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E: ISSN NO.: 2455-0817 *Remarking An Analisation*

Comparative seed coat anatomy of three Closely Related Bean Genera *Dolichos lablab*, *Mucuna pruriens* and *Canavalia gladiata* to Find out the Basic or Key Anatomical Features

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Abstract

Anatomical variations in seed structure are generally found at species, sub-species and botanical variety level. Several workers have done extensive study on seed coat anatomy and comparative histogenesis on various seed tissues of members of family Fabaceae emphasizing the importance of comparative anatomy and structure of seed as aid to phylogenetic and taxonomic consideration in various angiosperm families. In present investigation the structure of seeds and seed coat in legumes has been studied by microtomy because work in pulses deserves special mentions here which have given a key for identification of Indian pulses with the help of seed structure and seed coat anatomy.

Keywords: Seed coat, Microtomy, Anatomy and identification.

Introduction

The three bean genera *Dolichos lablab, Mucuna pruriens* and *Canavalia gladiata* had been unprivileged legumes and not much work has been done despite of their immense utility as food, fodder, medicine, green manure and conservation in tropical environments with a summer rainfall etc. On the basis of study of leguminous crops reported that genus *Dolichos* includes about sixty species of mostly large trailing plants distributed throughout the tropics. The most important is *Dolichos lablab* which occurs in India (Cobley, 1956).In present investigation the emphasis had been given on anatomical features of seed coat in three well known but unprivileged bean genera, *Dolichos lablab*, *Mucunap ruriens* and *Canavalia gladiata* and tried to find out the basic or key anatomical features which could be used as identification features in the three bean genera. The anatomical features of macrosclerids, osteosclerids and hour glass cells formed the base of distinction in three bean genera under consideration.

Material & Methods

Anatomical studies were made by section cutting of developing and mature seeds with the help of Rotary Microtome. Procedure for preparing microtome slides is given below:

The pods at early and late stages of development were taken from each genotype on bright sunny days and cut into small pieces to submerge in formalin acetic acid alcohol (FAA) prepared in the following proportion:

Ethyl alcohol 70% = 90 ml.
↓
Formaldehyde solution = 5 ml.
↓
Glacial acetic acid = 5 ml.

Selected pieces of pods fixed in FAA were subjected to further dehydration in alcohol-xylol series to remove moisture and alcohol respectively.

After dehydration the pieces of pods containing developing and mature seeds were placed in the infiltration tubes containing xyline and

paraffin wax of low melting point (52 C). These tubes before transferring into the oven at 60-70 C were placed in sunlight or under the table lamp to make a homogenous solution of xyline and wax.

Paper boats were used for embedding of infiltrated material. Folding up of herbarium paper sheets made these boats. Paraffin wax 60 C melting point was poured in to the paper boat coated with glycerin and thereafter; infiltrated pods and seeds were immediately arranged in linear manner.

The pieces of paraffin along with material were mounted on the block holder with the help of a warm scalpel and dipped in cold water, when the paraffin became completely hard the blocks were trimmed using a sharp edged razor blade and excess paraffin from around the blocks was removed one after the other.

For microscopic observations cut sections were stained in haemotoxylin and light green stain (Johensen, 1940) using cold method.

Mounting was done in Canada balsam, which is diluted by adding tertiary butanol. Xyline was used to dip cover glass before putting on slide for perfect spreading of Canada balsam.

Aim of the Study

The present study was carried out to use the anatomical structure of seed and fruit as tool of identification in *Dolichos lablab*, *Mucuna pruriens* and *Canavalia gladiata* cultivars.

Results and Discussion

Anatomical variations in seed structure are generally found at species, sub-species and botanical variety level. Several workers have done extensive study on seed coat anatomy and comparative histogenesis on various seed tissues of members of family Fabaceae like Singh (1964), Wunderlich (1967) and Corner (1976) who had emphasized the importance of comparative anatomy and structure of seed as aid to phylogenetic and taxonomic consideration in various angiosperm families but the work of Chowdhury and Buth (1970) deserves special mention here who have given a key for identification of Indian pulses with the help of seed structure and seed coat anatomy. Structure of seeds and seed coat in legumes has peculiar characters and are stable in wide geographical and climatic conditions and hence amenable for identification as stated by Begchi and Tripathi (1986) after conducting scanning electron microscopy.

In present investigation the emphasis had been given on anatomical features of seed coat in three well known but unprivileged bean genera, *Dolichos lablab*, *Mucuna pruriens* and *Canavalia gladiata* and tried to find out the basic or key anatomical features which could be used as identification features in the three bean genera. The anatomical features of macrosclerids, osteosclerids and hour glass cells formed the base of distinction in three bean genera under consideration.

The seed coat hardness and thickness was maximum in *Canavalia gladiata* followed by *Mucuna prurines* and *Dolichos lablab* because of impregnation of tissues. The shape of palisade layer was almost same but the size of the palisade cells varied and was noticed maximum in *Canavalia gladiata* followed by *Dolichos lablab.* Palisade layer was confluent in *Canavalia gladiata* and it was absent in *Dolichos lablab* and *Mucuna pruriens.* Studies showed that cell walls of the palisade layer contribute to the mechanical strength of the seed coat. The findings are supported by Patel (1976) and Idu *et al*. (2002). The hourglass cells in *Dolichos lablab* and *Canavalia gladiata* had equal ends and in *Mucuna pruriens* palisade cells had unequal ends. The hourglass cells in the seed coat of *Dolichos lablab* were interesting in that they appear to serve as a reservoir for proteins. The presence of numerous starch grains in the hourglass cells indicated that during embryogenesis the seed coat could synthesize nutrients for the developing embryo.

According to Vijayambika *et al*. (2011) size and shape of rim-aril found useful in delimiting the bean taxa. In *Dolichos lablab* the rim-aril is cushion like and prolonged on the sides of lens. In *Mucuna lablab* wing shaped rim-aril is present where as curved and stout rim-aril was present in *Canavalia gladiata* Findings related to structure, morphology and vascular supply have also been described by Netolitzky (1926) and Corner (1951, 1976). The tracheid or vascular bar showed distinguishable differences in thickening patterns in tracheids. In *Dolichos lablab* tracheids bar was present below the hilar groove and the tracheids of the bar were highly irregular and had reticulate thickening.

The observation of microtomical sections of *Dolichos lablab*, *Mucuna pruriens* and *Canavalia gladiata* through seed coat showed the characteristics of typical legumenous seeds. However, no considerable differences were observed among accessions of the bean genera reported but at genera level considerable differences' were observed. The seed coat was having different layers as described below [Fig. 1(A-F)].

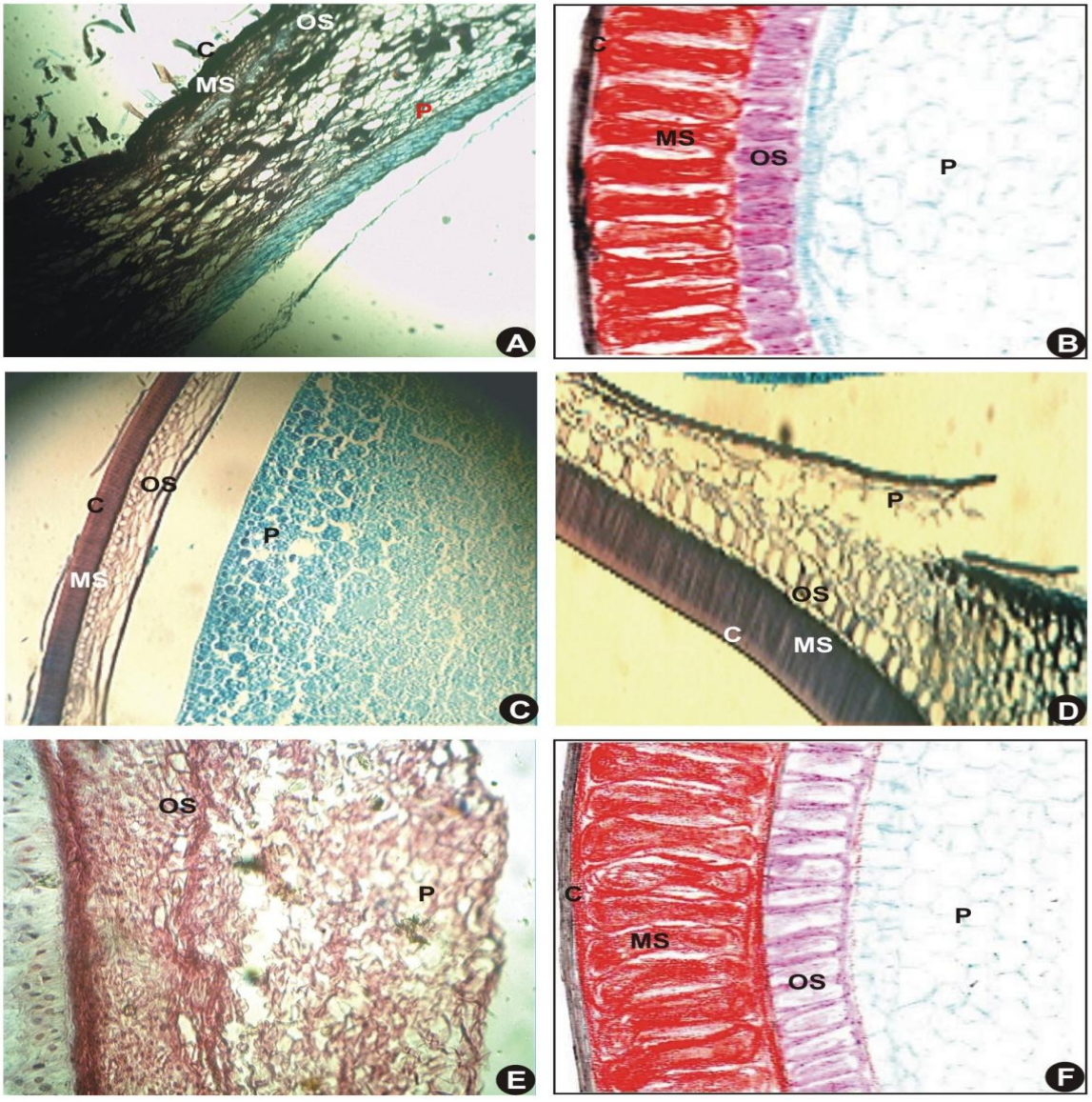
In *Dolichos lablab* (Fig.1 A-B), *Mucuna pruriens* (Fig. 1 C-D) and *Canavalia gladiata* (Fig. 1 E-F) species the outermost layer of the seed coat was thick cuticle (C) which was followed by palisade layer consisting of single layer of Macrosclereids (MS) except under the hilum, where two layers occurred. They are elongate perpendicular to the surface of the seed and had dumb-bell or oval shape. The macrosclereids are compactly arranged leaving no inter-sclereid spaces. The important feature of the palisade cells was that they were bulbous away from cuticle. However, the size of macrosclereids in all the three genera showed variation and their size was greater in *Canavalia* genotypes than the other two genera.

Next to macrosclerid (MS) a layer of bone shaped cells called osteosclereids (OS) cells was present which also called as hourglass cells. This layer seems to be arisen from the outer cell layer of the inner integument. They are usually larger than adjacent cell layers and are separated by wide intercellular spaces, except under the hilum cleft where they are absent. The hourglass cells in *Dolichos* and *Canavalia* genotypes had equal ends and in *Mucuna* genotypes palisade cells had unequal

ends. The hourglass cells in the seed coat *Dolichos* genotypes were interesting in that they appear to serve as a reservoir for proteins. They were found uniformly distributed throughout the seed coat, except in the area of the hilum, where a smaller number of layers can be distinguished. In mature seed coats, the interior parenchyma is often crushed or partially crushed as the embryo expands.

In *Mucuna pruriens* and *Canavalia* seed coats next to the osteoscerid layer (hourglass cells) parenchymatous layer was seen which is consisted of thin walled branched parenchyma cells called as stellate parenchyma which provide hardness and mechanical strength to seed testa and also maintain seed dormancy.

Fig.1 (A-F) T.S. of seed coat of *Dolichos lablab* (A-B), *Mucuna pruriens* (C-D) and *Canavalia gladiata* (28 E-F). MS-Macrosclereids, OS- Osteoscleids (Hourglas cells), P- parenchymatous and C- Cutile



Conclusion

In present investigation the emphasis was given given on anatomical features of seed coat in three well known bean genera, *Dolichos lablab,Mucuna pruriens*and *Canavalia gladiata* and tried to find out the basic or key anatomical features which could be used as identification features in the three bean genera. The anatomical features of macrosclerids, osteoscleridsand hour glass cells formed the base of distinction in three bean genera under consideration.

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