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COMPARATIVE INVESTIGATION OF CALCIUM OXALATE CRYSTALS RELATED TO STARCH STORAGE IN THREE VARIETIES OF *DIOSCOREA ALATA* LINN. OF WAYANAD DISTRICT

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ABSTRACT: Yam is one of the staple foods for several people, used as various forms such as flour and starchy paste. It is locally available, rich source of starch so tubers of *Dioscorea* ensure rural food security. The member of the family Dioscoreaceae is found throughout the tropical and warm temperate regions of the world. Calcium oxalate crystals and missile like raphides are reported in many Yam species especially *D. rotundata*. The presence of calcium crystals is related to starch storage. In the present study calcium oxalate crystals are found in the form of raphides are observed in three varieties of *Dioscorea alata* (*Ginger kachil, Red kachil* and *Neendi kachil*). Microscopical evaluation of stem is done with the help of Leica M 80 Stereomicroscope. The structure and position of raphides are different in each variety. Three varieties of *Dioscorea alata Ginger kachil*, *Red Kachil* and *Neendi kachil*, (these term were used by Pulaya Communities of Wayanad, based on the morphological features of stem tuber) are used for the present study. In *D. alata*, (*Ginger kachil*) raphides are only seen in pith along the parenchymatous wall. Rahides are found along the cortex in *D. alata (Red kachil*) in the form of vertical needle like structure. In *Red Kachil*, raphides are not observed in pith. In *D. alata Neendi kachil*, raphides are found along the cortex as well as pith. Two different types of raphides are observed in this variety, vertical form in pith and clumped form in cortex. Among the three varieties of *Dioscorea alata*, the quantity of raphides is more in *D. alata* var. *Neendi kachil*.

INTRODUCTION: Yam is one of the staple foods for several people, used as various forms such as flour and starchy paste. It is locally available, is rich source of starch so tubers of *Dioscorea* ensures rural food security. Tubers produced from *Dioscorea alata, D. bulbifera, D. caryensis, D. dumetorum, D. esculenta, D. hispida, D. opposita, D. rotundata and D. trifida* serving as food stuff for the people in the Far East [1].

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Dioscorea species are well known for the presence of steroidal sapogenin (Diosgenin) precursor for the synthesis of steroidal drugs. Diosgenin is a steroidal sapogenin used in oral contraceptives. There were nineteen species of *Dioscorea* has already been reported in Kalpetta, Wayanad from various studies [2, 3, 4]. More than 25 wild plant species/types in Wayanad are known for edible roots, tubers and rhizomes and are eaten by the tribe and non-tribe communities of the district. *Dioscorea* are rich in starch, but it have no better position in our food system because the place of yam was replaced by other tubers such as cassava, sweet potato *etc*. In South America the position and culture of yam has been replaced by other varieties such as wheat, corn, bean and rice varieties [5].

But now days few species of *Dioscorea* are cultivated as food crops such as *D. rotundata, D. alata, D. caryensis, D. dumetorum, D. esculenta, D. bulbifera etc. Dioscorea* are rich in starch [6, 7] and vitamins [8] *etc*. The members of the family Dioscoreaceae is found throughout the tropical and warm temperate regions of the world. Calcium oxalate crystals and missile like raphides are reported in many Yam species especially *D. rotundata*. The presence of calcium crystals is related to starch storage. In the present study calcium oxalate crystals are found in the form of raphides are observed in three varieties of *Dioscorea alata* (*Ginger kachil*, *Red kachil* and *Neendi kachil*). The role calcium oxalate crystals in each plant are different. Even in the different tissues, its function may vary. Its functions are removal of toxic compound; act as reservoir of calcium, plant pathogen defense, tissue support *etc*.[9, 10, 11].

MATERIALS AND METHODS: According to Benthem and Hookers classification, *Dioscorea* belongs to Monocotyledonae under the series Epigynae. Identification of different species of *Dioscorea* is very difficult. Its climbing patterns, nature of stipule, bracts, shape of tubers are main key characters for identification. Different varieties of *D. alata* are set up from Wayanad. They are *Chuvappu kachil, Ginger kachil, Neendi kachil, Thunnan kachil, Urullan kachil, Kuyikka vitthu, Quinten kachil, Kaduvakayyan kachil, Parichakodan kachil, Vazhavadakkan kachil* and *Kolli kachil.* The weight of each *dioscorea* varies based on ecological factors; rich fertile soil produces large tubers. So weight of tubers is not significant character throughout the identification.

During favourable condition, *ginger kachil* produces tubers as much as *Quinten kachil*. So weight of the tubers is not a criterion for identification. So the following taxonomic characters are used for identification; Climbing plants with a fleshy tuberous root stock, Leaves are opposite or alternate with reticulate leaves, Flowers regular, small and minute, usually unisexual flowers with inferior ovary, perianth tubular, male flower -stamens 3 or 6, trimerous, female flower - 3 or 6 staminodes, trimerous, ovary inferior, fruit berry or valved capsule. The morphology of tubers has a great role in identification.

The characters of *red kachil* are the outer part of this tuber is in color of beetroot. It is also called as *Chora kachil, Blue kachil*. It does not possess particular shape. When it is possessed in boiling water it gives a pleasant smell. But in the case of *Ginger kachil* tuber characters are different from that of *Red kachil*, it produces ginger like tubers; outer in yellow colour, where as inner part is white in colour. Each plant produces more than two tubers. *Neendi kachil* produces long tuber, which is brownish black in colour. Its more peculiar characters are climbing part is covered with spines. After proper identification plant materials are collected from Wayanad for microscopical evaluation.

Fresh plants of *Dioscorea alata* (three varieties *Ginger kachil*, *Red kachil* and *Neendi kachil*) were collected from various parts of northern Kerala. Transverse section of five different plants of same variety is used for the study. Sections were made using sharp blade from the fresh material. Climber is cut into serial sections, washed in double distilled water. Next step is staining, is done with the help of saffranin. Place a drop of glycerin on a specimen in order to avoid dehydration. Then pass a clean cover slip through spirit lamp flame and then place on the drop of glycerine. The stained sections were observed under Leica M 80 Stereo microscope.

RESULTS AND DISCUSSION: Transverse section of the three varieties is circular in outline. In *Ginger kachil*, TS of stem is circular with two wings (**Fig. 1A** & **B**). Where as in *Red kachil* and *Neendi kachil* TS of stem is 3 angular and 4 angular respectively (**Fig. 1C** & **Fig. E**). The cuticle is generally thin in nature. The epidermis is made up of thin parenchymatous cells. Hypodermis is composed of chlorenchymatous cells. Cortex is divided into two; outer cortex sclerenchymatous and inner cortex parenchymatous. Vascular bundles of the stem are arranged in two concentric circles. Outer and inner ring is composed of two metaxylem elements with phloem. The number of vascular bundle varies from varieties to variety (**Fig. 1A**, **C** & **E**).

The structure and position of raphides are different in each variety. In *D. alata*, (*Ginger kachil*) raphides are only seen in pith along the

parenchymatous wall (**Fig. 1A** & **B**). Rahides are found along the cortex in *D. alata (Red kachil*) in the form of vertical needle like structure. In *Red kachil*, raphides are not observed in pith (**Fig. 1C** & **D**). In *D. alata Neendi kachil*, raphides are found along the cortex as well as pith. Two different types of raphides are observed in this variety, vertical form in pith and clumped form in cortex (**Fig. 1E** & **F**). Among the three varieties of *Dioscorea alata*, the quantity of raphides is more in *D. alata* var. *Neendi kachil*.

The tubers of *Dioscorea* posses several medicinal properties such as antimicrobial, antioxidant and hypoglycemic activities. Due to these properties they are used against the diseases such as digestive disorder, diarrhoea, irritability, abdominal pain and wound burns *etc*. [12] The antifungal activity was also reported in two species of *Dioscorea* [13]. *Dioscorea* are considers as rich source of different secondary metabolites such as alkaloids, saponins, cholestrol, flavonoids *etc*. [13]

Based on morphology crystals are classified into four types crystal sand, raphide, druse, styloid and prismatic [9, 14, 11]. (These crystals are formed endogenously; first synthesizing oxalic acid and combined with calcium to produce calcium oxalate crystals [11]. Calcium oxalate crystals in the form of raphides are found in three varieties of *D. alata*.

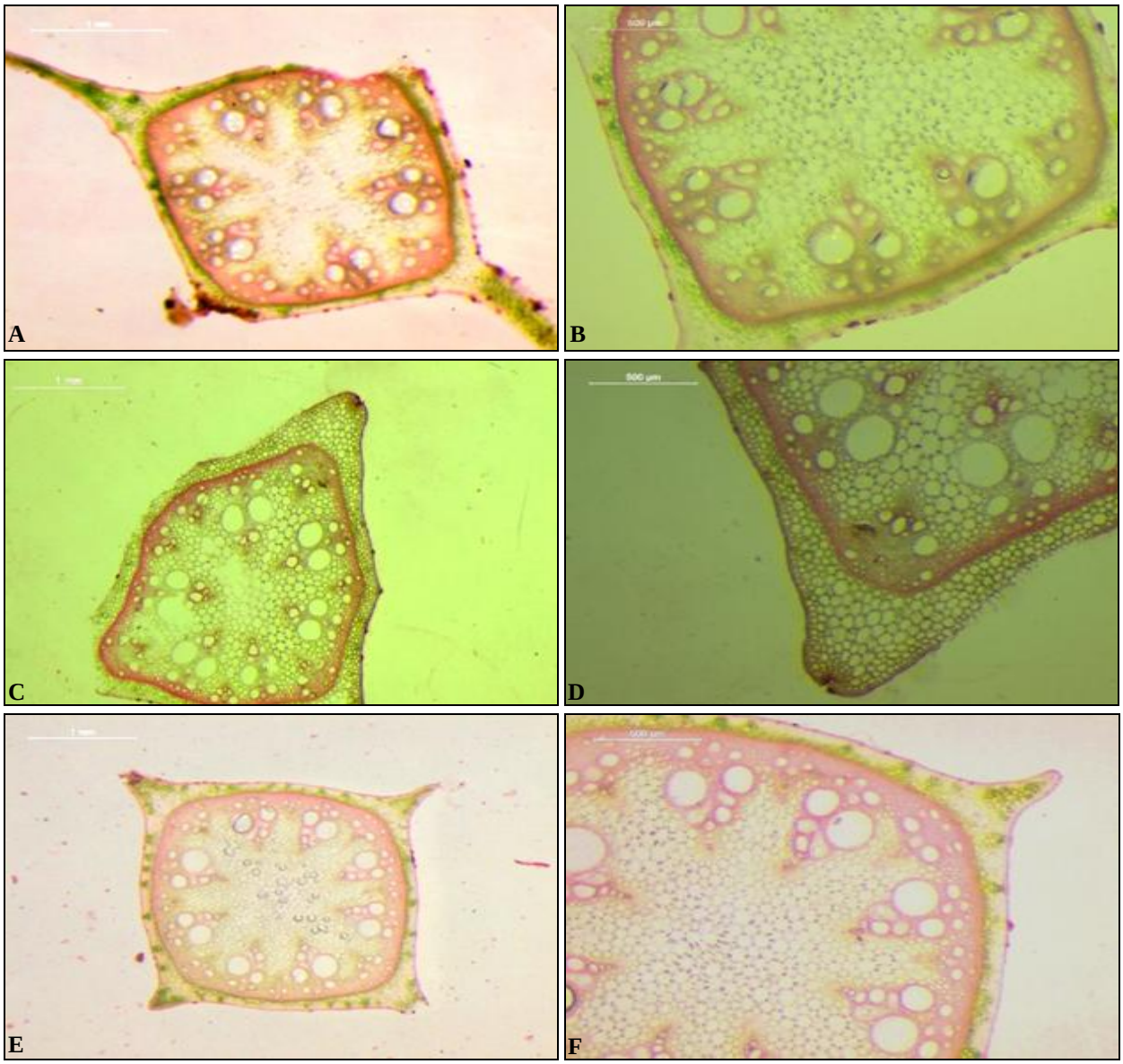

FIG. 1: A: STEM TS OF *D. ALATA GINGER KACHIL*; B: ENLARGED VIEW OF STEM TS; C: STEM TS OF *D. ALATA RED KACHIL*; D: ENLARGED VIEW OF STEM TS; E: STEM TS OF *D. ALATA NEENDI KACHIL;* F: ENLARGED VIEW OF STEM TS

CONCLUSION: The present study reveals that raphides are common in three varieties of *Dioscorea alata*, but differ in their structure and occurance. In the variety *Dioscorea alata var Ginger kachil*, raphides are absent in cortex, where as in *red kachil* raphides are absent in pith.In the case of *Dioscorea alata var*. *Neendi Kachil* raphides are present in both cortex and pith. Based on the shape, two different types of raphides are observed in *Dioscorea alata Neendi kachil* - clumped form as well as vertical needle like form.

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CONFLICT OF INTEREST: The authors declare that there is no conflict of interest regarding the above article.

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