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MORPHOLOGICAL, MICROMORPHOLOGICAL AND EDS STUDIES OF SOME LEGUMINOUS SEEDS OF CHANDRAPUR DISTRICT (M.S.), INDIA

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ABSTRACT: In this study morphological, micromorphological and Energy Dispersive X-Ray Spectroscopy (EDS) characterization of seeds belonging to 5 legume species *Albizia lebbeck, Cajanus cajan, Sesbania grandiflora, Crotalaria retusa* and *Crotalaria verrucosa* were investigated. Various morphological characteristics of seed like shape, size, surface texture, colour, hilum features, mass, etc. were studied. Biometric analysis of seeds was carried out to obtain seed length, width and mass. Micromorphological details, particularly seed coat ornamentation/ spermoderm pattern was studied with the help of Scanning Electron Microscopy (SEM). Using EDS, elemental analysis of sample seeds was carried out. Seeds of studied species differ in morphology, biometry, spermoderm micromorphology and elemental composition. The study revealed that these differences can be useful in the identification of species.

Key words: - legume, morphology, biometry, micromorphology, scanning electron microscopy, energy dispersive x-ray spectroscopy..

INTRODUCTION :

Leguminosae or Fabaceae is the third largest and most economically important family of flowering plants with approximately 19,400 species (Lewis *et al.,* 2005). It comprises vegetables, crops, ornamental trees, timber, fodder crops and weeds plants (Yahara *et al.,* 2013). Family Leguminosae has been divided into the following three subfamilies: Caesalpinioideae, Mimosoideae, and Papilionoideae (Du Puy *et al.,* 2002). Leguminosae members are characterized by the distinctive fruit, called a legume, which gives the family its original name. The family produces a broad variety of seeds with many valuable taxonomic characteristics.

Seeds have been found to possess characteristics for the analysis of taxonomic relationships not only at the level of genera and species but have shown to be diagnostic for families (McClure, 1957). Seeds exhibit great diversity in their morphological features such as shape, size, surface sculpturing, texture, colour and chemical constituents. All these features help in the understanding of seed nature, origin, occurrence and ecological condition, evolution and may also be used as a tool in the identification and classification of taxa. Among these morphological features of seeds have been used in various scientific studies to establish taxonomic relationships in a variety of plant families (Takhtajan, 1991; Zhang *et al.*, 2005; Gontcharova *et al.,* 2009). In addition to the gross morphology of seeds, seed coat micromorphological (sculpture) details are quite variable between different species and can be of systematic importance (Gohary and Mohammed, 2017). Exomorphic features revealed by scanning electron microscopy (SEM) provide a great tool to achieve more accurate seed identification (Brisson and Peterson, 1976; Heywood, 1971). Spermoderm pattern analysis

observed under the SEM has been well recognized as a reliable approach for establishing phenetic relationships and identification of species or taxa (Barthlott, 1981; Javadi and Yamaguchi, 2004). Elemental compositions of seeds may be considered as another criterion to differentiate the species. The elemental composition of seeds can be studied with the help of Energy Dispersive X-Ray Spectroscopy (EDS/ EDX) analysis (Memecylon *et. al.* 2021). Among the plants, Leguminosae is an extremely diverse family of flowering plants. Various seed morphological studies of leguminous taxa have been employed from time to time (Salimpour *et al.* 2007; Al-Ghamdi and Al-Zahrani, 2010). The present work has been formulated to study the diversity in morphology, micromorphology and elemental composition of leguminous seeds belonging to 5 legume species *Albizia lebbeck, Cajanus cajan, Sesbania grandiflora, Crotalaria retusa* and *Crotalaria verrucosa* growing in Chandrapur district of Maharashtra, India.

MATERIALS AND METHODS:

Fresh plant samples of 5 legume species *Albizia lebbeck, Cajanus cajan, Sesbania grandiflora, Crotalaria retusa* and *Crotalaria verrucosa* were collected from different sites of Chandrapur district (M.S.) Identification of collected plant specimens was done by referring to different floras, books and relevant journal articles, and also help from the expertise available in the relevant field will be sought. About 15-20 mature seeds of each species were obtained by manually separating them from a legume. For further study, seeds were cleaned with alcohol to avoid any alternation in the micromorphological features. Various morphological characteristics of seed like shape, size, surface texture, colour, hilum features, mass, etc. were studied. A biometric analysis of seeds was performed to determine mass and size. Micromorphological details, particularly seed coat ornamentation/ spermoderm pattern was studied with the help of JOEL-JSM-7610F Scanning Electron Microscopy (SEM). Terminologies used to describe seed coat ornamentation/ spermoderm patterns are that of Lersten (1981). Using EDS, elemental analysis of sample seeds was carried out.

RESULTS AND DISCUSSIONS:

Seed morphological features of studied taxa (Table-1) showed variation in shape, size, surface texture, colour, hilum features, mass etc. Variation in seed shape was observed in investigated taxa i. e. ovate, orbicular and oblong in *Albizia lebbeck, Cajanus cajan* and *Sesbania grandiflora* respectively. While in *Crotalaria retusa* and *Crotalaria verrucosa* it was characteristically kidney-shaped. The texture of the seed was found glabrous in all studies species. The colour of seeds has diagnostic and systematic interest among the taxa. Three different colours were noted i.e. light brown, brown and creamer. In *Albizia lebbeck* it was light brown, in *Cajanus cajan, Sesbania grandiflora* and *Crotalaria retusa* it was brown while creamer in *Crotalaria verrucosa.* Hilum shape in studied taxa was found oval, oblong, circular and sunken. The colour of the hilum varies from dark brown, creamer to white. Central hilum position was noted in all the seeds studied. *Albizia lebbeck, Cajanus cajan* and *Sesbania grandiflora* were found to have rounded hilum poles while in *Crotalaria retusa* and *Crotalaria verrucosa* acute hilum poles were recorded. The highest mass of the seed of seeds was found in *Albizia lebbeck* (150 mg) while the lowest mass was found in *Crotalaria retusa* (10 mg) (Fig. 1). *Albizia lebbeck* was found to have the largest seed (12 × 7.7 mm) among the taxa examined followed by *Cajanus cajan* (8 × 7mm). Length and width ratio of *Sesbania grandiflora, Crotalaria retusa* and *Crotalaria verrucosa* was found 6 × 5 mm, 5.4 × 3.9 mm and 5.1 × 3.7 mm respectively (Fig. 2).

The study of seed coats revealed many important micromorphological characteristics of systematic importance. In the present work scanning electron microscopic (SEM) study was used to reveal micromorphological details. Micromorphological features among studied taxa show three basic seed coat patterns (Table-2). In *Albizia lebbeck* (Fig.3. A, B and C) and *Cajanus cajan* (Fig.4. A, B and C) it was found regulate. Seeds of *Sesbania grandiflora* showed a multi-reticulate seed coat pattern (Fig.5. A, B and C). In *Crotalaria retusa* (Fig.6. A, B and C) and *Crotalaria verrucosa* (Fig.7. A, B and C) levigate was noted.

Elemental analysis of seeds by Energy Dispersive X-Ray Spectroscopy (EDS/ EDX) of examined taxa revealed variation in elemental concentration (Table-3; Fig. 8-12). Carbon (C) and oxygen (O) were noted in the large amount in all the seed samples examined. In *Albizia lebbeck* C, O, Ca, K, S and Cl (Fig. 8), *Cajanus cajan* C, O, Ca, K, Cl, and Na (Fig.9), *Sesbania grandiflora* C, O, Ca and K (Fig.10), *Crotalaria retusa* C, O, Ca, K and Mg (Fig. 11), *Crotalaria verrucosa* C, O, Ca, K,Cl, Na and Mg (Fig.12) elements were detected.

In this study, we observed variation in seed morphology, micromorphology of seed coat and elemental composition in the seeds of examined taxa. Skvortsov and Rusanovitch (1974) stated that the seed coat surface features are genetically determined and are the main source of intra- or interspecific variation. According to Lersten (1981), the spermaoderm pattern reflects epidermal configuration and cuticular deposition as influenced by seed expansion. Gandhi *et al.* (2011) concluded that seed coat patterns may be use for species identification. Morphological and SEM studies revealed that seed coat diversity among different species to be characteristics of each species. The present study provides certain useful characteristics which may be useful for delimiting species. Morphological features, SEM features and EDS analysis may prove helpful in the accurate identification of seeds.

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Table 1: Morphological features of seeds.

Seed feature	Plant species				
	Albizia lebbeck	***Cajanus cajan***	***Sesbania grandiflora***	***Crotalaria retusa***	***Crotalaria verrucosa***
Shape	Ovate	Orbicular	Oblong	Kidney	Kidney
Texture	Glabrous	Glabrous	Glabrous	Glabrous	Glabrous
Colour	Light brown	Brown	Brown	Brown	Creamer
Hilum shape	Oval	Oblong	Circular	Sunken	Sunken
Hilum colour	Dark brown	Creamer	Creamer	White	White
Hilum position	Central	Central	Central	Central	Central
Hilum poles	Rounded	Rounded	Rounded	Acute	Acute
Mass (mg)	150	130	70	10	20
Size (mm)	12 × 7.7	8 × 7	6 × 5	5.4 × 3.9	5.1 × 3.7

Table-2. Seed coat pattern observed in examined seeds.

Species	Testa topography patterns observed
Albizia lebbeck	Rugulate
Cajanus cajan	Rugulate
Sesbania grandiflora	Multi-reticulate
Crotalaria retusa	Levigate
Crotalaria verrucosa	Levigate

Table-3 Comparative Wt % of detected elements in EDS spectra of examined seeds.

Species	Wt % of Detected elements								
	C	O	Ca	K	S	Cl	Na	Mg	Total
Albizia lebbeck	59.2	37.3	1.3	1.2	0.6	0.4	--	--	100
Cajanus cajan	55.3	40.9	1.0	0.7	--	1.0	1.1	--	100
Sesbania grandiflora	53.2	43.8	1.6	1.4	--	--	--	--	100
Crotalaria retusa	47.5	46.4	1.2	3.8	--	--	--	1.0	100
Crotalaria verrucosa	44.5	39.6	3.8	8.7	--	0.8	0.8	1.7	100

Fig.1 Comparative mass of seeds in milligrams.

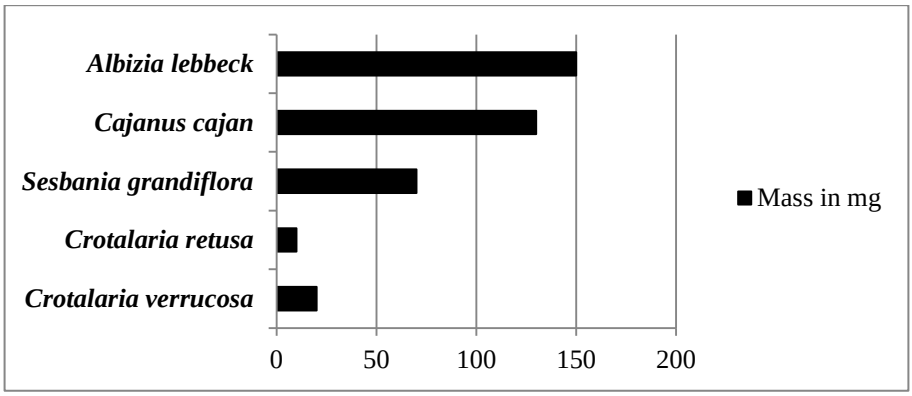

Fig.2 Comparative size of seeds and thickness in millimetres.

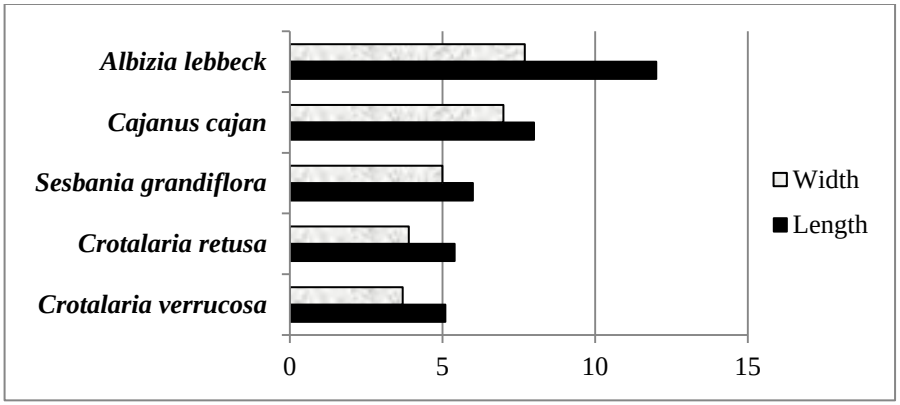

Fig. 3. Scanning electron microscopy study of legume seed *Albizia lebbeck* (A, B and C).

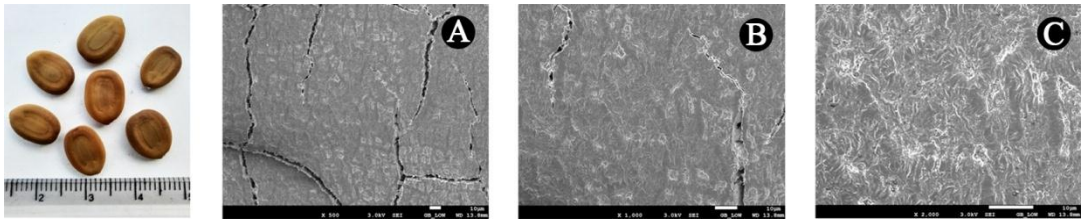

Fig. 4. Scanning electron microscopy study of legume seed *Cajanus cajan* (A, B and C).

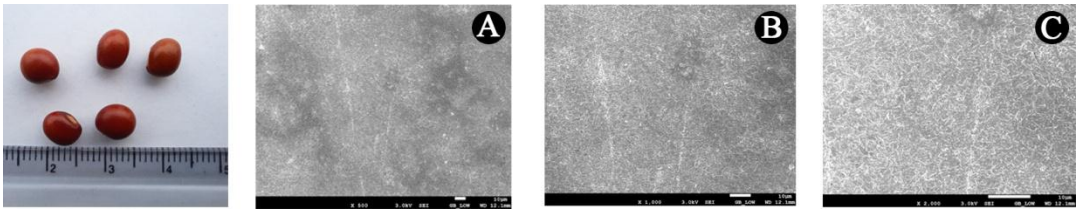

Fig. 5. Scanning electron microscopy study of legume seed *Sesbania grandiflora* (A, B and C).

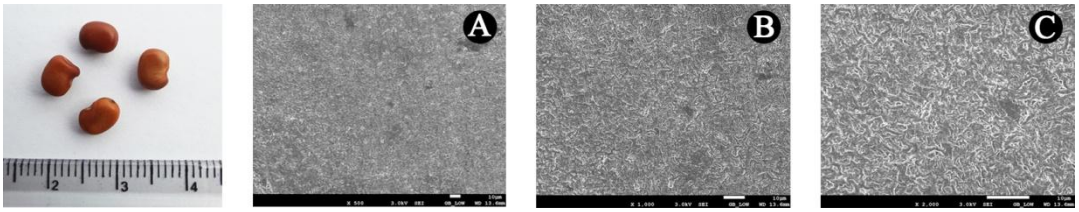

Fig. 6. Scanning electron microscopy study of legume seed *Crotalaria retusa* (A, B and C).

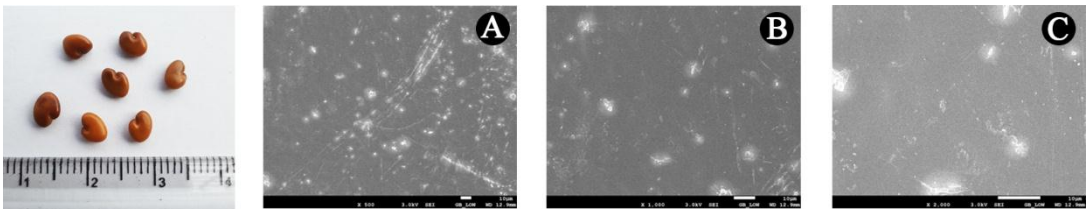

Fig. 7. Scanning electron microscopy study of legume seed *Crotalaria verrucosa* (A, B and C).

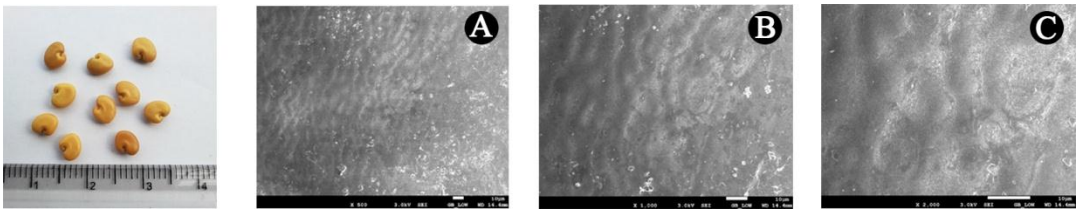

Fig. 8. EDS spectrum of *Albizia lebbeck* seed.

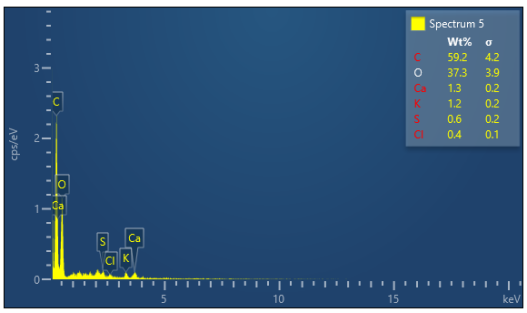

Fig. 9. EDS spectrum of *Cajanus cajan* seed.

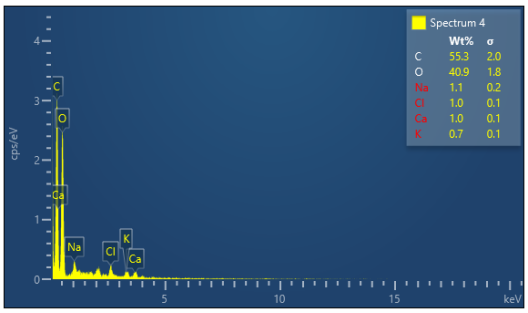

Fig. 10. EDS spectrum of *Sesbania grandiflora* seed.

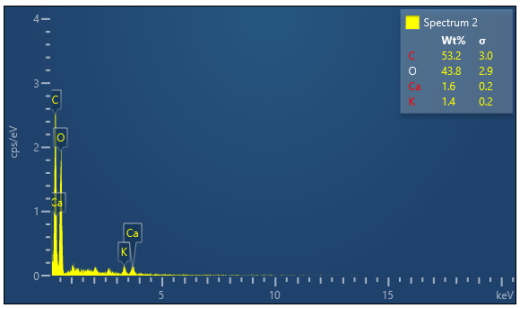

Fig. 11. EDS spectrum of *Crotalaria retusa* seed.

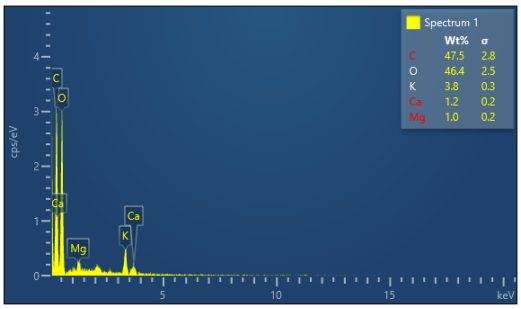

Fig. 12. EDS spectrum of *Crotalaria verrucosa* seed.