Application of Scanning Electron Microscopy Unravelling the Seed Coat Micro Morphology and FESEM-EDAX Characterization of Selected Species of the Genus *Rotala* L.(Lythraceae) in India

Suvarnika Vellamthatta[1], Raji. R Varangumchuvattil[2] & Binu Thomas[3]

[1]Govt. College, Vidyanagar, Kasaragod (Affiliated to Kannur University) , Kerala, India

[2]St. Mary's College, Sulthan Bathery (Affiliated to the University of Calicut), Wayanad, Kerala, India

[3]Department of Botany, St. Joseph's College, Devagiri (Autonomus), Kozhikkode (Affiliated to the University of Calicut), Kerala, India

DOI: https://doi.org/10.51244/IJRSI.2026.1306000315

Received: 12 June 2026; Accepted: 18 June 2026; Published: 09 July 2026

ABSTRACT

The taxonomic importance of seed coat micromorphological and macromorphological traits among 10 species of the genera Rotala, was investigated by using Scanning Electron Microscope (SEM). The study aimed to identify unique ultra-structural characteristics of seed coats that would be useful for identification. helping to accurately determine and clarify their taxonomic position. This study explored the ultrastructure and micromorphological diversity among the examined genera, Morphological characters, including colour, texture, shape, hilum position, anticlinal and periclinal shape of seed coat were investigated. The highest seed length/width ratio was observed in *R. occultoflora*, 2.576 mm, whereas the lowest seed length/width ratio was found in *R. malabarica*, 1.154 mm.The most prominent seed coat sculpture is reticulate type. Hilum tip is acute in all studied genera except *R, kasaragodensis* where it is round in shape,These characteristics proved valuable for distinguishing species within the studied genera and provided strong taxonomic insights. Our findings highlight that SEM-based examination of seed coat micromorphology can serve as a reliable tool for identifying plant taxa at both genus and species levels, offering potential for new taxonomic discoveries.

Keywords: Identification; India; Lythraceae; Morphology; Rotala; Scanning Electron Microscope; Seed Coat

INTRODUCTION

The genus *Rotala* L. (Lythraceae) is distributed tropically and subtropically. It is represented globally by 44 species (Mabberley, 2005), with the highest distribution in tropical Asia (Cook, 1979). After Cook's revision, it is represented, as per the revised estimate, in India by 29 species, of which 24 are from Peninsular India (Joseph &Sivar 1988, 1989; Pradcep & al, 1990;Mathew & Lekshminarasimhan, 1 990;Yadav & al.2010; Prasad & al. 2012; Gaikwad & al. 2013; Prasad & Raveendran 2013 a, b; Sunil & al. 2013; Anto & al. 2014; Ratheesh Narayanan & al. 2014; Lemiya & Pradeep 2015; Prasanth & Sardesai 2022. Species of the genus Rotala are grouped under two categories, based on the nature of their habitat, viz., obligate aquatics, which grow in shallow water, and semi aquatics or terrestrials, which thrive in marshy lands. Most of the aquatic species are characterized by what is called 'Hippuris syndrome' (Cook 1978): erect, unbranched stems with simple, elongate leaves borne in symmetrical whorls, "heterophylly manifesting itself as variation in some leaves in each whorl and individual leaf shape and size." Among the Indian species, this syndrome is displayed by *R. verticillaris* L., *R. mexicana* (only where it grows as an emergent aquatic), *R. cookie* K, T. Joseph and *R, vasudevanii* K,T, Joseph & Sivar. Most other species have deccussate leaves, while *R. floribunda* (Wight) Koehne has alternate ones, and *R. occultiflora* has leaves disposed in whorls of 3. Leaf and flower arrangements have been taken as an important taxonomic character in this genus by many authors. Koehne

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VI June 2026

(1903) divided the genus into two sections based on these characters: Sect. Hippiuridum with whorled/alternate leaves and flowers,and Sect. Enantiorotala with decussate leaves and flowers, but species like *R. mexicana*. and *R. myriophylloides* Welw, ex Hierndisplay that both their characteristics depending upon whether they are growing as aquatics or terrestrials. Consequently,Cook (1978, 1979) considered these characters to be direct responses to the aquatic environment and that these Hippurismimics' do not constitute a single phyletic group. Cook (1979) has also found that the genus is very uniform and does not yield a satisfactory subgeneric classification into natural subgeneric groups. Seed coat characteristics have been successfully employed to solve taxonomic problems and elucidate phylogenetic relationships. Seed micromorphological studies using FESEM and EDAX have proven to be powerful tools for investigating features of taxonomic significance. Seed micromorphologicål characters are conserved and stable and thus used to solve many taxonomic ambiguities. Anatomical and seed micromorphological features can be used in taxonomic analysis for the identification of plants and establishing genetic relationships to solve taxonomic disputes.

The genus Rotala includes annual herbaceous plants that inhabit permanent and seasonal lateritic pools, depressions of lateritic rocks with rich humus deposits, paddy fields, and marshy areas. Leaves are alternate, opposite, or whorled. It may be petiolate or sessile, lanceolate or narrow needle-like, or bimucronate at apex, margins are entire, and the colour ranges from bright green to reddish, depends on light intensity and nutrient availability in the habitat Seeds are oval, spherical or ellipsoidal. Flowers are bracteate and bracteolate, with calyx lobes 3-5, small, corolla lobes 3-5 or absent in some, and nectar scales are seen in most species. Stamens 3-5, inserted at the base of the calyx tube. Ovary ellipsoid, style short, stigma capitate, capsule globose, seeds ovoid, brownish, 10-15 per fruit. The seed micromorphological features of *Rotala* have not been fully explored so far; hence, the present study mainly focused on the seed coat characterization or selected species of *Rotala* from India to fill this gap.

METHODOLOGY

COLLECTION OF SAMPLES

Rotala species were collected from various locations in Kerala, for seed morphological studies from June 2024 to January 2026 (Table l). The plant taxa selected for the present study include *R. cheruchakkiensis densiflora*, *R. indica, R. kasaragodensis, R. macrandra, R. malabarica, R. malampuzhensis, R. meenkulamensis, R. mexicana and R. occultiflora*. Identification of selected taxa was done by observing their morphological characteristics with the help of available floras and literature and it was also compared with authenticated herbarium specimens deposited in the herbarium of University of Calicut (CALI), the voucher specimens were also deposited in the herbarium of St. Joseph's College (Autonomous), Devagiri, Calicut (DEV) for future reference. Fresh plant specimens were preserved in formalin and FAA for further morphological characterization.

The plant species investigated in this study were collected from their natural habitats within the study region, based on the availability of material. To obtain seeds, mature seeds of *Rotala* plants were removed from fruits, collected during field visits, in the fruiting season, Seed debris were removed by treatment with distilled water, followed by air drying. A detailed list of the plant species studied, along with their collection sites and voucher numbers, is provided in Table l . In total, 10 species of *Rotala* were investigated. From each species, ten seeds were analyzed and evaluated using the most employed morphological descriptors (Table 2).

LIGHT MICROSCOPIC ANALYSIS OF SEEDS

Mature seeds of plants were repeatedly washed with ethanol to dissolve waxes, debris and also to sterilize the surface. External colour, shape, size (length and width), and hilum were observed by using a stereo microscope (Olympus SZX2-ALLT) equipped with a Magnus Magcam DC 10 camera., placed in Plant Anatomy Lab, Department of Botany, Govt. College, Vidyanagar, Kasaragod, Kerala, India, Seed shape was identified by Seed Identification Manual (Alexander C. Martin & William D. Barkley, 1961).

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VI June 2026

SCANNING ELECTRON MICROSCOPIC ANALYSIS OF SEEDS

Seed samples were prepared for the SEM study following the protocol of Usma ct al, (2019). using SEM (Model JEOL/FE, VERSION 4, JSM IT 800) ornamentation of seed has been observed. Collected seeds were separated from the plant immediately on stubs by using tape. These samples were then sputter-coated with gold—palladium and examined by SEM. The examined specimens were identified and snapped by Polaroid P/N 665 film, The diagnostic features of seed coat such as seed shape and surface, testa features (ie, epidermal cells and cell wall pattern) were described according to the terminologies of (Barthlott, 1981 In all cases, seeds of 10 samples of each species were analyzed.

ANALYTICAL TECHNIQUES FOR FESEM AND EDAX ANALYSIS

Field Emission Scanning Electron Microscopy (FESEM) is employed to explore the micro structural characteristics of *Rotala* species in conjunction with Energy Dispersive X-ray Analysis (EDAX) to delineate the elemental composition of its seeds. The imaging provided by FESEM facilitates high-resolution seed coat morphology; whereas the EDAX analysis permits the identification of critical minerals. Through this comprehensive methodology, we aspire to elucidate the structural and elemental features that are fundamental to the plant's ecological resilience and pharmacological capabilities, thereby offering significant contributions to the fields of conservation biology.

CHEMICALS AND SAMPLE PREPARATION

In this investigation, a diverse array of chemical agents was employed to prepare seed samples of *Rotala* species for Field Emission Scanning Electron Microscopy (FESEM) and Energy Dispersive X-ray Spectroscopy (EDAX) analyses, thereby enhancing the clarity and resolution of specific structural and elemental features,

SPUTTER-COATING TECHNIQUE

To facilitate the preparation of samples for FESEM analysis, a sputter-coating technique was implemented, which necessitated the uniform deposition of a thin layer of gold or platinum onto the surfaces of the samples. This conductive coating effectively mitigated charging artifacts and enhanced image quality under high vacuum conditions, thereby enabling precise visualization of surface characteristics such as trichomes and cellular textures at the microscopic scale. Regarding EDAX profiling, no supplementary chemicals were directly introduced to the samples, as this technique is predicated on the detection of characteristic X-rays emitted from the elemental constituents of the samples when subjected to an electron beam bombardment. This analytical approach permitted a non-destructive evaluation of vital minerals including carbon (C), oxygen (O), calcium (Ca), magnesium (Mg), potassium and trace metals, which are pivotal for elucidating the elemental composition of seed coats of *Rotala* species.The integration of these chemical preparations and analytical methodologies facilitated a holistic understanding of the seed coat surface and elemental characteristics of the plant, bolstering insights into its prospective medicinal and ecological adaptations.ln the current study, seed coat morphology and FESEM-EDAX of ten seeds of *Rotala* species was studied, *R. cheruchakkiensis, R. densiflora, R. indica, R. kasaragodensis,R. macrandra, R. malabarica,R. malampuzhensis, R. meenkulamensis, R. mexicana and R. occultiflora* (Table I).lmages of studied *Rotala* species are shown in Fig. 1&Fig. 2

STATISTICAL ANALYSIS

MORPHOMETRICS

Statistical research based on the seed morphological traits of the studied taxa was performed by morphometric methods traits, including seed color, shape, sculpture, hilum shape, seed length, seed width seed L/W ratio, trichomes was performed. Five measurements were made of each seed's width and length, The length-to-width ratio is calculated using the L/W formula, where L is the seed length W, is its width (Table. 2).

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VI June 2026

Table l . Collecting data on the Rotala species selected for the seed coat micromorphology and FESEM-EDAX studies.

No.	Taxa	Habitat	Geographical coordinates	Details of plant specimens studied
	R. cheruchakkiensis Anto P.V., Devikrishna C.S,, Jacob Abraham Pulickal. Varghese C. D, and Ignatius Antony	wet lateritic soil	12º28′87″ N 75º23′80″ E	Karinthalam, Kasaragod District. Kerala State, Suvarnika.V., 10409, DEV, 17/07/2024
2	*R. densiflora* (Roth ex Roem. &Schult.) Koehne	Dry paddy field	11º 84′67″ N 76º06′31″ E	Kattikkulam, Wayanad District, Kerala State, Suvarnika.V.,10406,DEV, 10/01/2024
3	*R. indica* (Willd.) Koehne	Wet paddy field	12º22'35" N 75º04′28″ E	Thannott, Ravaneshwar, Kasaragod District, Kerala State, Suvarnika.V.,10410,DEV,13/11/2025
4	*R. kasaragodensis* K.S. Prasad & Raveendran	Seasonal lateritic pool	12^0 14'25"N 75^0 13'55 $^{i'}$ E	Velichamthodu,Cheemeni,Kasaragod District, Kerala State, Suvarnika.V.,10403,DEV,13/09/2024
5	*R. macrandra* Koehne	Wet paddy field	12º39′69″ N 75º39′69″ E	Vellamthatta, Ravaneshwar,Kasaragod District' Kerala State, Suvarnika.V., 10408, DEV, 06/01/2025
6	*R. malabarica* Pradeep, K.T. Joseph &Sivar.	Seasonal lateritic pool	12º28′87″ N 75º23′80″ E	Karinthalam, Kasaragod District, Kerala State, Suvarnika.V.,10404,DEV,10/10/2024
7	*R. malampuzhensis* R.V. Nåir ex C.D.K. Cook	Seasonal lateritic pool	1226'49"N 75^0 18'37" E	Kayyur, Kasaragod District, Kerala State, Suvarnika.V., 10402, DEV, 25/09/2024
8	*R. meenkulamensis* K.S. Prasad & K. Ravi	Seasonal lateritic pool	12º28′87″ N 75º23′80″ E	MeenkuIam,Pyyannur Kannur District, Kerala State,Suvarnika.V., 10411, DEV,15/10/2025
9	*R. mexicana* Schltdl. & Cham	Wet lateritie soil	12º14′25″ N 75º13′55″E	Velichamthodu,Cheemeni, Kasaragod District,Kerala State, Suvarnika.V.,10405,DEV,24/09/2024
10	*R. occultiflora* Koehne	Seasonal lateritic soil	12º28′87″ N 75º23′80″ E	Karinthalam, Kasaragod District, Kerala State,Suvarnika.V., 10403, DEV, 13/09/2024

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VI June 2026

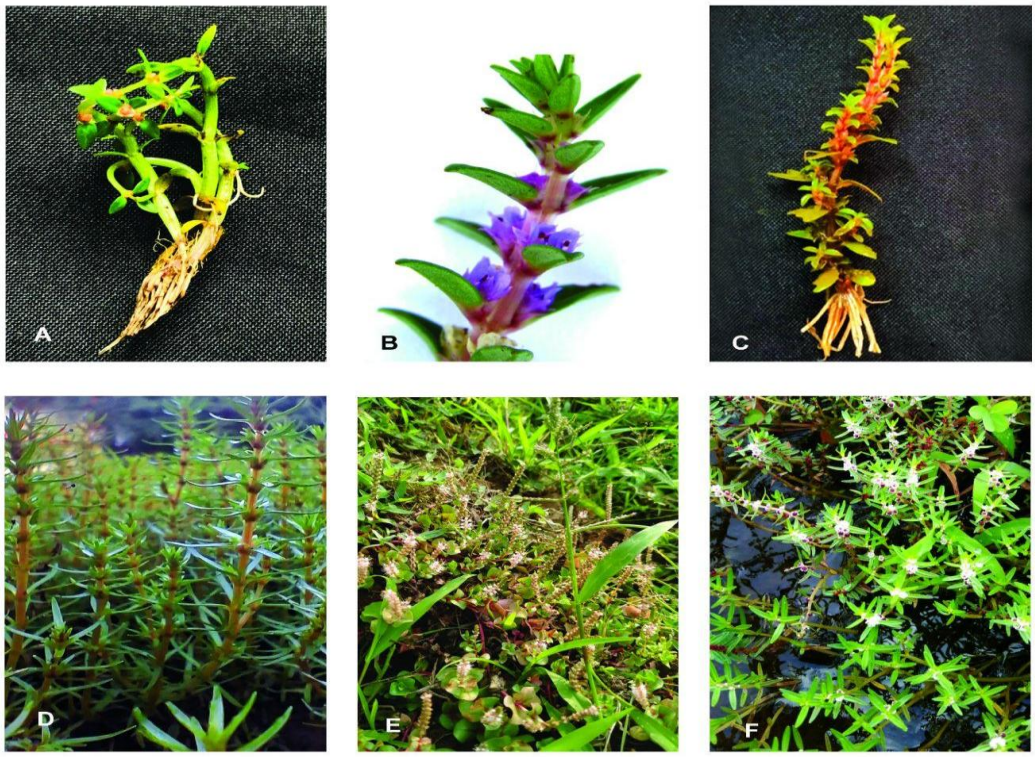

Fig. 1. Habit or selected *Rotala* spp. from the study area:

A: *R. cheruchakkiensis;* B*: R densiflora;* C*: R indica;* D*: R. kasaragodensis;* E*: R. macran&a•.* F*: R malabarica*

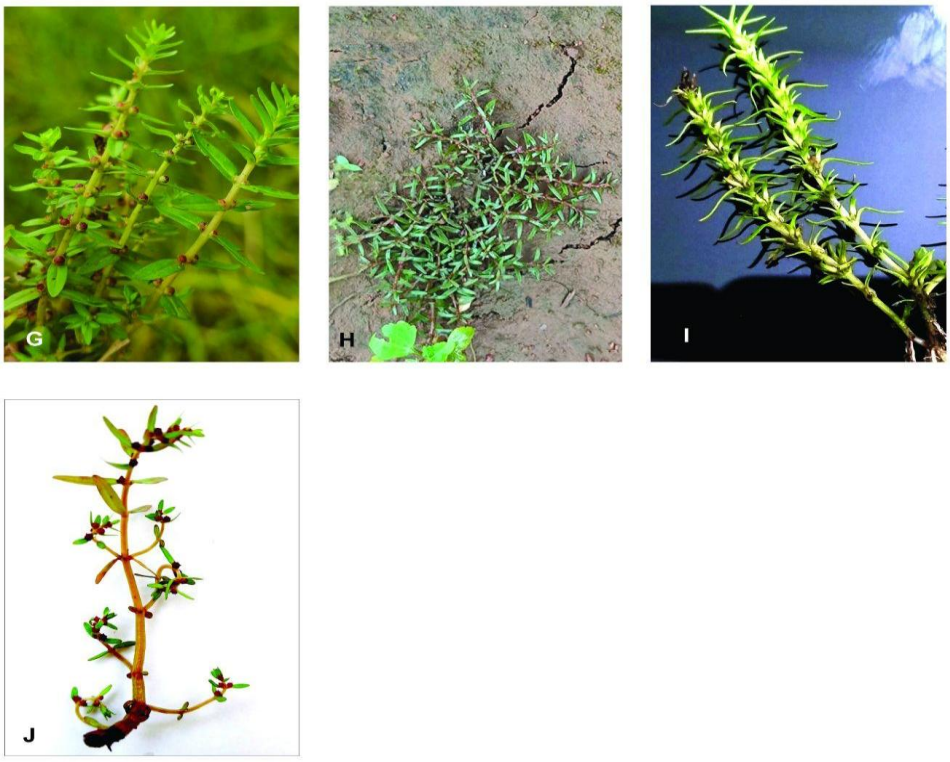

Fig. 2. Habit of selected *Rotala* spp. from the study area: **G***: R. malampuzhensis;* **H***: R. mexicana;* **I:** R. occultiflora; **J:** *R. meenkulamensis*

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VI June 2026

RESULT

General seed coat characters of the genus *Rotala* are discussed below:

The dominant seed shape was ovate and obovate (fig. 3). Seeds of all the studied species were found with smooth surface and with terminal hilum. The seed of *R. occultiflora* was observed with a maximum length of 297.8 mm to width of 1 15.6 mm. Length to width ratio of seeds was also calculated (Table, 2), and it was found that the maximum L/W ratio was observed in *R. occultiflora* i.e., 2.576mm.

Table 2. Seed micro morphological characteristics of studied *Rotala* spp.

Species	Seed shape	Seed colour	Seed length	Seed width	Seed LAV ratio	Hilum	Trichome	Seed coat texture
R. cheruchakkiensis	Round elliptic	Golden brown	452.1 mm	334.8mm	I .350mm	Acute	Present	Reticulate
R. densiflora	Oval	Pale brown	440.5 mm	359.1 mm	I .226mm	Acute	Absent	Reticulate
R. indica	Elliptic	Pale brown	425.2 mm	335.4mm	1267mm	Acute	Present	Reticulate polygonal
R. kasaragodcnsis	Obovate	Pale brown	3.824 mm	3.022mm	I .265mm	Rounded	Absent	Wrinkled
R. macrandra	Obovate	Pale brown	374.0 mm	199.2mm	1.877mm	Acute	Absent	Reticulate
R. malabarica	Ovate	Dark brown	692.9 mm	600.2mm	1.154mm	Acute	Absent	Reticulate polygonal
R. malampuzhensis	Obovate	Dark brown	637.7 mm	518.4mm	1.230mm	Acute	Absent	Irregular polygonal
R. meenkulamensis	Ovate	Pale brown	670.3 mm	576.2mm	1.163mm	Acute	Absent	Irregular polygonal
R. mexicana	Elliptic	Pale brown	269.2 mm	170.4mm	1.579mm	Acute	Absent	Striate
R. occultiflora	Elliptic	Dark brown	297.5 mm	1 15.6mm	2.576mm	Acute	Absent	Striate

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VI June 2026

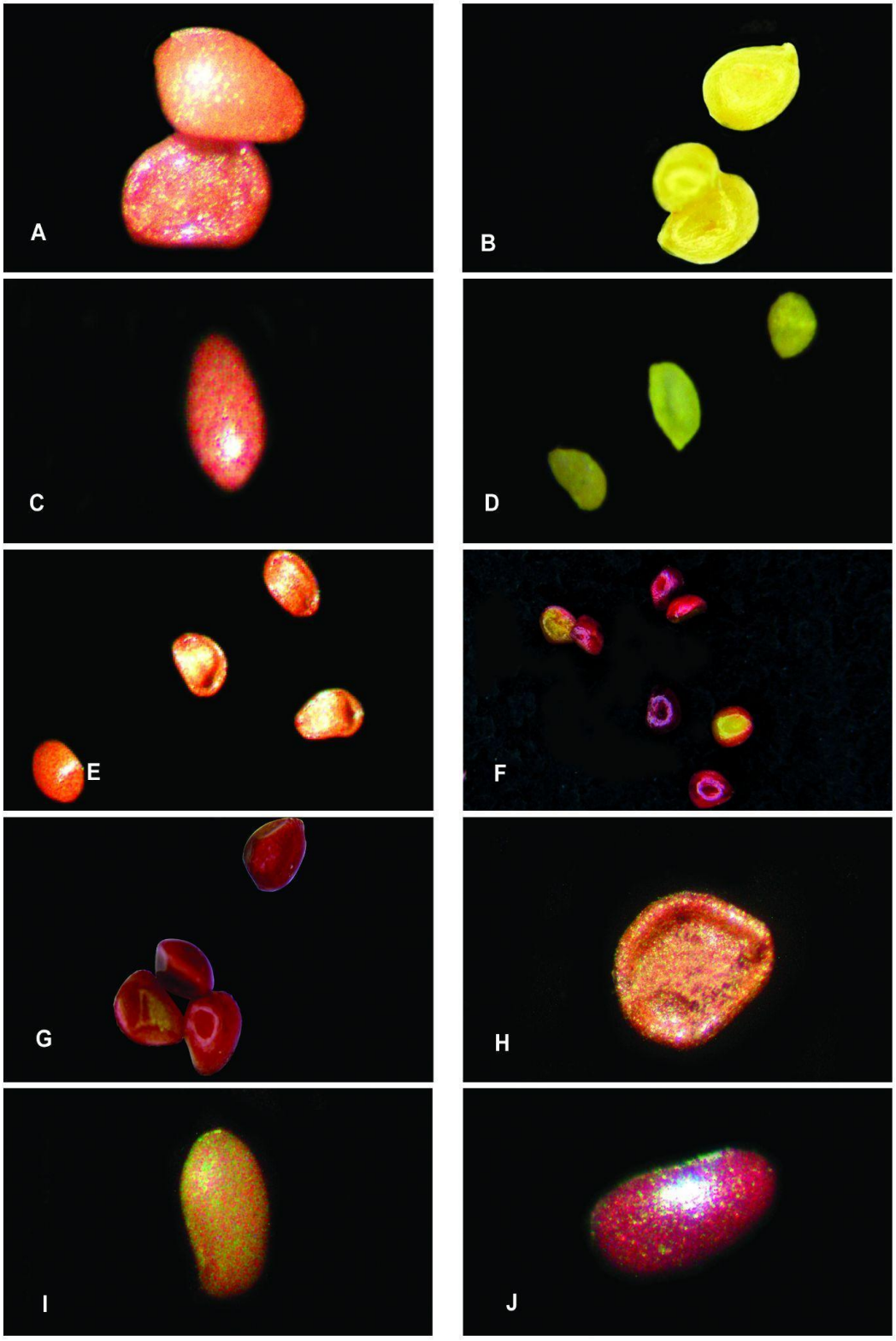

Fig. 3. Stereomicroscopic images of seeds of selected *Rotala* spp.:

A: *R. cheruchakkiensis:* B: *R densiflora:* C: *R. indica:* D: *R. kasaragodensis:* E: *R macrandra,*

F: *R, malabarica;* G: *R malampuzhensis;* H*: R. meenkulamensis;* l: *R. mexicana;* J*: R. occultiflora*

1.1. *R. cheruchakkiensis* Anto P. V. , Devikrishna C.S., Jacob Abraham Pulickal, Varghese C. D, and Ignatius Antony

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VI June 2026

R. cheruchakkiensis is the smallest species in the genus *Rotala*, with a height of about 3cm. Golden to brownish coloured seeds are seen. The number of seeds per fruit are 8-9.The seed shape was flattened oval and the texture was smooth. The compression was lateral, while the hilum was terminal. The length of the seed was 452.1 mm, whereas the width of the seeds was 334.8 mm, while length to width ratio of 1.350mm was recorded. Fig.(4) represent the FE-SEM results of *R. cheruchakkiensis* that indicate smooth seed sculpturing with epidermal hairs. Seed coat ornamentation is reticulated. Wings are absent on the seed coat surface. Outer epidermal cell shape and anticlinal wall shape are irregular; anticlinal wall texture is folded. The anticlinal wall is thickened and its level is shallow. The periclinal wall is thickened. convex shaped and its texture is reticulate.

The morphological variation of the seeds seems to reflect the geographical distribution of taxa, but adaptation may have played an important evolutionary role. *R. cheruchakkiensis* is considered as highly endemic, it emerges during the first rainy seasons of the month June and completes its life cycle within 1-2 months.

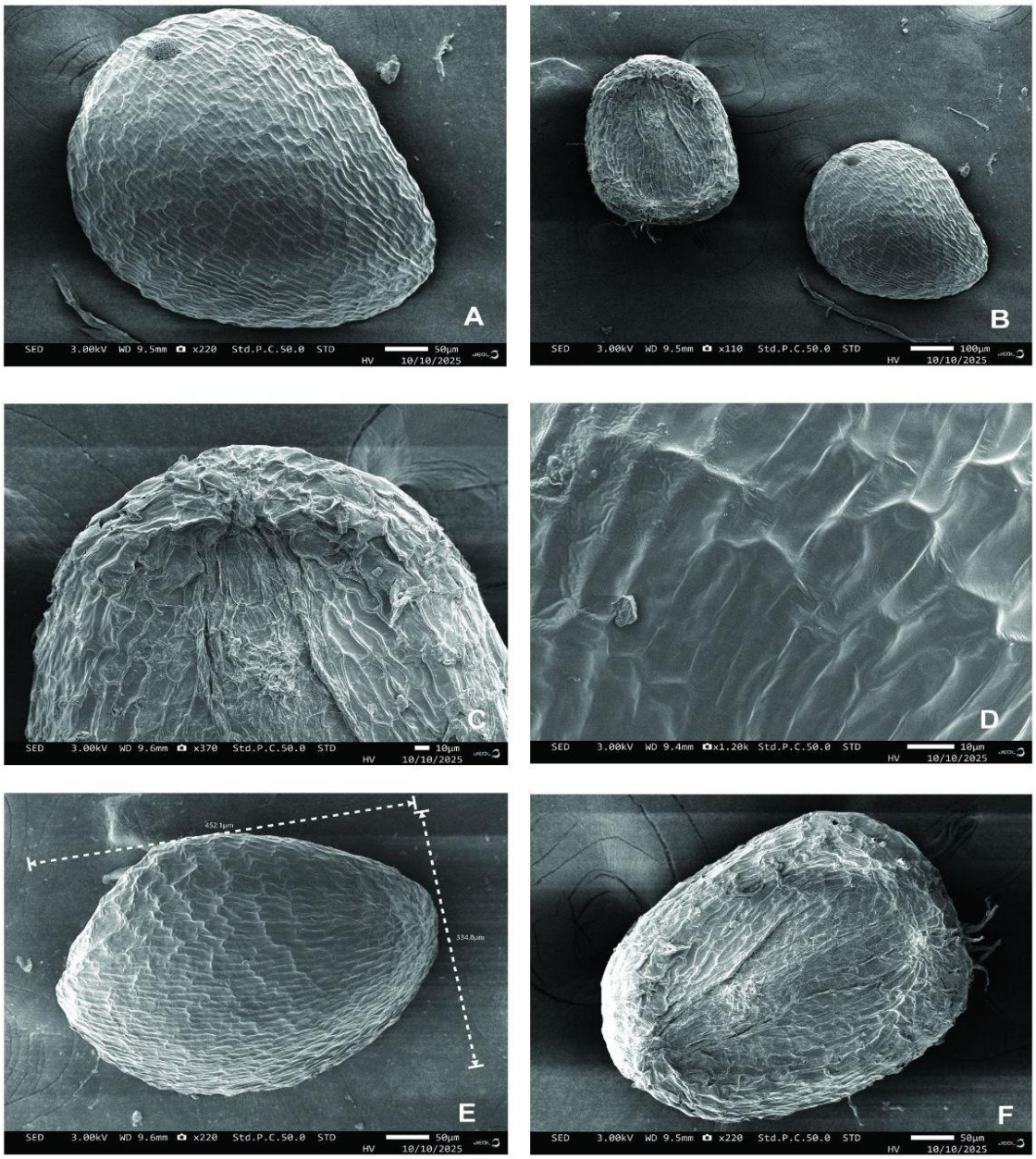

Fig. 4. SEM micrographs of seeds of *R. cheruchakkiensis* (scale bar= 50µm).
A: antiraphal face; B: raphal and anti raphal face; C: hilum; D: seed coat surface; E: length and width F: raphal face with evaginated trichomes

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VI June 2026

1.2. *R. densiflora* (Roth ex Roem. &Schult.) Koehne

R. densiflora is usually growing in paddy fields and marshy areas. The stem is highly branched and produces dense flowers towards the stem apex. The number of seeds per fruit are 10-12, which are pale brown in colour.The seed shape was triangular ovoid and the texture was smooth. The hilum was terminal. The length of the seed was 440.5 mm, whereas the width of the seeds was 359.1 mm, while length to width ratio was 1.226mm. Fig.(5) represent the FE-SEM images of *R. densiflora*. seed sculpturing or seed coat ornamentation was reticulate and without epidermal hairs. Wings are absent on the seed coat surface. Outer epidermal cell shape and anticlinal wall shape are irregular; anticlinal wall texture is folded. The anticlinal wall is thickened and its level is shallow. The periclinal wall is thickened, convex shaped and its texture is reticulate.

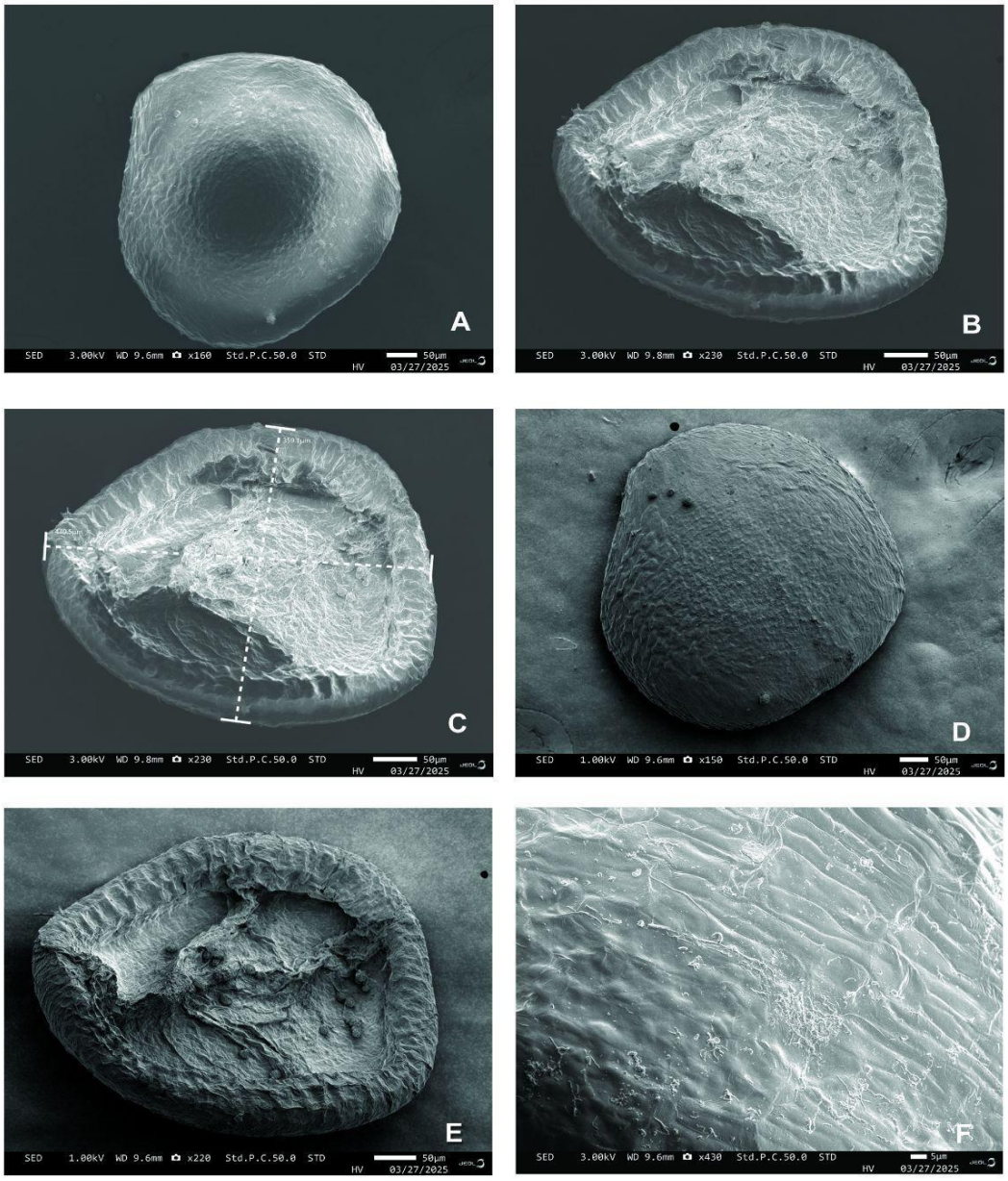

Fig. 5. SEM micrographs of seeds of *R. densiflora* (scale bar= 5μm & 50μm). A&D: antiraphal face; B&E: raphal face; C: length and width; F: seed coat surface.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VI June 2026

1.3. *R. indica* (Willd.) Koehne

R. indica in habits paddy field and temporary lateritic pools, The plants may have white to reddish colour according to the habitat. Flowers are light rose in colour.The number of seeds per fruit are 10-12.The seed shape was oval; seed coat ornamentation reticulated. The compression was lateral, while the hilum was terminal. The length of the seed was 425.2 mm, whereas the width of the seeds was 335.4 mm, while length to width ratio of 1.267mm was recorded. Fig.(6) represents the FE-SEM results of *R. indica*, which indicates smooth seed sculpturing with epidermal hairs. Seed coat ornamentation is reticulated. Wings are absent on the seed coat surface. The outer epidermal cell shape was polygonal; the anticlinal wall texture was reticulate. The anticlinal wall was thickened and shallow. The periclinal wall was thickened with reticulate texture; convex level. Trichomes present on seed coat.

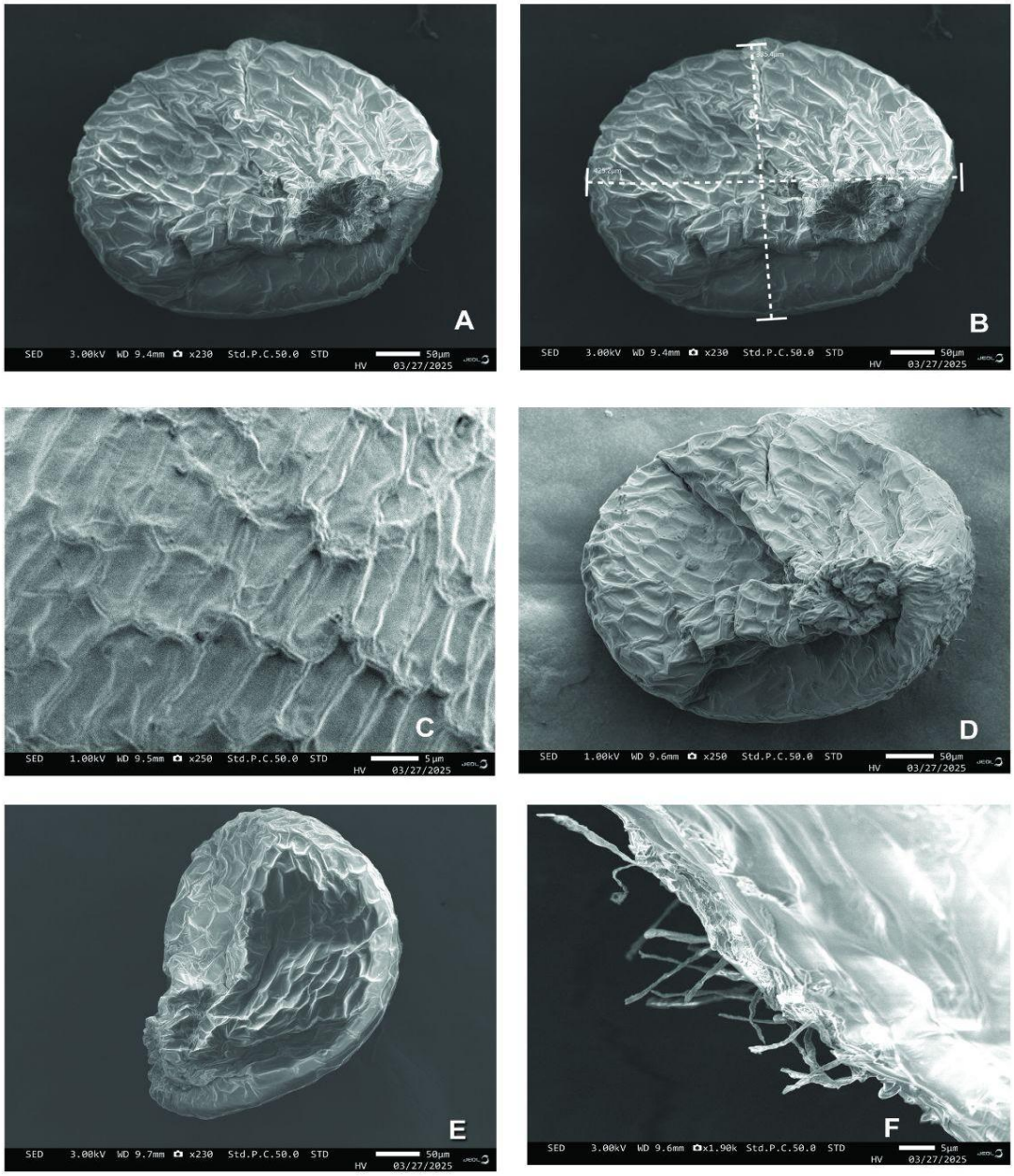

Fig. 6. SEM micrographs of seeds of *R. indica* (scale bar= 5µm & 50µm).

A&D: antiraphal face; B: length and width; C: seed coat surface; E: hilum

F: seed coat surface with evaginated trichomes.

1.4. *R. kasaragodensis* K.S. Prasad & Raveendran

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VI June 2026

R. kasaragodensis is usually found in the emergent marshy areas of temporary lateritic pools and paddy fields. Stem was erect and leaves arranged in clusters. Companulate, pink coloured flowers are produced singly from nodal regions, leaves are narrow and needle like. The capsule was oblong with 10 ten seeds, which were pale brown in colour.The seed shape was obovatc and the texture was wrinkled. The hilum was terminal and rounded. The length of the seed was 3.824 mm, whereas the width of the seeds was 3.022 mm, while length to width ratio of 1.265mm was recorded (Fig.(7)). Epidermal hairs are usually absent. Seed coat ornamentation was wrinkled, Wings are absent on seed coat surface. Outer epidermal cell shape and anticlinal wall shape are irregular; anticlinal wall texture is folded. Anticlinal and periclinal walls are thickened.

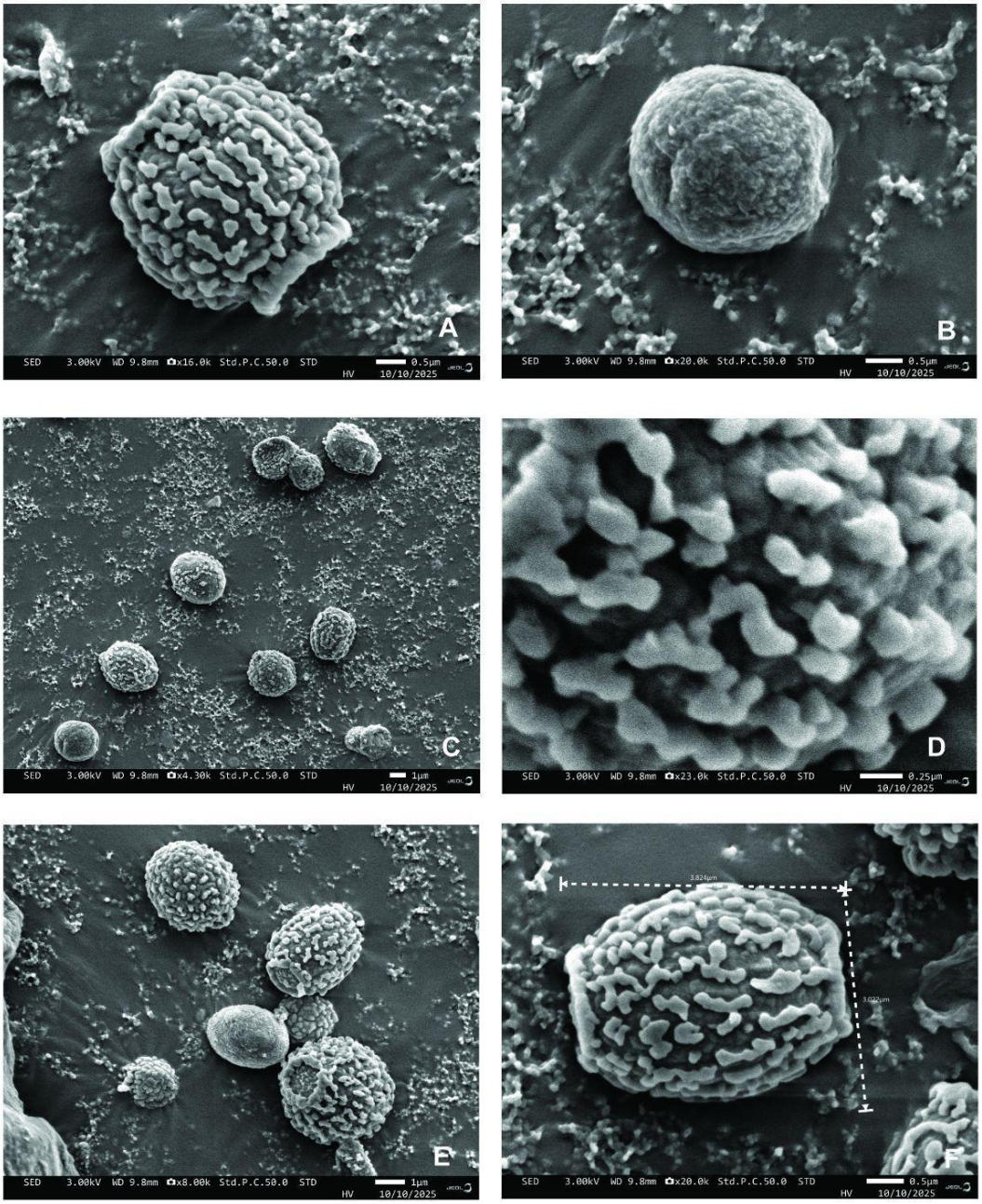

Fig. 7. SEM micrographs of seeds of *R. kasaragodensis* (scale bar=0.25µm,0.5µm &1µm). A,B,C&E: raphal and antiraphal face; D: seed coat surface; F: length and width.

1.5. *R. macrandra* Koehne

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VI June 2026

R. macrandra is growing in lateritic pools and marshy areas. Dimorphic leaves are seen in this species; the stem is white to reddish in colour. Flowers arc pink and anthers exceed the corolla tube. Golden to brownish coloured seeds are seen. The number of seeds per fruit are 8-9. The seed shape was obovate and seed colour was pale brown, hilum was terminal. The length of the seed was 374.0 mm, whereas the width of the seeds was 199.2mm, while length to width ratio of 1.877mm was recorded. Fig. 8. represents the FE-SEM results of *R. macrandra*. Seed coat surface shows reticulate ornamentation. Epidermal hairs or trichomes are absent, Seed coat ornamentation is reticulated. Wings are absent on the seed coat surface. Outer epidermal cell shape and anticlinal wall shape are irregular; anticlinal wall texture is smooth. The anticlinal wall is thickened and its level is shallow. The periclinal wall is thickened; convex shaped.

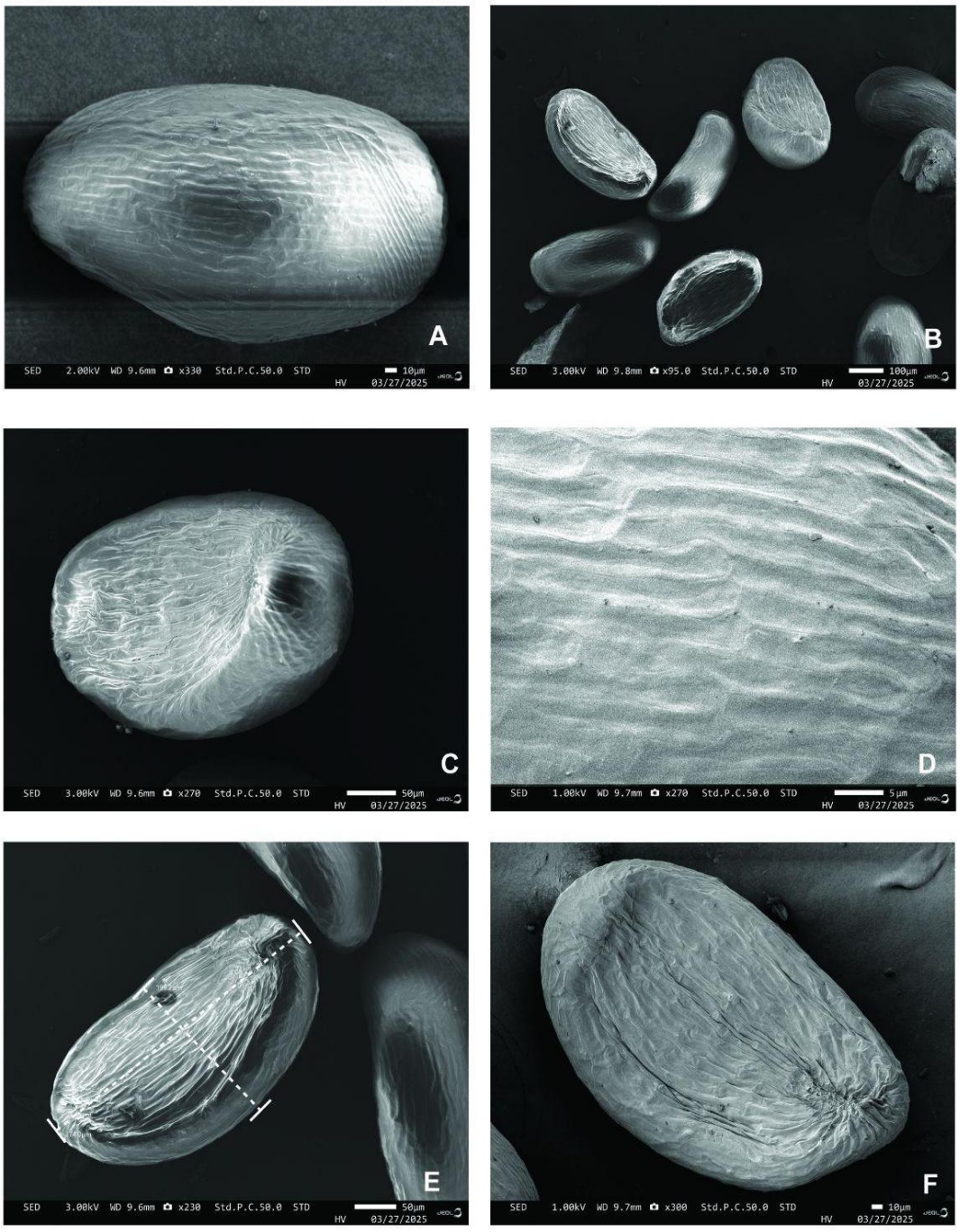

Fig. 8. SEM micrographs of seeds or *R. macrandra* (scale bar= 5,µm 10,µm, 50µm & 100µm). A: antiraphal face; B: raphal and antiraphal face; C&F antiraphal face; D: seed coat surface; E: length and width

1.6. *R. malabarica* Pradeep, K.T., Joseph &Sivar.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VI June 2026

*R. malabaric*a is a herbaceous plant growing in seasonal laterite pools. Its stem is quadrangular, and leaves bimucronate. flowers are pentamerous. The seeds are dark brown in colour. The number of seeds per fruit are 10-12. The seed shape was oval and the texture was smooth. The compression was lateral, while the hilum was terminal. The length of the seed was 692.9 mm, whereas the width of the seeds was 600.2 mm, while length to width ratio of l.154mm was recorded. Fig. 9 & 10 represents the FE-SEM results of *R. malabarica*. Seed coat surface is irregular polygonal. Wings are absent on the seed coat surface. Outer epidermal cell shape and anticlinal wall shape are irregular; anticlinal wall texture is smooth. The anticlinal wall is thickened and its level is shallow. The periclinal wall is thickened, convex shaped and its texture is reticulate.

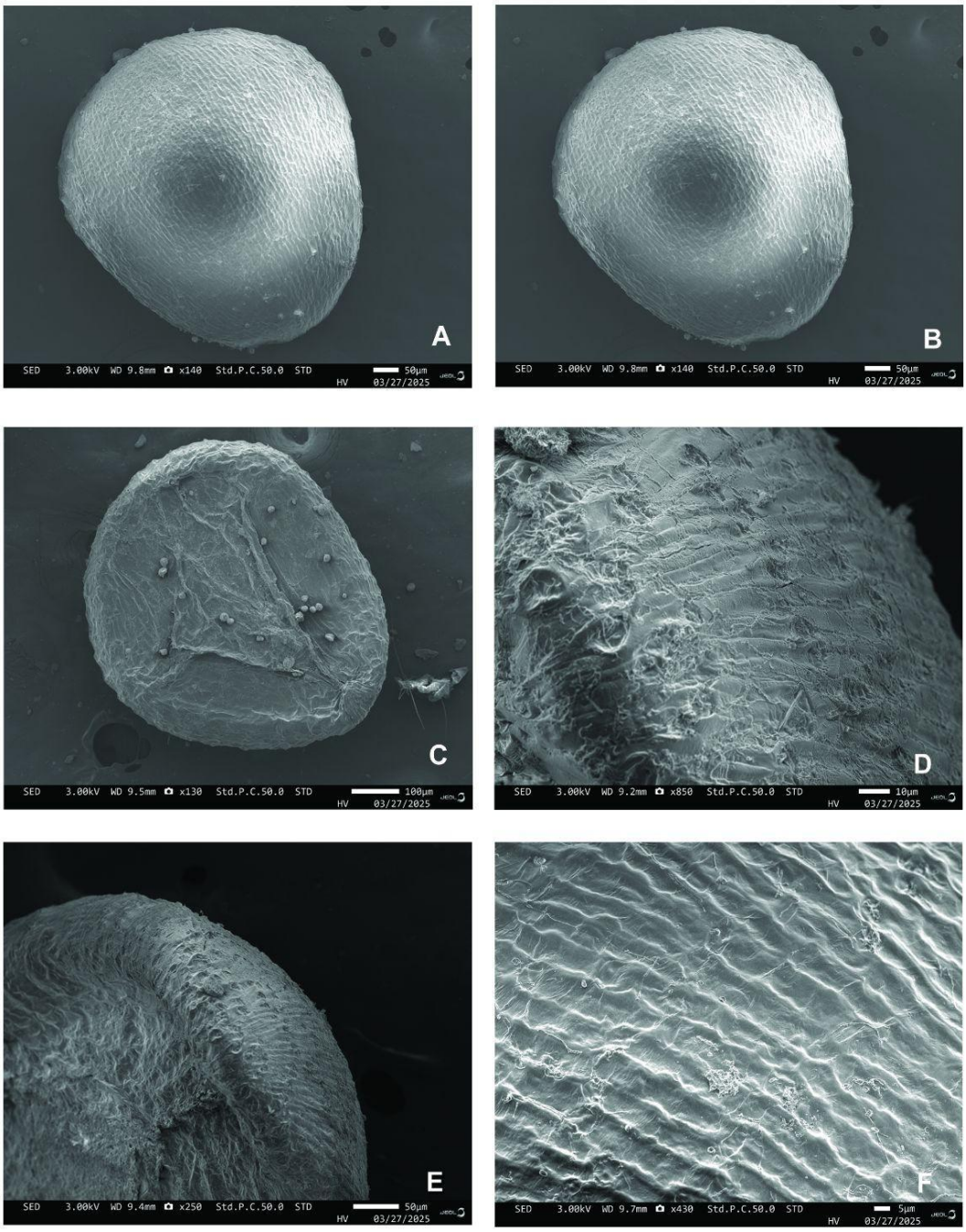

Fig. 9. SEM micrographs of seeds of *R. malabarica* (scale bar= 5µm 10µm, 50µm& 100µm). A.&B: antiraphal face; C: raphal face; D,E&F: seed coat surface.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VI June 2026

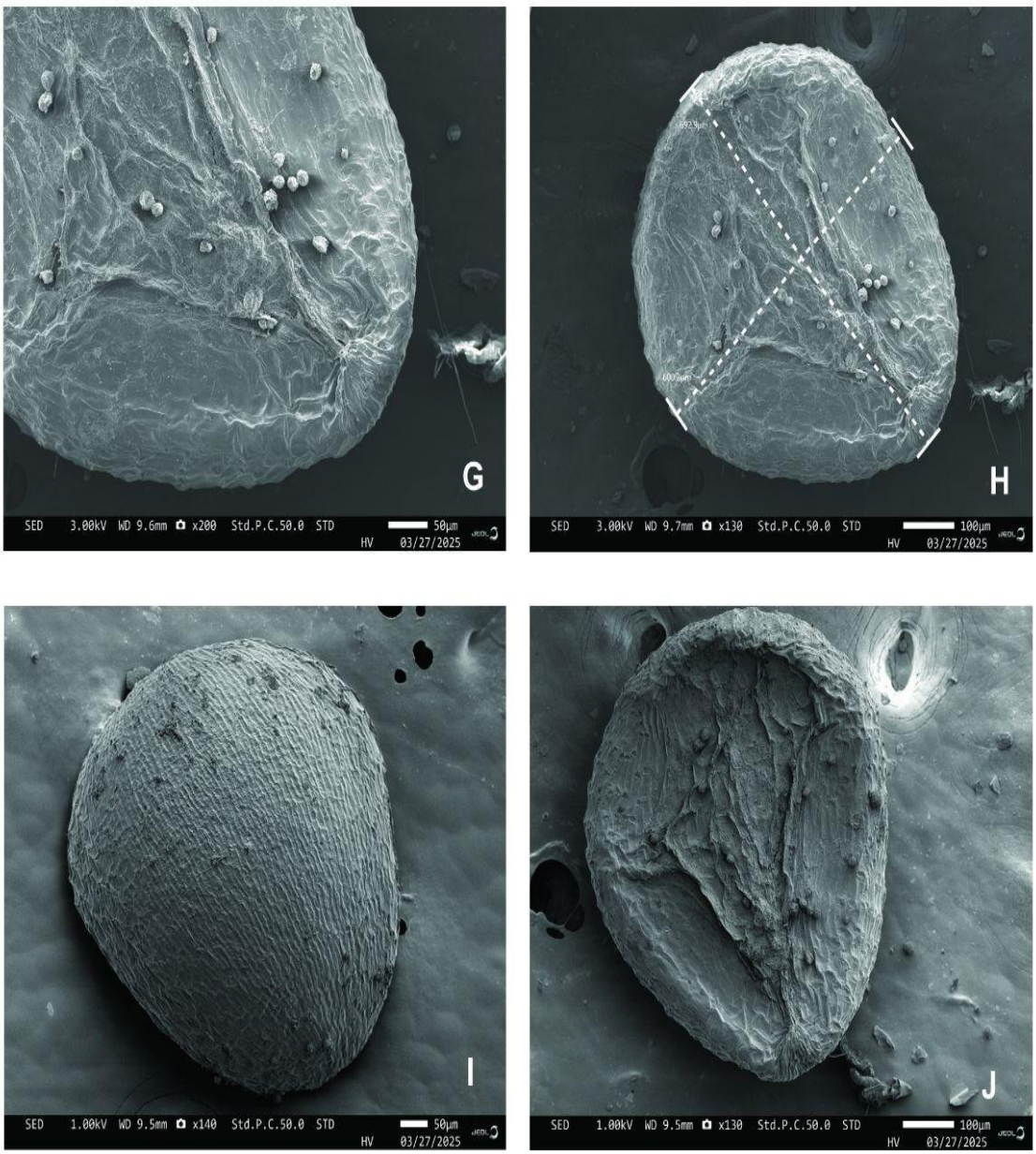

Fig. 10.SEM micrographs of seeds of *R. malabarica* (scale bar= 50 µm& 100µm). G: hilum; H: length and width; l: antiraphal face; J: raphal face.

1.7. *R. malampuzhensis* R.V. Nair ex C.D.K. Cook

R. malampuzhensis is growing in seasonal lateritic pools. Its stem is cylindrical, leaves lanceolate and flowers are trimerous. The seeds are dark brown in colour. The number of seeds per fruit are 8-10. The seed shape was obovate and the texture was smooth. The hilum was terminal and acute. The length of the seed was 637.7mm, whereas the width of the seeds was 518.4 mm, while length to width ratio of 1.230mm was recorded. Fig. 11 represents the FE-SEM results of *R. malampuzhensis*. Seed coat surface is irregular polygonal. Wings are absent on the seed coat surface. The outer epidermal cell shape is irregular polygonal and the anticlinal wall is thin; texture is smooth. The periclinal wall is thick, convex and its texture is reticulate. Trichomes are absent on the seed coat surface.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VI June 2026

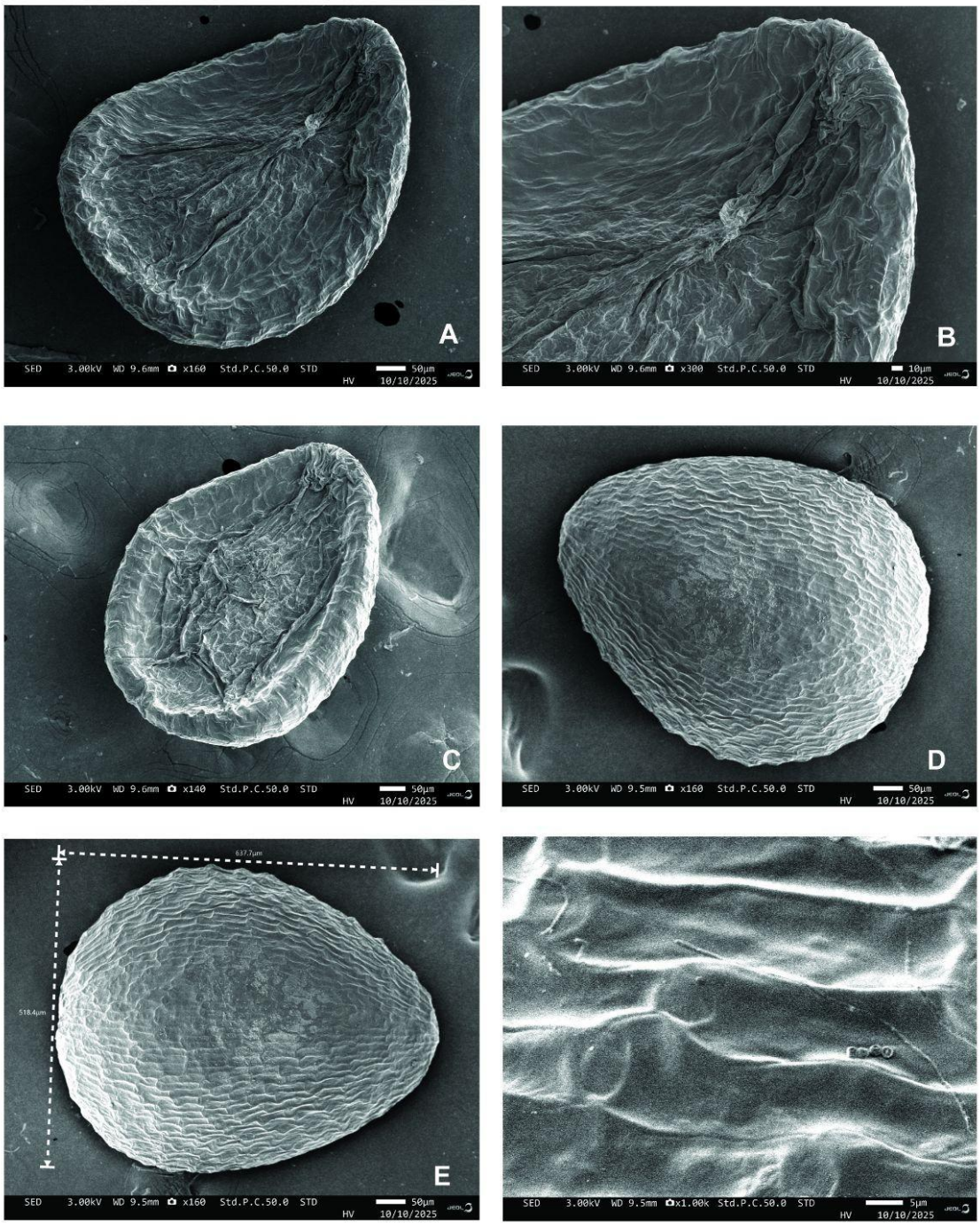

Fig. 11. SEM micrographs of seeds of *R. malampazhensis* (scale bar=5µm,10 µm &50µm). A&C: antiraphal face. B: hilum; D: raphal face; E: length and width F: seed coat surface.

1.8. *R. meenkulamensis* K.S. Prasad & K. Ravi

R. meenkulamensis is a highly endemic species growing in seasonal lateritic pools with high humus content. Its stem is quadrangular and flattened at the base; flowers are pentamerous. The seeds are pale brown in colour. The number of seeds per fruit are 10-12.The seed shape was oval and the texture was smooth. The hilum was terminal. The length of the seed was 670.3mm, whereas the width of the seeds was 576.2 mm. while length to width ratio of 1.163mm was recorded. Fig. 12 represents the FE-SEM results of *R. meenkulamensis*. Seed

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VI June 2026

coat surface is irregular, wings and trichomes are absent on seed coat surface. Outer epidermal cell shape and anticlinal wall shape are irregular; anticlinal wall texture is smooth. The anticlinal wall is thin and its level is shallow. The periclinal wall is thickened, convex shaped and its texture is reticulate.

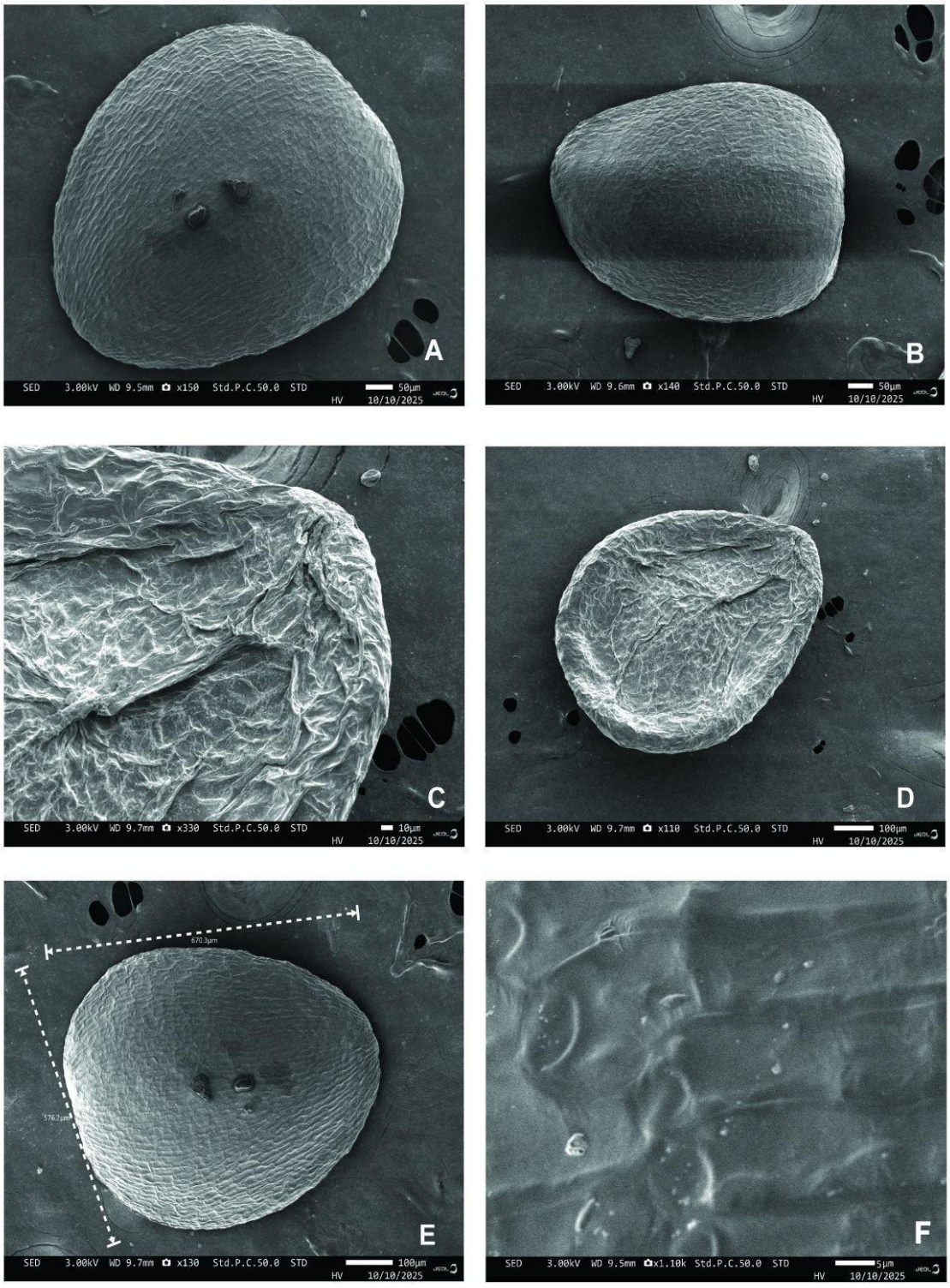

Fig. 12. SEM micrographs of seeds of *R meenkulamensis* (scale bar=5, 10µm ,50µm &100µm). A&B: antiraphal face; C: hilum; D: raphal face; E: length and width F: seed coat surface

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VI June 2026

1.9. *R. mexicana* Schltdl. & Cham

R. mexicana is a herbaceous plant growing in wet lateritic soils. Its stem is cylindrical, leaves linear, narrow and opposite in phyllotaxy. Flowers are Pink coloured. Seeds are elliptic and pale brown in colour.

The number of seeds per fruit are 10-12.The seed shape was oval and the texture was smooth. The compression was lateral, while the hilum was terminal. The length of the seed was 269.2mm, whereas the width of the seeds was 170.4 mm, while length to width ratio of 1.579mm was recorded. Fig. 13 represents the FE-SEM results of *R. mexicana*. Hilum is acute, seed coat surface is striate. Wings and trichomes are absent on the seed coat surface. Outer epidermal cell shape and anticlinal wall shape are irregular; anticlinal wall texture is smooth. The anticlinal wall is thin and its level is shallow. The Periclinal wall is thickened; convex shaped

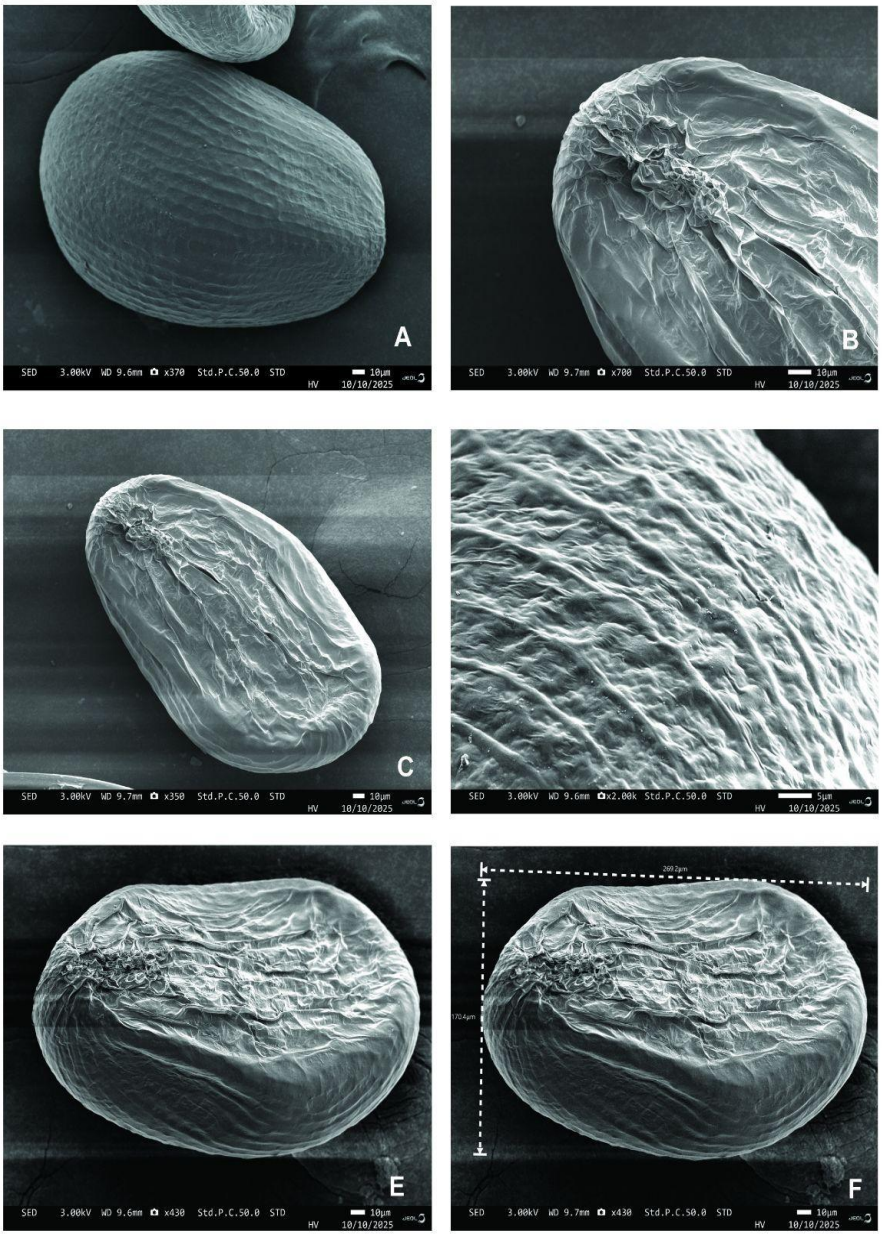

Fig.13. SEM micrographs Of seeds of *R. mexicana* (scala bar=5μm &10μm)

A: antiraphal face; B: hilum; C&E: raphal face; D: seed coat surface; F: length and width

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VI June 2026

1. 10. *R. occultiflora* Koehne

This plant is growing in lateritie seasonal pools. Stem is white to reddish according to the habitat.Leaves are narrow and linear. Flowers are whitish, companulate with 4 lobes. Fruit is a capsule, 8-10 seeds per fruit, seed shape is elliptical to pear shaped and dark brown in colour. The seed shape was oval and the texture was smooth. The compression was lateral, while the hilum was terminal. The length of the seed was 297.8mm, whereas the width of the seeds was 115.6 mm, while length to width ratio of 2.576mm was recorded. It shows the largest seed length to width ratio, among the studied species of *Rotala* (Fig. 14). Hilum is acute, seed coat surface is striate. Wings and trichomes are absent on the seed coat surface. Outer epidermal cell shape and anticlinal wall shape are irregular; anticlinal wall texture is smooth. The anticlinal wall is thin and its level is shallow. The periclinal wall is thick with striate texture; convex shaped.

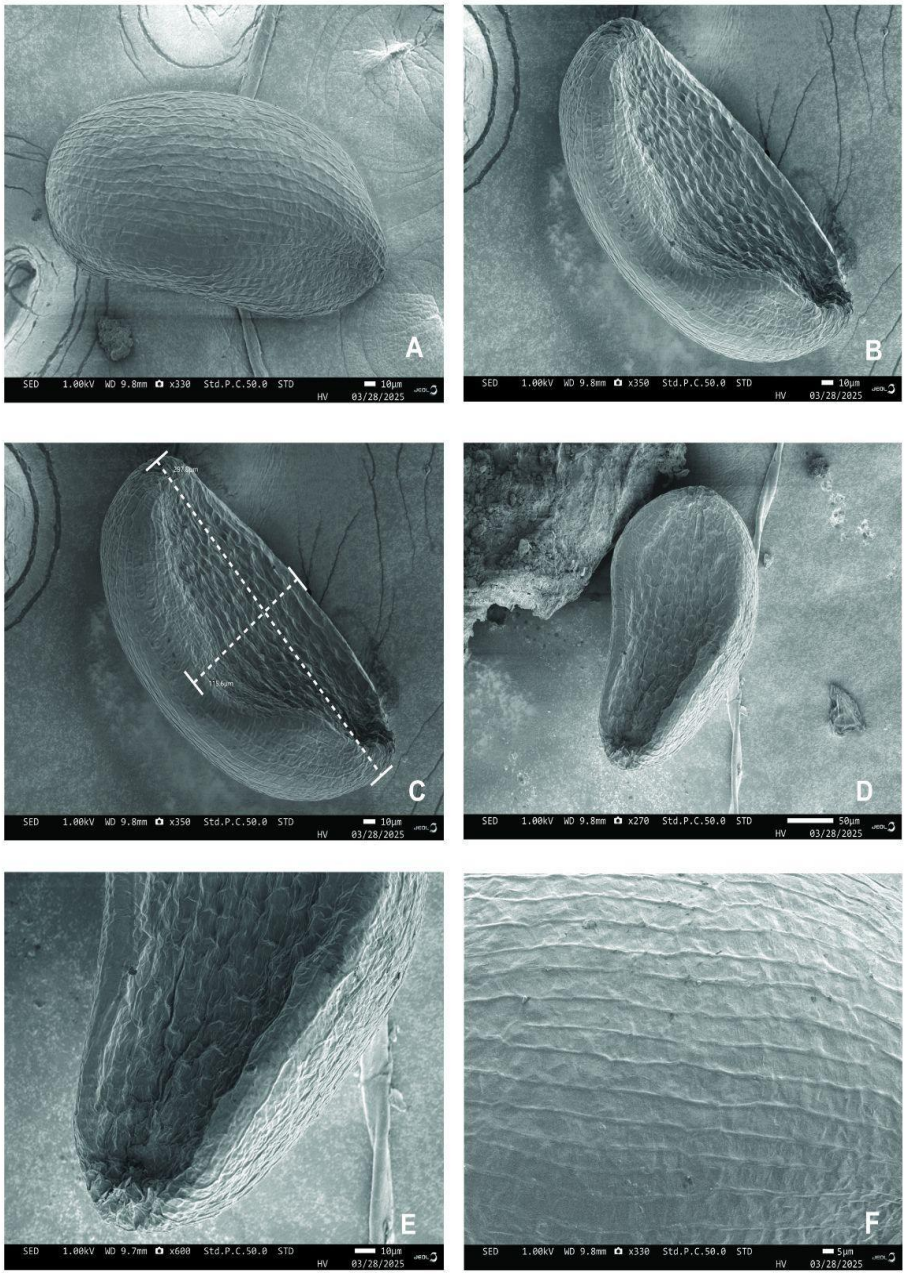

Fig. 14. SEM micrographs of seeds of *R. occultiflora* (scale bar=5µm , 10µm & 50µm). A: antiraphal face; B&D: raphal face. C: length and width; E: hilum; F: Seed coat surface

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VI June 2026

DISCUSSION

In this study, we found that, in most species, the seed coat is reticulate type. *R. indica* and *R. cheruchakkiensis* show the presence of trichomes on seed coat surfaces. In the present study, We also noticed that a limited number of seeds, 10-12, are seen in all the studied species and almost all are difTerent sized. The species like *R. kasaragodensis*, *R. mexicana* and *R. meenkulamen*sis are highly endemic and distributed in specific localities of lateritic area. Seeds of *R. kasaragodensis* are spherical and 12 in number in each fruit. Placentation is axile in all studied species; the seed coat surface is smooth in appearance.

EDAX ELEMENTAL PROFILE

The EDAX (Energy Dispersive X-ray Analysis) elemental profiling of *Rotata* seeds elucidates a quantitative distribution of vital minerals and elements (Fig. 15-24). The EDAX spectrum delineates significant elements, with pronounced peaks for Carbon (C), Oxygen (O), Magnesium (Mg), Potassium (K), alongside trace quantities of Sodium (Na), Aluminium (Al), Silicon (Si), Sulfur (S) and Calcium (Ca). Carbon and Oxygen acts as vital regulators of seed dormancy, longevity and seed germination. Calcium and Potassium give structural stability and initial nutrition to the seed coat. Potassium is present at intermediate levels and is recognized for its role in the regulation of turgor pressure in seed coat. Micronutrients. including Silicon and Aluminium, although present in diminished concentrations, are essential for providing structural rigidity and cellular health. Chlorine controls chemical scarification and also releases dormancy.

These observations, represented in spectra from four distinct regions (Area l, Area 2, Area 3, and Area 4, etc.,)accentuate the variability in elemental distribution, thereby providing insights into the functional adaptations and mineral storage capacities of the seed. The EDAX spectra furnish a spatial analysis of mineral composition, illustrating how elemental concentrations fluctuate across the seed surface, which may correlate with specific physiological or ecological functions of *Rotala* species.The collective findings of these analyses demonstrate that *Rotala* species possesses both structural and biochemical adaptations that are highly conducive to its indigenous habitat.The microscopic examinations and FESEM (Field Emission Scanning Electron Microscopy) investigations of trichomes, seed coat configurations, and hilum shape in conjunction with the EDAX elemental profiling, present a holistic perspective on the seed's functionality. The FESEM analysis further elucidated the distribution of essential elemental profiles across the seed surface, indicating pathways for crucial nutrient interactions.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VI June 2026

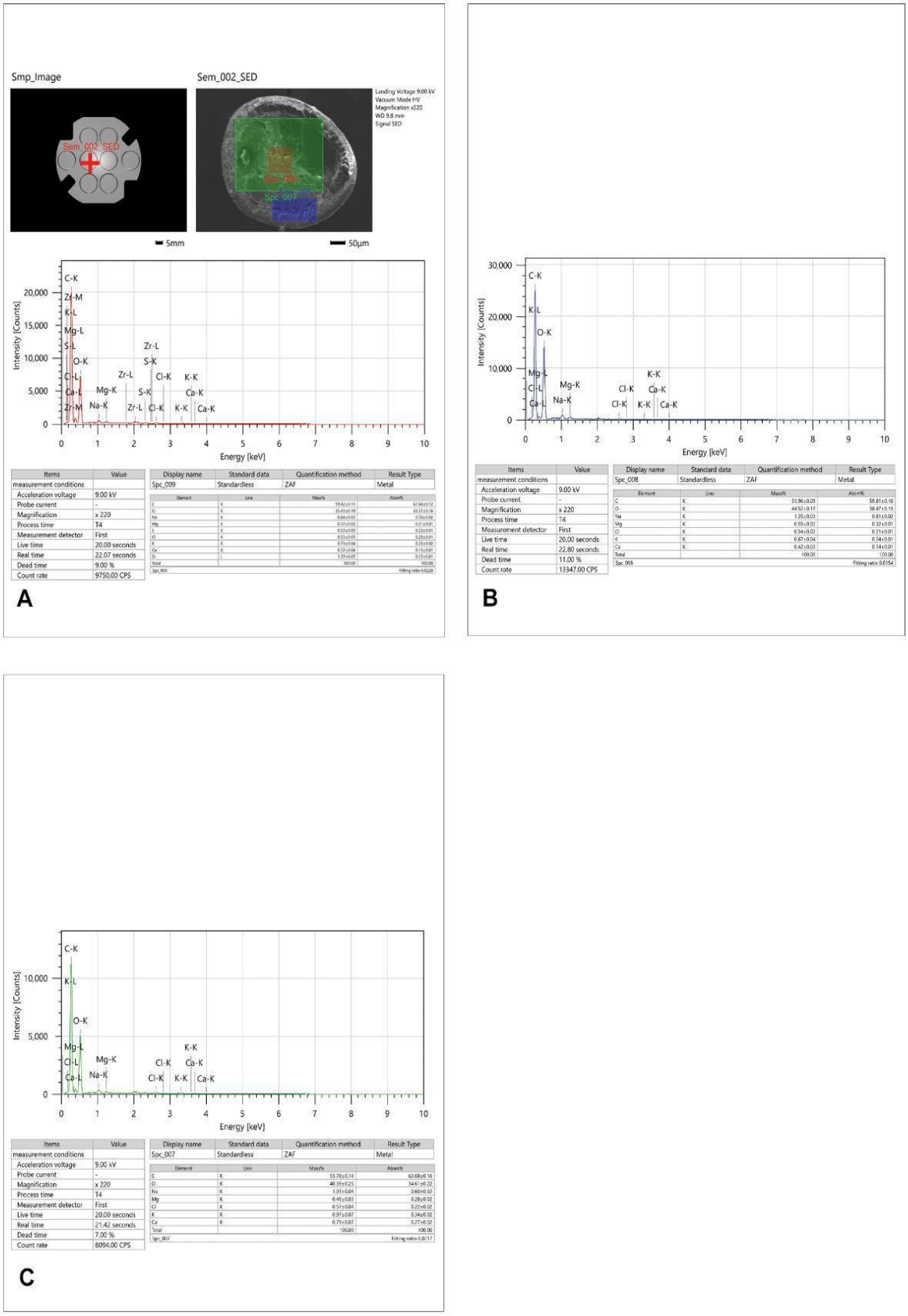

Fig. I5. EDAX profiling of seeds of *R. cheruchakkiensis* (scale bar= 100µm)

A: Spc-011; B: Spc-012; C: Spc-013

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VI June 2026

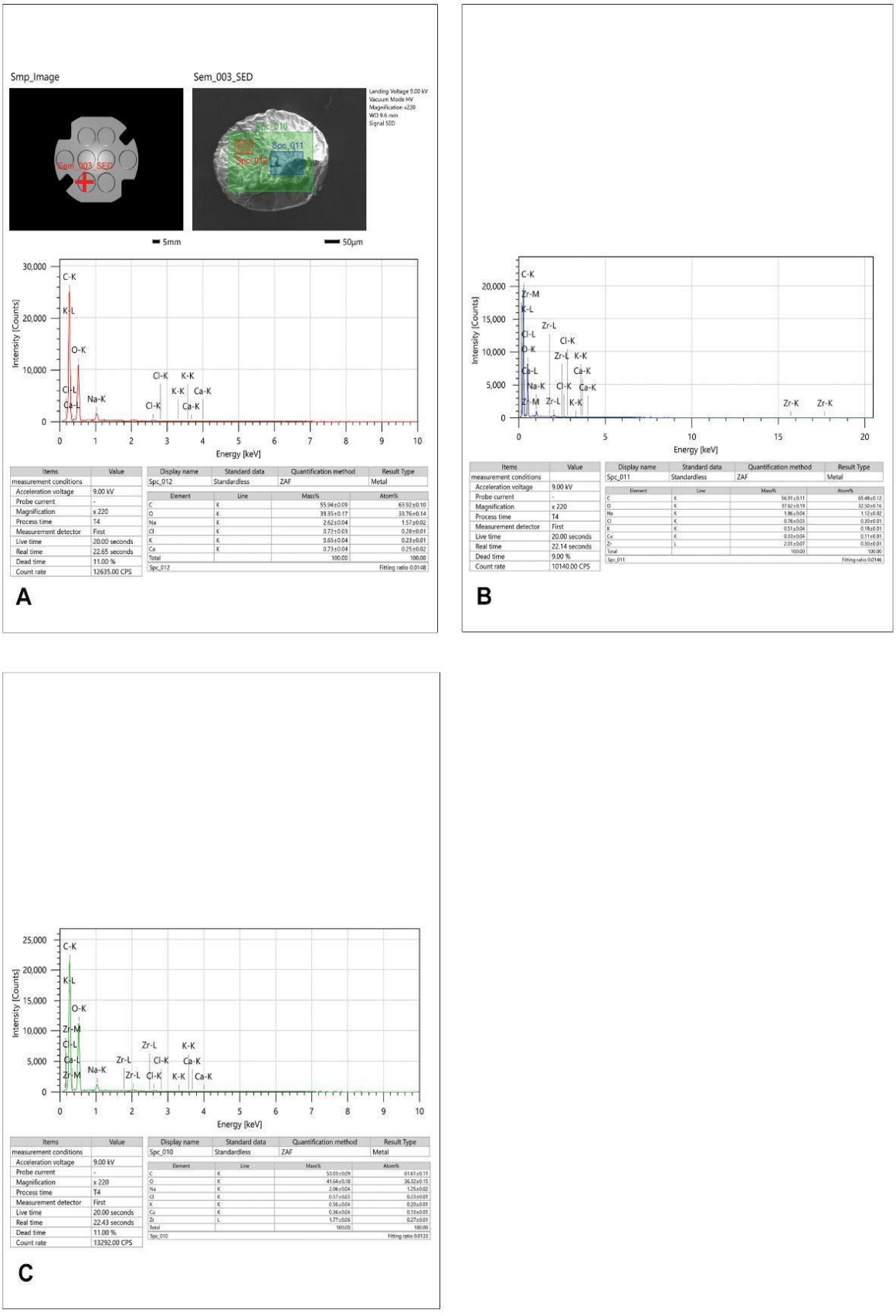

Fig. 16. EDAX profiling of seeds of *R. densiflora* (scale bar= 50µm)

A: Spc-009; B: Spc-008; C: Spc-007

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VI June 2026

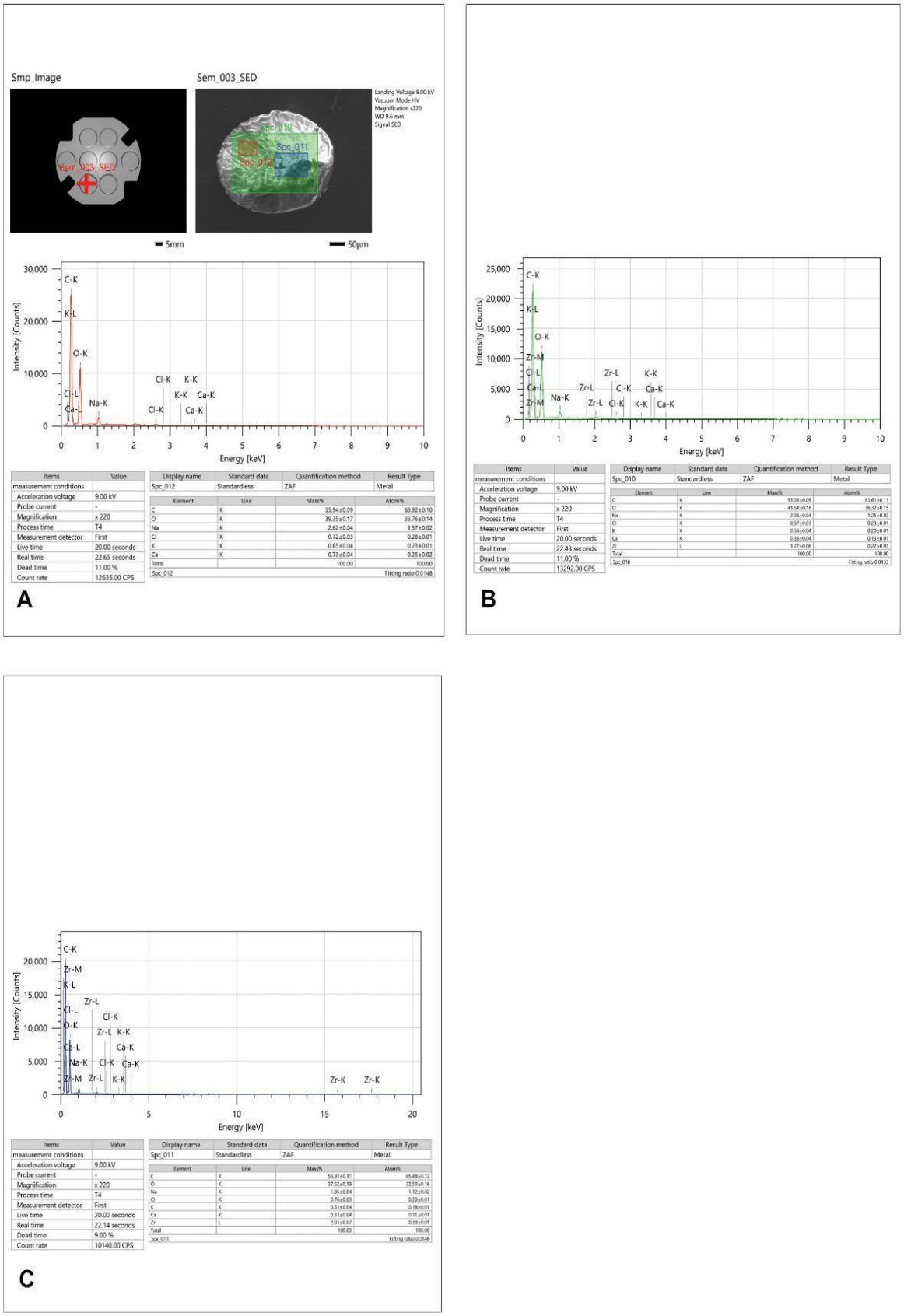

Fig. 17. EDAX profiling of seeds of *R. indica* (scale bar= 50µm)

A: Spc-012; B: Spc-011; C: Spc-010

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VI June 2026

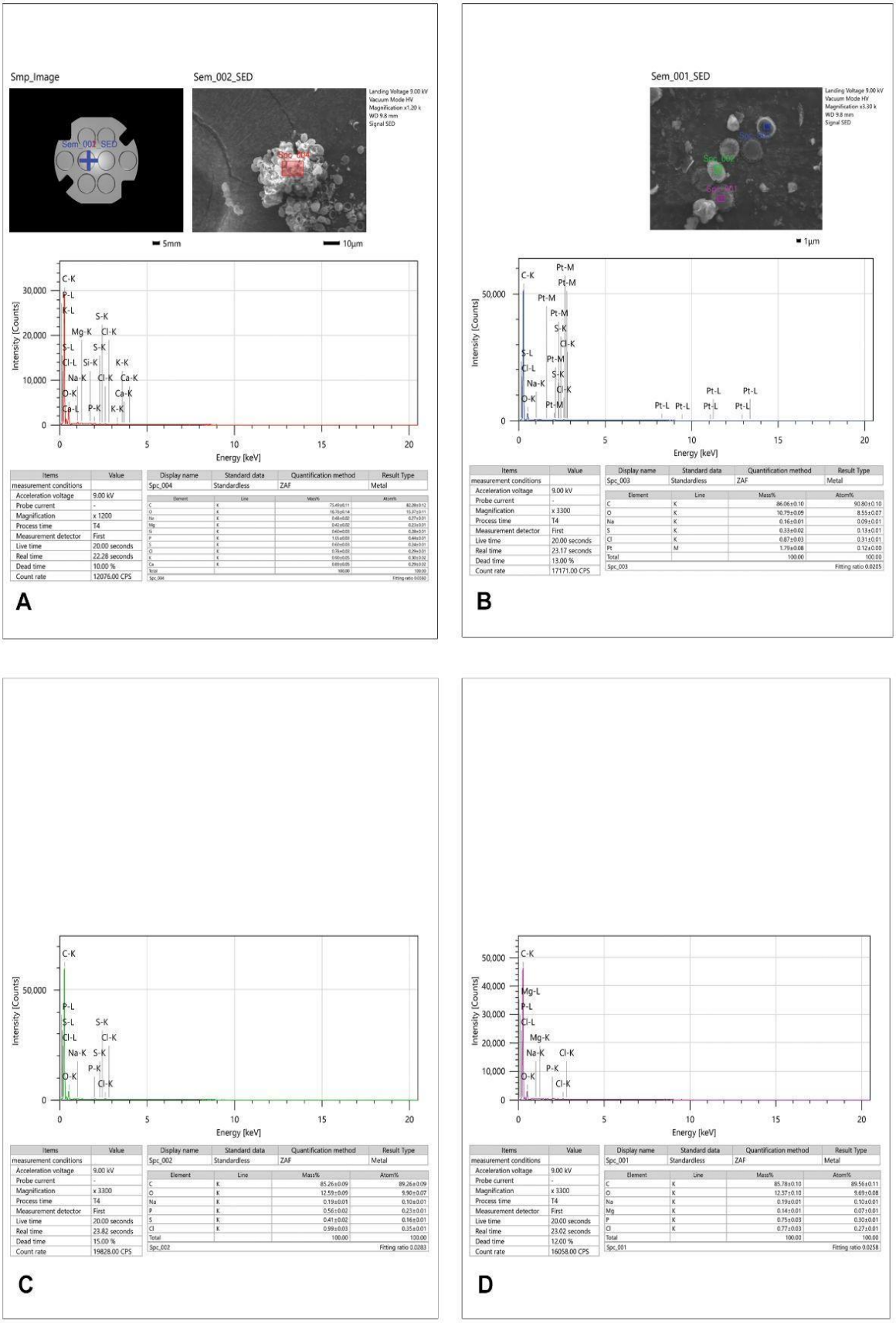

Fig. 18. EDAX profiling of seeds of *R. kasaragodensis* (scale bar= 10µm)

A: Spc-004; B: Spc-003; C: Spc-002; D: Spc-001

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VI June 2026

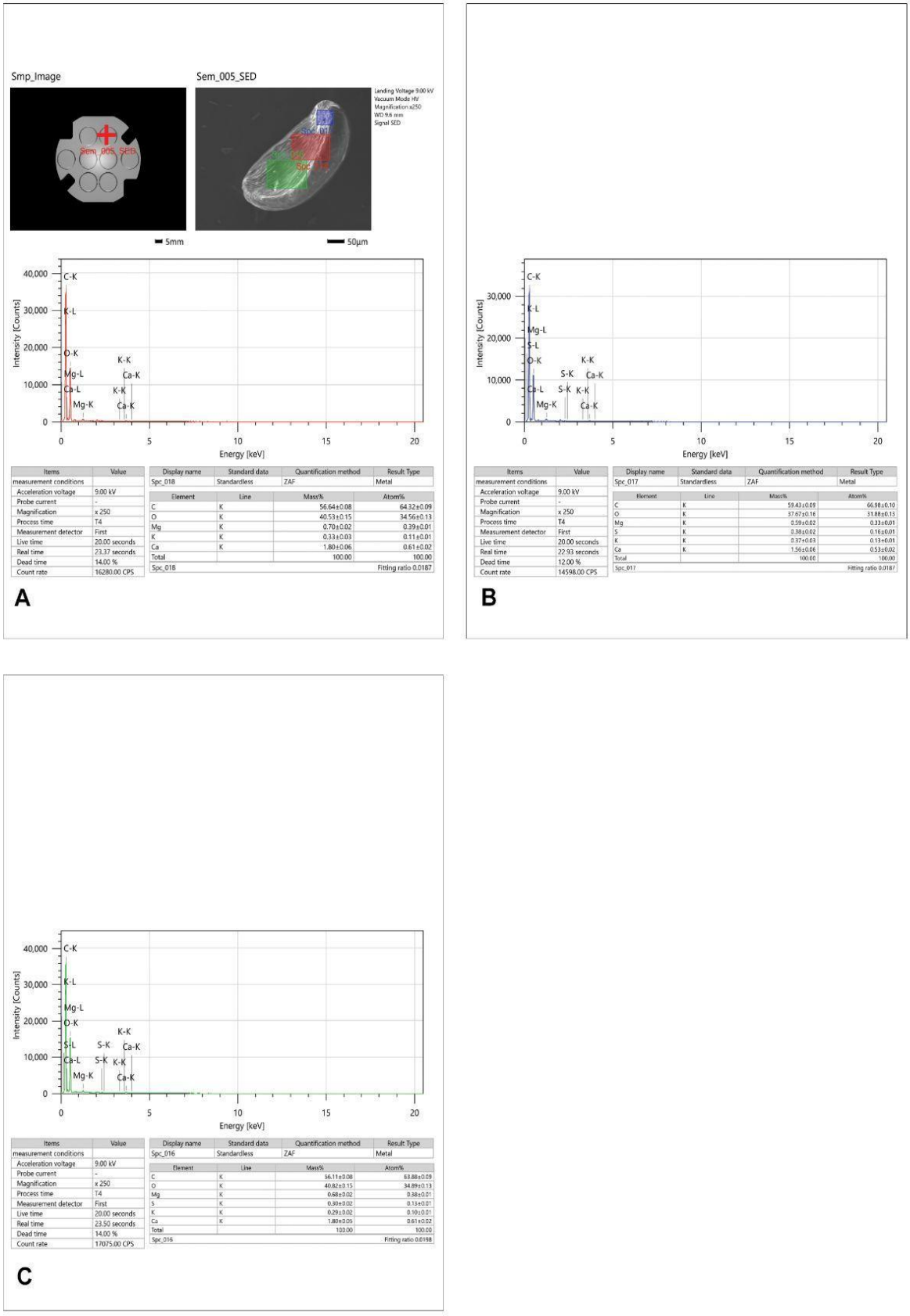

Fig. 19. EDAX profiling of seeds of *R. macrandra* (scale bar= 50µm)

A: Spc-018; B: Spc-017; C: Spc-016

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VI June 2026

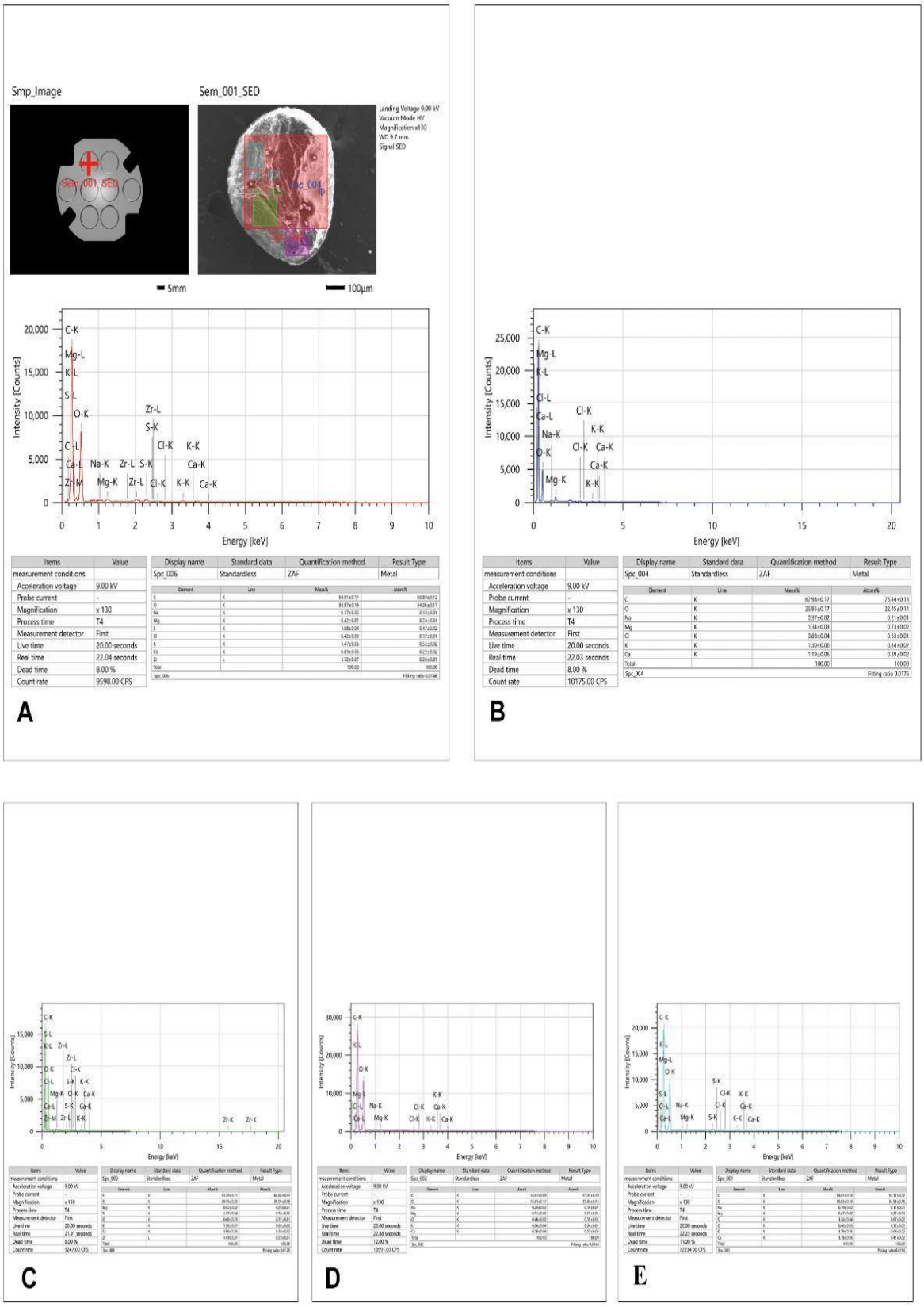

Fig. 20. EDAX profiling of seeds of *R. malabarica* (scale bar= 100µm)

A: Spc-006; B: Spc-004; C: Spc-003; D: Spc-002; E: 001

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VI June 2026

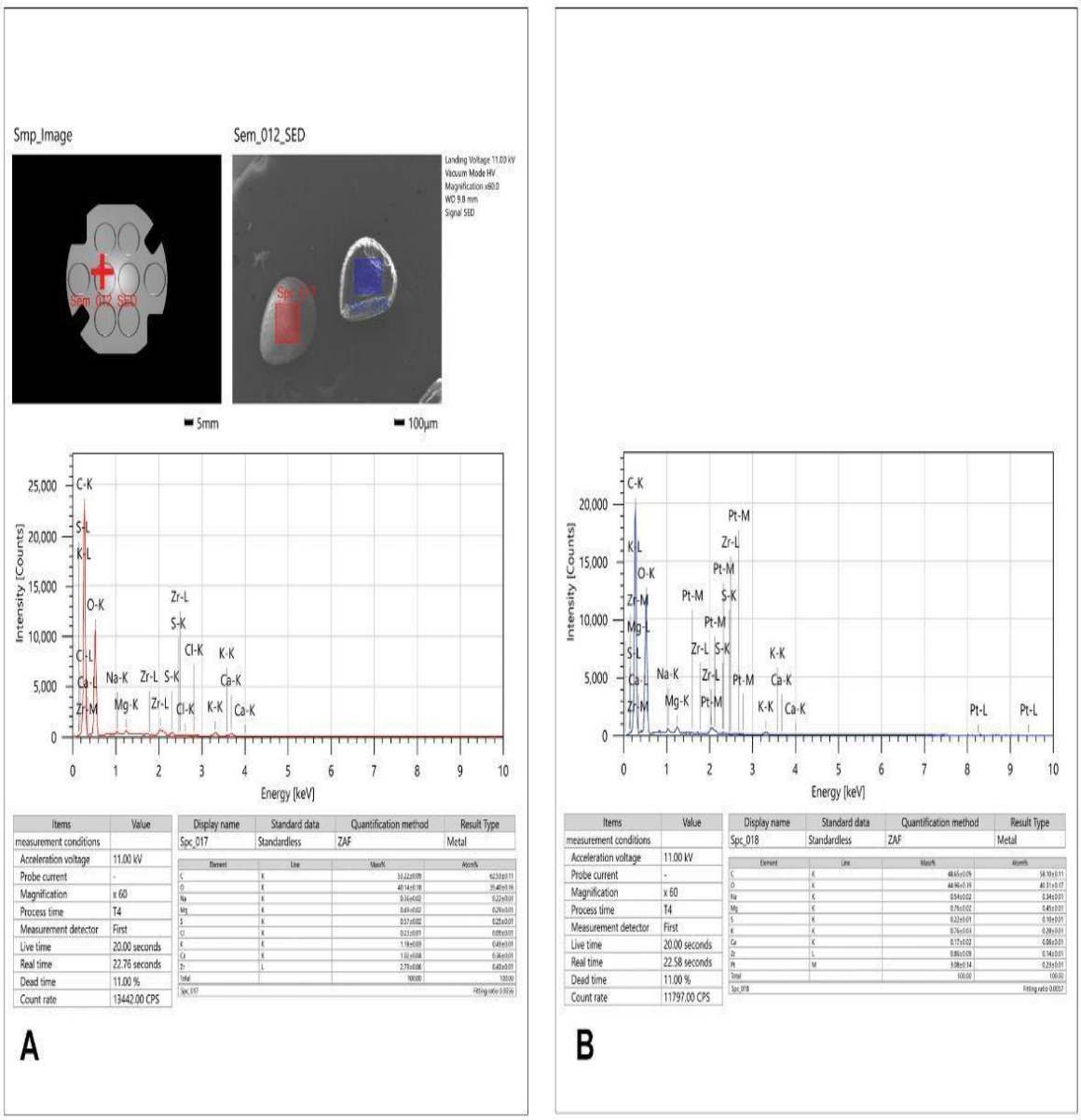

Fig. 21. EDAX profiling of seeds of *R. malampuzhensis* (scale bar= 100µm)

A: Spc-017; B: Spc-018

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VI June 2026

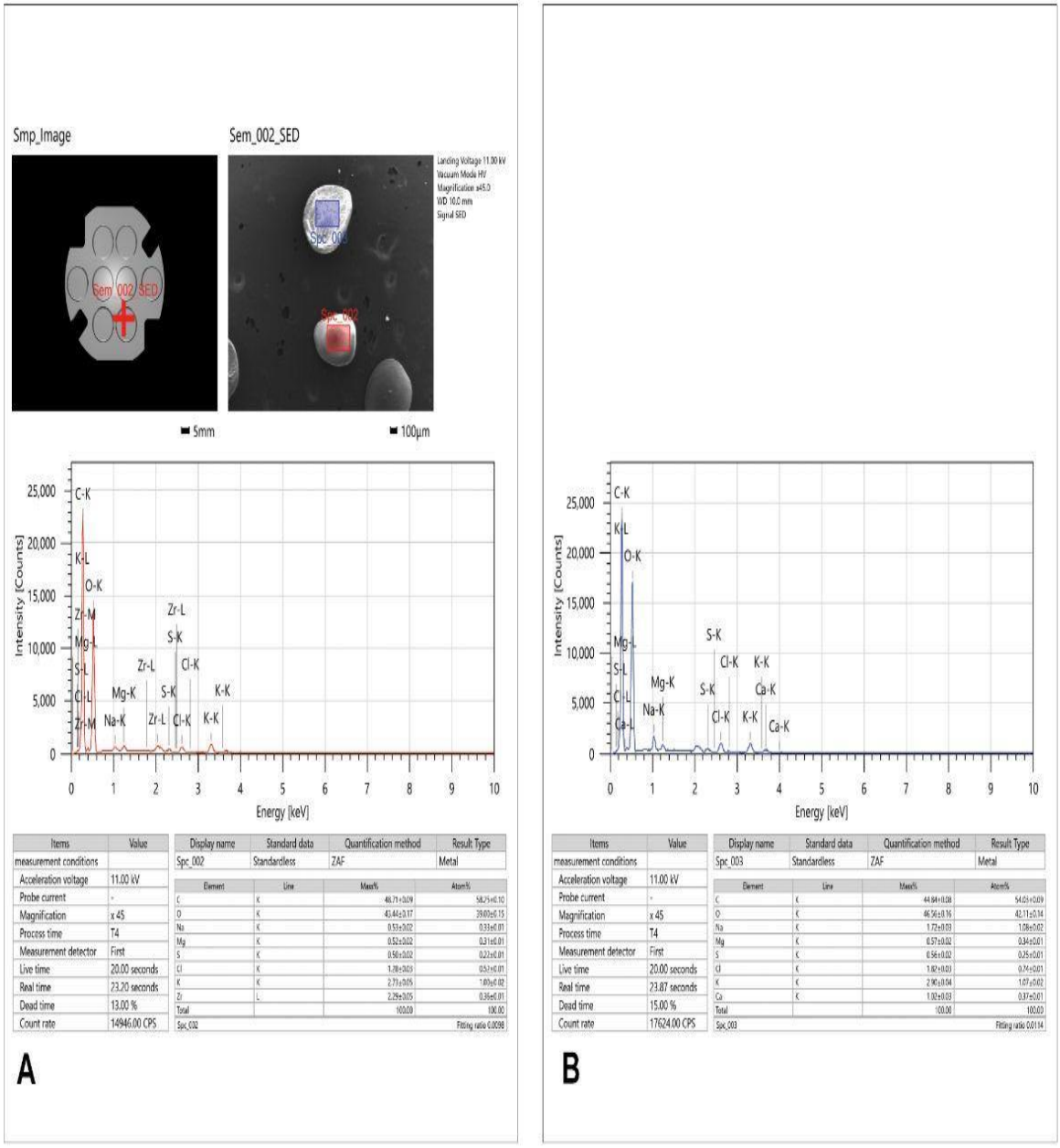

Fig. 22. EDAX profiling of seeds of *R. meenkulamensis* (scale bar= 100µm)

A: Spc-002; B: Spc-003

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VI June 2026

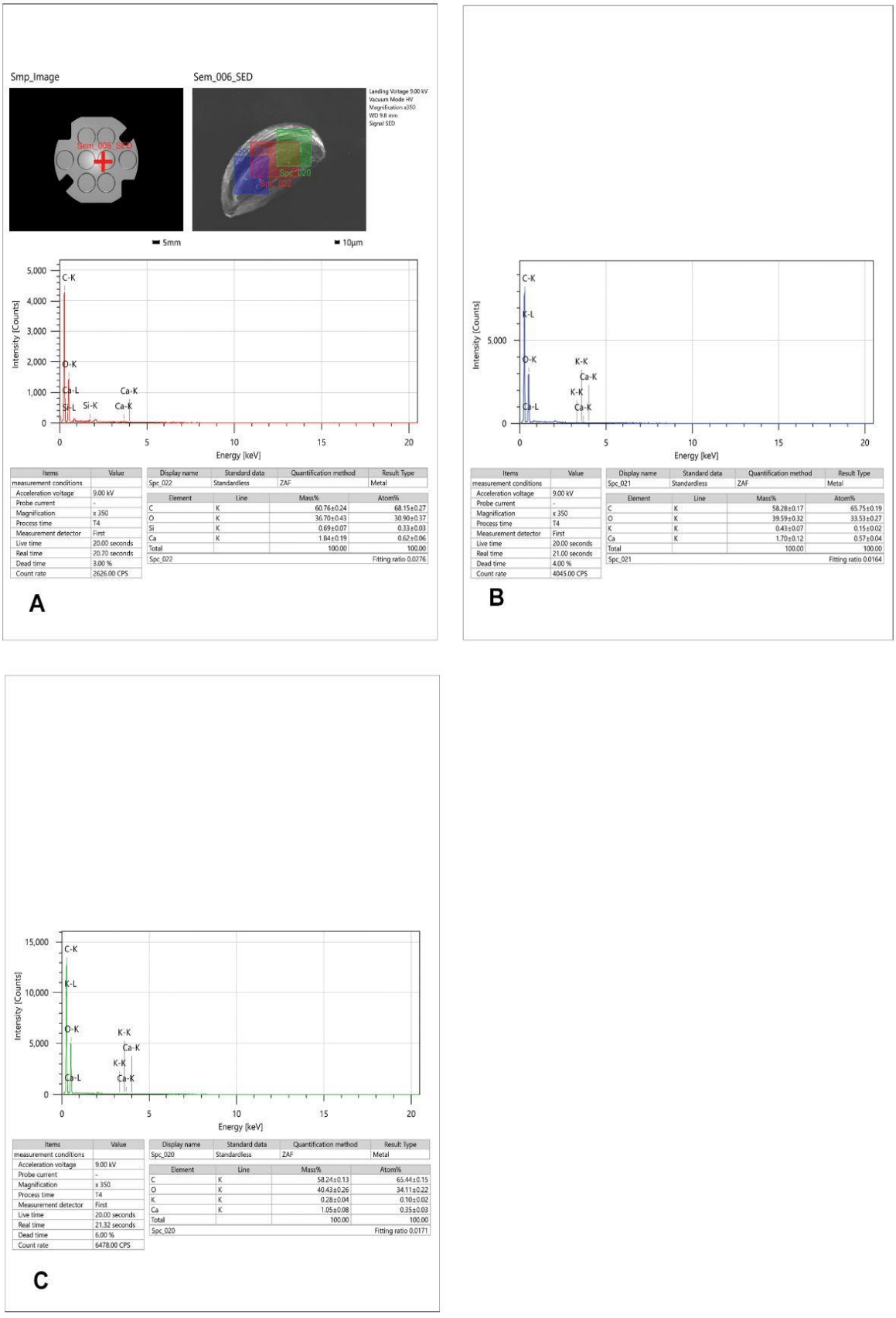

Fig. 23. EDAX profiling of seeds of *R. mexicana* (scale bar= 10µm)

A: Spc-022; B: Spc-021; C: Spc-020

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VI June 2026

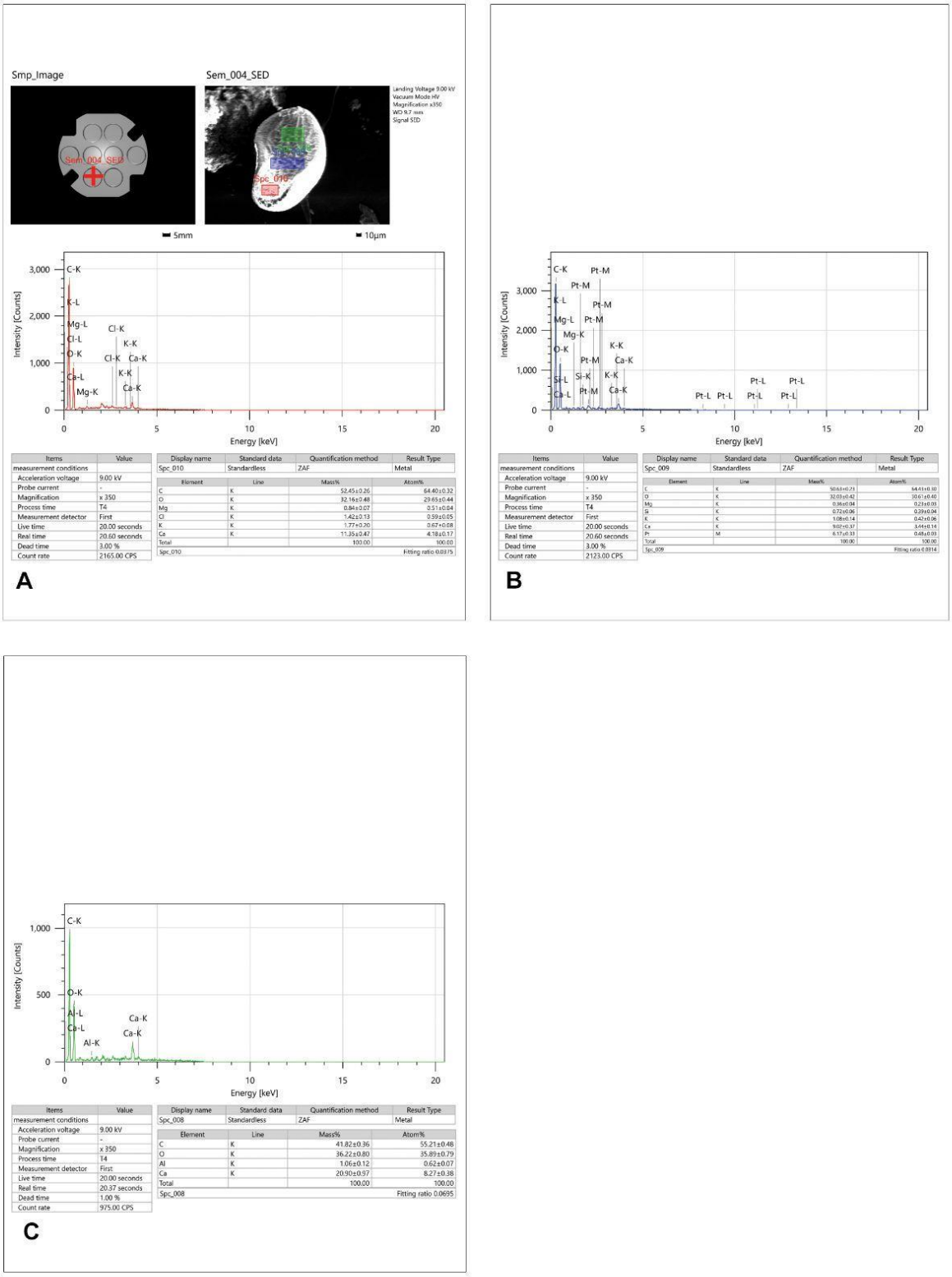

Fig. 24. EDAX profiling of seeds of *R. occultiflora* (scale bar= 10µm)

A: Spc-010; B: Spc-009; C: Spc-008

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue VI June 2026

CONCLUSION

From the above results, it can be seen that a clear cut distinction can be made between taxa based on the main external morphology of seeds. Seed morphological characters provide an aid in distinguishing various species and support their placement in different clades. The present study indicates that the use of seed morphological characters is a useful parameter for species identification in the genus *Rotala*. In addition to the morphological characters of seeds, texture and sculpturing of features of the external seed coat show variations among species and can be proved essential systematically. It can be concluded from the findings of the current studies that macro and micromorphological characteristics of seeds are considered diagnostic at both generic and specific level of plant species among the wide range of taxa. We recommend that future studies on *Rotata* plants with special reference to macro and micromorphological characters of seeds will provide a new perspective for understanding the habitat and dispersal features of the species. Moreover, the elemental composition of seeds of *Rotala* is significantly impacted by environmental variables, including the quality of water and soil. seed coats act as a source of various essential and nonessential elements, which may show slight variation in quantity according to the plant species. Lateritic soil is acting as a source of various elements. It may also affect the plant as it is usually growing in lateritic pools and marshy areas.

ACKNOWLEDGEMENTS

I express my gratitude to the authorities of St. Joseph's College, Devagiri(Autonomous), Kozhikkode, Kerala and Govt. College, Vidyanagar, Kasaragod. Kerala for providing essential laboratory facilities. I also extend my thanks to Dr. Biju P, Professor, Department of Botany, Govt. College, Vidyanagar, Kasaragod, Kerala for providing proper guidance during plant collection and identification.

REFERENCES

1. Alexander C. Martin & William D, Barkley, 1961. Seed Identification Manual.
2. Anto, P.V., Jacob, C.S., Abraham, P., Varghese, M.C. & Antony; I. 2014: A new species of Rotala L. (Lythraceae) from the lateritic hills of Thrissur district, Kerala, India. International Journal of Advanced Research 2(11): 532-535.
3. Barthlott, W. (1981). Epidermal and seed surface characters of plants: systematic applicability and some evolutionary aspects. Nordic Journal of Botany, l: 345-355.
4. Cook, C D K., 1978: The Hippuris syndrome; in Essays in plant taxonomy (ed. H E Street (London: Academic Press). pp. 163-176. Cook, C.K. 1979: A revision of the genus *Rotala* (Lythraceae). -Boissiera 29: 1-156.
5. Gaikwad, S.P., Sardesai, M.M. & S. R. Yadav. 2013: *Rotala sahyadrica* sp. nov. (Lythraceae) From Western Ghats, India. -Nordic Journal of Botany 32(5): 575-577. https://doi.org/10.1111/j.1756- 1051.2013.00322.x
6. Joseph, K.T. &Sivarajan, V.V. 1988: *Rotala cookii*: A new species of Lythraceae from India Showing *Hippuris* Syndrome. -Plant Systematics and Evolution 159: 141-144. https://doi.org/10.1007/BF00937431
7. Joseph, K. T. &Sivarajan, V.V. 1989: *Rotala* Linn. (Lythraceae) in peninsular India. Proc. Ind. Acad. sci. (Plant.Sei.) 99: 179-197. https://doi.org/ 10.1007/bf03053593 *Rotala*Linn. (Lythraceae) in peninsular India. Proc.
8. Koehne, A. 1903: *Rotala* in Das Pflanzenreich (ed, A Engler) (Lipzig: W Engelmann) 17. Heft (IV. 216), pp. 22-42.
9. Lemiya, K.M. & Pradeep, A.K. 2015: A new species of *Rotala* (Lythraeeae) from Kerala, India. — Rheedea 25: 159-163. https://dx.doi.org/10.22244/rheedea.2015.25.02.17
10. Mabberley, D.J. 2005: The Plant Book. Cambridge Univ. Press. https://doi.org/10.1017/9781316335581
11. Majeed, S., Zafar, M., Ahmad, M., Zafars S.. Ghufran, A., Ayoub, M., Sultana, Sh.s Yaseen, G., Raza, J., and Nabila, Morpho-palynological and anatomical studies in desert cacti (Opuntia dillenii and Opuntia monacantha) using light and scanning electron microscopy, Microsc. Res.

Tech.,2022, vol. 85, no. 8, pp. 2801-2812. https://doi.org/10.1002/jemt.24129

12. Mathew, S.P. &Lakshminarasimhan, P. 1990: *Rotala andamanensis*— A new species of Lythraceae from the Andaman Islands. -Bulletin of the Botanical Sunrey of India 32: 189-191.
13. Pradeep, A.K., Joseph, K. T. &Sivarajan, V.V. 1990: *Rotala malabarica*, A new species of Lythraceaefrom India. -Botanical Bulletin of Academia Sinica 31 59-6L
14. Prasad, K.S., Biju, P., Raveendran, K. & Bhat, K.G. 2012: (Lythraceae) from Kerala, India. -Nordic Journal of Botany 30: 59-60. *Rotala tulunadensis* sp. nov. https://doi.org/10.1111/j.1756-1051.2011.01275.x
15. Prasad, K.S. & K. Raveendran. 2013a: A New species of *Rotala* L. (Lythraceae) from Kerala, India. – Taiwania-58(2): 104-107. https://doi.org/10.6165/tai.2013.58.104
16. Prasad, K.S. & Raveendran, K. 2013b: *Rotala kasaragodensis*(Lythraceae), a new species from Kerala, India. -Edinburgh J. Botany 70(3): 451-454. https://doi.org/10.1017/S0960428613000140
17. Prasanth, A, &Sardesai, M,M, 2022: *Rotala biglandulosa*(Lythraceae), A new species of macrophyte from high-level ferricrctes of the northern Western Ghats, India, -Folia Geobotanica57(3): 201-212, https://doi.org/10.1007/s12224- 022-09418-x
18. Sunil, C.N., Shaju, T., Nandakumar, M. K., Sivadasan, M. &Alfarhan, A.H. 2014: *Rotala dhaneshiana*, a new species of Lythraceae from India. -Phytotaxa 188(4): 227-232. https://doi.org/10.11646/PHYTOTAXA.188.4.5
19. Sunil, C.N., Ratheesh Narayanan, M.K., Nandakumar, M.K., Jayesh, P.J., Abdul Jaleel, V. & Anil Kumar, N. 2013: *Rotala khaleeliana* sp. nov. (Lythraceae), A new species fromLateritic hills of Kannur, Kerala,India. -International Advanced Research 1(2): 14- 16.
20. Usma, A., Ahmad, M., Zafar, M., Ali, I.M., Kilic, O., Ozdemir, A.F., Sultana, S., Nazir, A.,Anjum, F., Kalsoom, N., 2019. Taxonomic significance of caryopsis in subfamily Panicoideae (Poaceae) using scanning electron microscopy and light microscopy. Microsc. Res. Tech. 82 (10), 1649–1659. https://doi.org/10.1002/jemt.23331
21. Watson, K.M. & Pradeep, A.K. 2015: A new species of *Rotala* (Lythraceae) from Kerala, India. - *Rheedea*, 25(2): 159-163. https://doi.org/10.22244/rheedea.2015.25.02.17
22. 22. Yadav, S.R., Malpure, N.V. &Chandore, A.N. 2010: *Rotala belgumensis*(Lythraceae) from the Western Ghats, India. -Nordic Journal of Botany 28: 499- 500. https://doi.org/10.1111/j.1756- 1051.2009.00612.x