, *Ocecophylla smaragdina* which transport the entire seed in several meters making nest called bivouac and storing lipid rich appendages along with seed in the leaf nest cavity. The other behaviour was the storage of seed in the gap junctions of trees' nest cavity. The consumption of aril occurs whenever the energy necessity occurs. In *C. laevis*, *Anoplolepis gracilipes* seed is dispersed (Fig. 3C) directly in mud and also in leaf nest cavity. The veil-type aril was directly consumed by *Ocecophylla smaragdina* in *C. woodsonii*. The mechanical forces applied during this consumption help the species for seed dispersal directly from dehisced fruit to the mud. Later no dispersal syndrome was noticed. In *H. speciosa* two types behaviour were observed. In open areas the aril was directly consumed by *Camponotus irritans* and later on the seeds were dispersed by mechanical force of seed dispersers. In forest sites, the seeds were primarily dispersed by wind and secondarily by the ant *Odontomachus* sp. (Fig. 3F) which transports seed into the nest in mud. No dispersal syndrome was noticed in *T. ananassae* that show indehiscent capsular fruit. Earlier study by Schemske (1983) reported that birds were recognized for seed dispersal in *C. laevis*.

However, the present results emphasize that the arils in studied taxa are evolved convergently with regard to myrmecochorous syndrome. Lipid bodies also observed in the inner endosperm, perisperm and embryonic cells are most probably meant for the developmental stages of seed. In the present work, all seeds of the studied taxa were arillate. Tomlinson (1956) mentioned the importance of aril characters and stated that aril character alone can be used for the separation of family Costaceae from Zingiberaceae. Among all studied taxa *T. ananassae* occupies a distinct micromorphological and anatomical seed characters which ensure taxonomic identity of *T. ananassae* as a separate species than the other species studied.

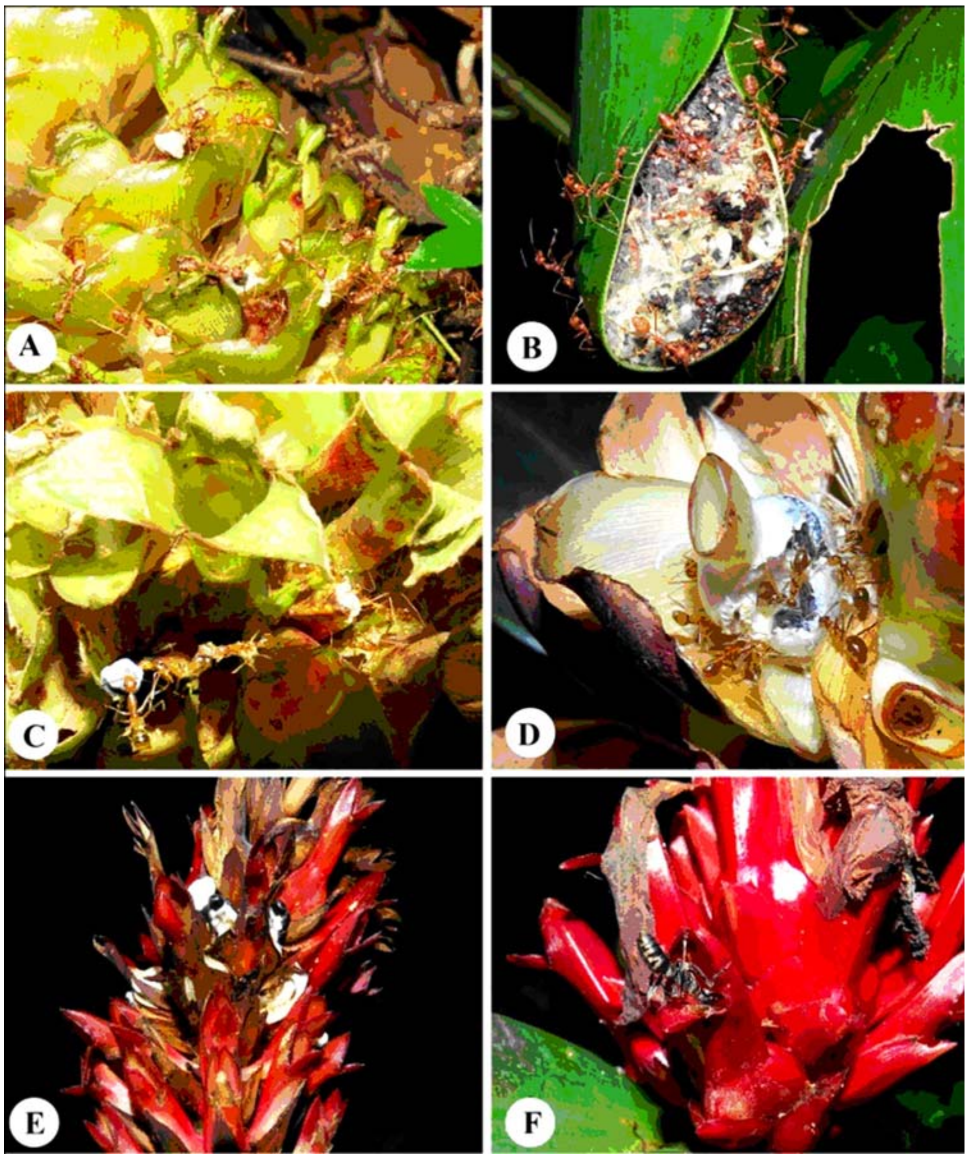

Fig. 3. Seed dispersal in examined taxa of Costaceae. A - B: *Ocecophylla smaragdina* in *C. dubius*, C: *Anoplolepis gracilipes* in *C. laevis*, D. *Ocecophylla smaragdina* in *C. woodsonii*, E: *Camponotus irritans* in *H. speciosa* and F. *Odontomachus* sp. in *H. speciosa*.

Key to the species of examined Costaceae based on morphological and anatomical characteristics

1a.	Aril lacerate wide type	2
1b.	Aril lacerate veil type	*C. woodsonii*
2a.	Endotesta sclerenchymatous	3
2b.	Endotesta absent	*C. laevis*
3a.	Seeds angular	4
3b.	Seeds ellipsoid	*C. dubius*
4a.	Seeds surface striate with linear anticlinal wall and aril cell is areolate with prominent nucleus	*H. speciosa*
4b.	Seeds surface striate and round dotted with continuous septate anticlinal wall and aril cell is areolate without prominent nucleus	*T. ananassae*

The present study supports a better understanding that the morphological and anatomical characteristics of seeds are useful tool for species recognition within Costaceae. In the current work, the seeds of five species of Costaceae showed distinct variation in morphological and anatomical characters and are relevant for the taxonomic identity of species, in particular *T. ananassae* within the family Costaceae. Detailed anatomical study of seed specially aril anatomy revealed the presence of oil drops in aril cell and regarded as a co-evolved trait for myrmecochory. But different ant species were recognized for seed dispersal syndrome in all species. But in *C. laevis* which share both bird and ant seed dispersal syndrome that seeks the detailed analysis of aril chemistry with regard to specialized seed dispersers.

Acknowledgements

The authors are thankful to Dr. Santhosh Shreevihar, Consultant Entomologist, Prof. TCN Trust for Animal Taxonomy, Calicut for identification of the ant species. The work was supported by Kerala State Council for Science, Technology and Environment (KSCSTE) by providing necessary funds for research work. (Order No. 402/2015/KSCSTE dated 18.08.2015),

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(Manuscript received on 17 March, 2017; revised on 22 December, 2017)