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ANATOMY AND HISTOCHEMICAL LOCALIZATION OF CALCIUM OXALATE CRYSTALS IN PETIOLES OF FIVE VARIETIES OF *COLOCASIA ESCULENTA* (L.) SCHOTT

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ABSTRACT
Anatomy and histochemical localization of calcium oxalate crystals in petioles of five varieties of *Colocasia esculenta* (L.) Schott present in Anambra State, Nigeria were carried out using standard methods; with the intention of revealing their anatomical characters and occurrence of calcium oxalate crystals, which can serve as important taxonomic characters in delimitation of the species. The arrangement of the pores/vessels of all the varieties were exclusively solitary, with the exception of pores of 'ogeriobosi' which were in clusters. The size of the vessels varied. Crystal sand was found in petioles of *C. esculenta* var. *esculenta* and 'nwine'. Raphides were present in all the varieties, with the exception of 'nwine'. Styloid was only found in *C. esculenta* var. *antiquorum*. Prismatic oxalate crystals (rhombohedral) were detected in all the varieties. Druses and rosettes were only present in 'kochuo'. The findings indicated that the anatomy and occurrence of calcium oxalate crystals in petioles of varieties of *C. esculenta,* can be employed as additional taxonomic information in delineating the species.

KEYWORDS: Crystal sand, druses, raphides, rosettes, styloids, eddoe, dasheen, 'kochuo', 'nwine', 'ogeriobosi'.

INTRODUCTION

Colocasia esculenta (L.) Schott of the genus *Colocasia* belonging to the family Araceae is a tuber crop cultivated as a staple food in Anambra State, Nigeria. It is a starchy food of many varieties. The cultivars vary in their vegetative features and in the way they are processed or consumed, based on acridity and taste. However, some of the varieties such as 'kochuo' and 'nwine' do not require prolong cooking before consumption. Acridity in plants is believed to be connected with the occurrence of calcium oxalate (Osuji, 2013). Calcium oxalate is one of the ergastic substances in plants, which are presumed to serve as defensive mechanisms that deter herbivory. Uno *et al*. (2001) stated that ergastic substances in plants are known to be objects of defensive mechanism for warding off or discouraging herbivores.

Almost all members of the aroid family contain minute crystals of calcium oxalate distributed throughout their tissues, which may be implicated in the irritating quality found in many Araceae species *(*Okeke *et al*., 2009). Unlike phytoliths, which vary considerably in size and shape across families, calcium oxalate crystals are generally restricted to five basic morphological types. They include: needle shaped raphides, rectangular or pencil shaped styloids, mace head shaped aggregates called druses, block shaped aggregates called crystal sand, variously shaped prisms (Horner and Wagner, 1995). Araceae have numerous druses, multi-crystal druses and needle shaped raphides crystals of calcium oxalate (CaOx) present in the tissue (Franceschi and Nakata, 2005). The irritating activity is associated with raphides, and both defensive and non defensive raphide idioblasts, have been reported (Saadi and Mondal, 2012ab). In addition, druses may have a similar defensive function to that of raphides, as they also have sharp points resulting in considerable irritation if eaten (Prychid and Rudall, 1999). The presence or absence of micro-characters in plant system like calcium oxalate crystals has been used for understanding the evolutionary relationships of plant species (Saadi and Mondal, 2011). The frequency of occurrence, quantity and distribution of oxalates of calcium are important taxonomic characters, which have been clearly used to delimit cultivars as well as characterize plant germplasm (Osuji *et al*., 1997; Osuji, 2006). The taxonomy of *C. esculenta* has not received adequate attention. In addition, the anatomical and histochemical knowledge of varieties of this species are still scarce, and they can enhance wide acceptance of plant as food. The objectives of this study, therefore, were to determine the anatomical features as well as the occurrence and variability of calcium oxalate crystals in the petiole of varieties of this species, with a view to understanding the distinctions and affinities existing among them, thereby shedding light on varietal relationships; which would probably serve as important taxonomic information in delimitation of this species of plant.

MATERIALS & METHODS

Sources of Materials

Five varieties of *C. esculenta*, namely, *C. esculenta* var. *antiquorum* (eddoe), *C. esculenta* var. *esculenta* (dasheen), 'kochuo', 'nwine' and 'ogeriobosi' were obtained from the local farmers from Agulu in Anaocha LGA (Anambra North); Umuikwu-Anam in Anambra West LGA (Anambra Central) and Uga in Aguata LGA (Anambra South), which are the three senatorial districts of Anambra State. They were then grown at a common garden in Uga. The specimens' identification and the voucher specimens' authentication were performed by C.A. Ezeabara in liaison with Prof. C.U. Okeke, Plant Taxonomists, and deposited in the herbarium of Department of Botany, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

Anatomical Study

Fresh petioles of three-month old plants, grown in March, 2014 were used for anatomical study. They were collected and then free-hand-sectioned transversely, with a new sharp razor blade. For investigation of each specimen section, a drop of water was placed on a clean slide; the specimen section was mounted on it, and then stained with a drop of 0.1 % safranin solution and counter stained with a drop of 1 % alcian blue solution. The specimen was then carefully covered with a coverslip and observed under an OLYMPUS (XSZ-107BN, China) light microscope at X40 magnification. They were studied and photomicrographs taken with digital camera (Sony DSC-W230, China).

Histochemical Localization of Calcium Oxalate Crystals

Fresh petioles of the five varieties of *C. esculenta* planted in March, 2014 were obtained in August, 2014 and fixed in freshly prepared FAA (1 part formalin, 1 part glacial acetic acid and 18 parts 70 % ethanol v/v) for 24 hrs. The materials were rinsed twice in deionized water before sectioning. They were then free-hand-sectioned transversely. The sections were lightly stained with 0.1 % safranin solution, and counter stained with a mixture of 6 % w/v hydrogen peroxide (H_2O_2) and silver nitrate ($AgNO_3$) in bright light supplied by 100 W electric bulb fastened in a clamp stand. The slides were left in the bright light source for 30 minutes. A drop of oil immersion was placed on each slide and covered with coverslips. They were observed under an OLYMPUS (XSZ-107BN, China) light microscope at X100 magnification. Photographs of the informative sections were taken with digital camera (Sony DSC-W230, China).

RESULTS & DISCUSSION

The result of anatomical investigation showed that the vesssels were not occluded with tyloses (Fig. 1A-E). This is probably a specific character of *C. esculenta* and indicated a close affinity among the varieties. This agrees with Olerodo (1984) who stated that plant taxon has its characteristic features though there are also the unifying marks for highest groups. In addition, the arrangement of the pores/vessels of all the varieties were exclusively solitary (Fig. 1 A-D), with the exception of pores of 'ogeriobosi' which were in clusters; otherwise known as tangential arrangement (Fig. 1E).

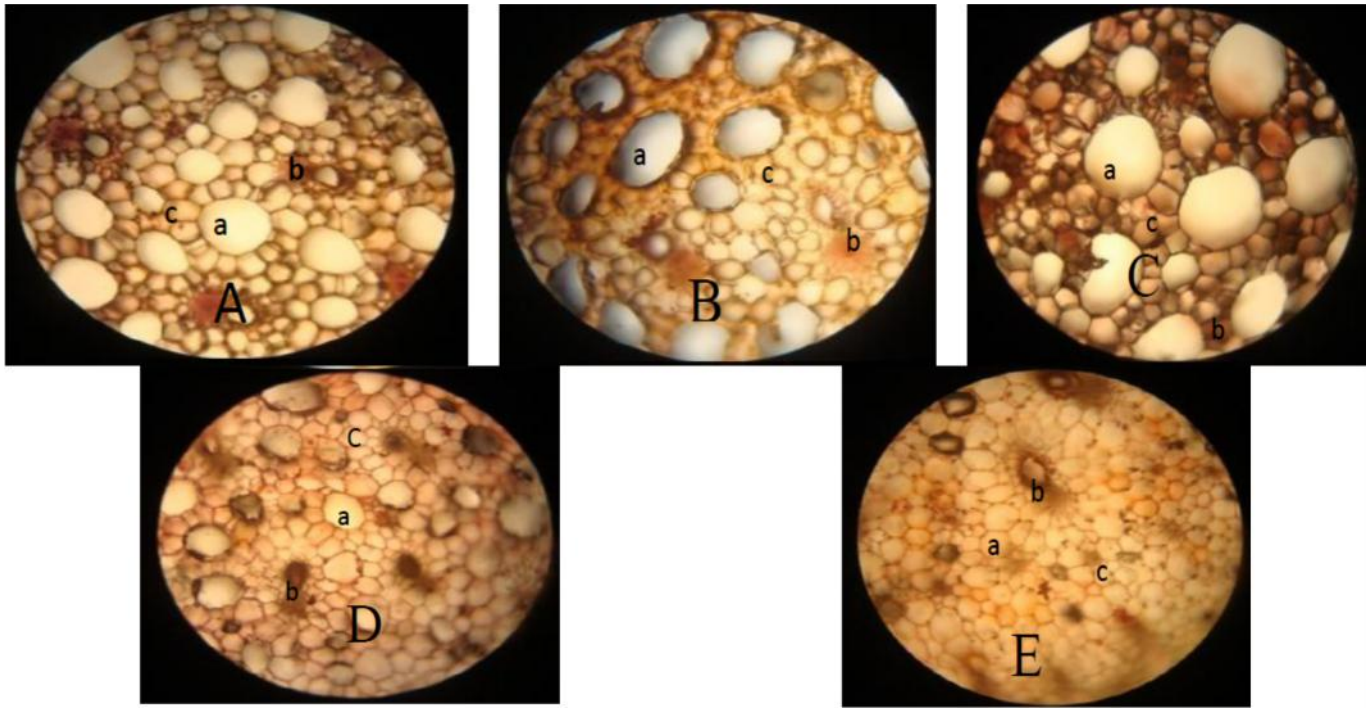
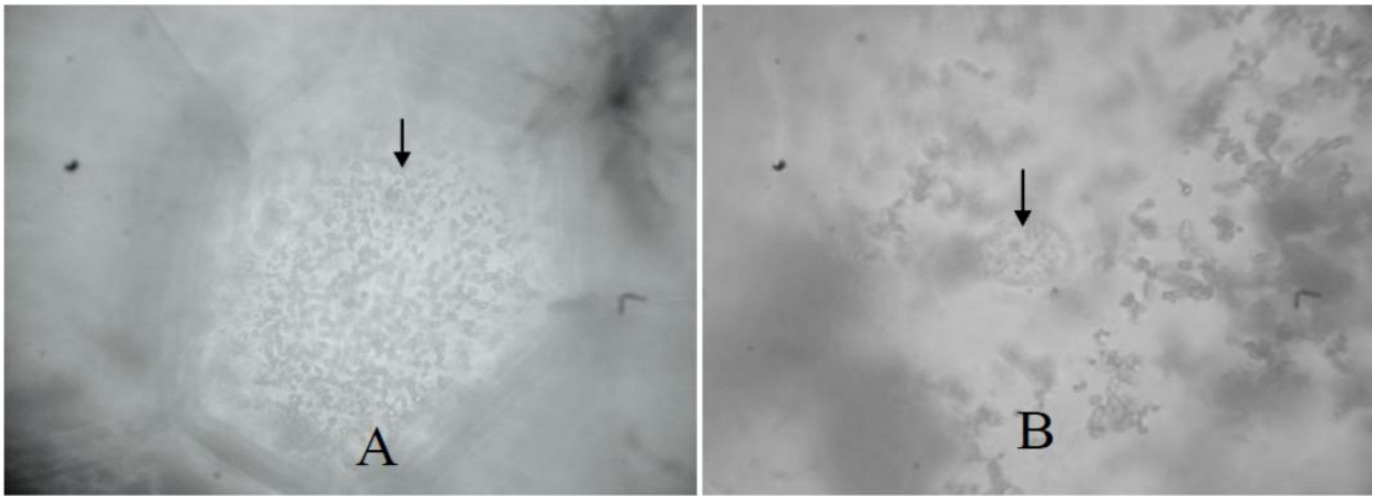


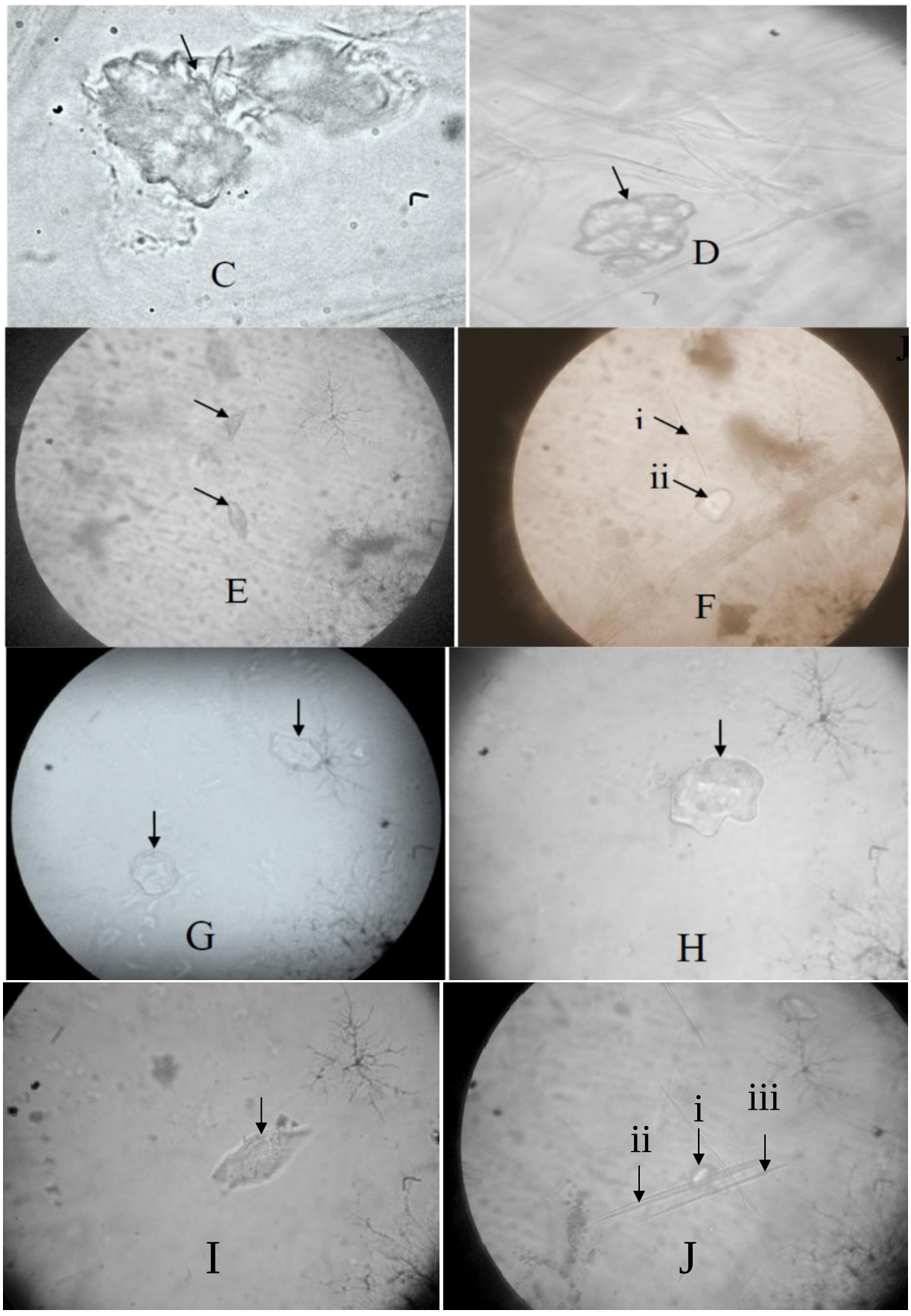
FIGURE 1: Transverse section (T/S) of petioles of varieties of *Colocasia esculenta* X400.1A. *Colocasia esculenta* var. *antiquorum.* 1B. *Colocasia esculenta* var. *esculenta*. 1C. 'kochuo'. 1D. 'nwine'. 1E. 'ogeriobosi'.
a=pore/vessel, b=vascular bundle, c=parenchyma

This suggested a close affinity among *C. esculenta* var. *antiquorum*, *C. esculenta* var. *esculenta*, 'kochuo' and 'nwine', while the arrangement of vessels in the petiole can be applied as a diagnostic character for 'ogeriobosi'. The pores of *C. esculenta* var. *antiquorum*, 'nwine' and 'ogeriobosi' were round (Fig. 1A, D and E), while those of *C. esculenta* var. *esculenta* and 'kochuo' were oval (Fig. B and C) in shape. These suggested a close affinity among *C. esculenta* var. *antiquorum*, 'nwine' and 'ogeriobosi', and between *C. esculenta* var. *esculenta* and 'kochuo'. Sharma (1993) reported that anatomical characters are most useful in determining relationship among taxonomic categories. The sizes of all pores varied (Fig. 1A-E); largest vessels were present in 'kochuo' (Fig. 1C), whereas the smallest were found in 'ogeriobosi' (Fig. 1E). This feature could be used as a delimiting parameter among the varieties of *C. esculenta*.

Generally, there were diverse forms of calcium oxalate crystals in petioles of all the varieties of *C. esculenta* (Fig. 2A-M). This suggested that occurrence of different forms of calcium oxalate in plant parts could be genetically controlled. Hence, some workers were of the opinion that calcium oxalate crystals may have taxonomic potential for both botanist and taxonomist (Horner and Wagner, 1995; Saadi and Mondal, 2011). In addition, occurrence of calcium oxalate crystals in the petioles of all the varieties of *C. esculenta*, in the form of crystal sand, druses, raphide bundle, rhombohedral, rosettes and styloid, indicated that a group affinity existed among them, and might be characteristics of members of Araceae family. It was reported that, oxalate crystals can be of several forms in the plant tissues where they exist, including raphides, conglomerate, cystoliths, (Okoli, 1988; Osuji, 2013); as well as druses, styloids, prisms and crystal sand (Franceschi and Nakata, 2005; Meric, 2009). Besides, Prychid and Rudall (1999) reported that three main types of calcium oxalate crystal occur in monocotyledons. They include: raphides, styloids and druses, although intermediates are sometimes recorded and more than one type may be present in a species. In addition, Araceae is the only family in which all three main crystal types are recorded. Furthermore, it has extensively been reported that oxalate crystal can form in any organ or tissue within the plants, including in stems, petiole, leaves, roots and tubers, and have a variety of function including calcium storage, defense and providing structural strength (Franceschi and Horner, 1980; Nakata, 2003; Saadi and Mondal, 2011).

Crystal sand was present in petioles of *C. esculenta* var. *esculenta* and 'nwine' (Fig. 2A and B). This implied that a close affinity existed between the two varieties. Druses and rosettes were only found in petioles of 'kochuo' (Fig. 2 C and D).These characters could be used as differential characters for distinguishing 'kochuo' from other varieties. Prismatic oxalate crystals (rhombohedral) were found in all the varieties (Fig. 2E-Ji). Possession of these common features appeared to be the specific identities of *C. esculenta*. The resemblance of rhombohedral calcium oxalate crystals in *C. esculenta* var. *antiquorum* and 'ogeriobosi' (Fig. 2E and I) suggested a close affinity between them. Osuji *et al.* (1997) showed that crystals of calcium oxalate could play a taxonomic role since their quantity, frequency of occurrence and distribution could distinguish between related species and cultivars.

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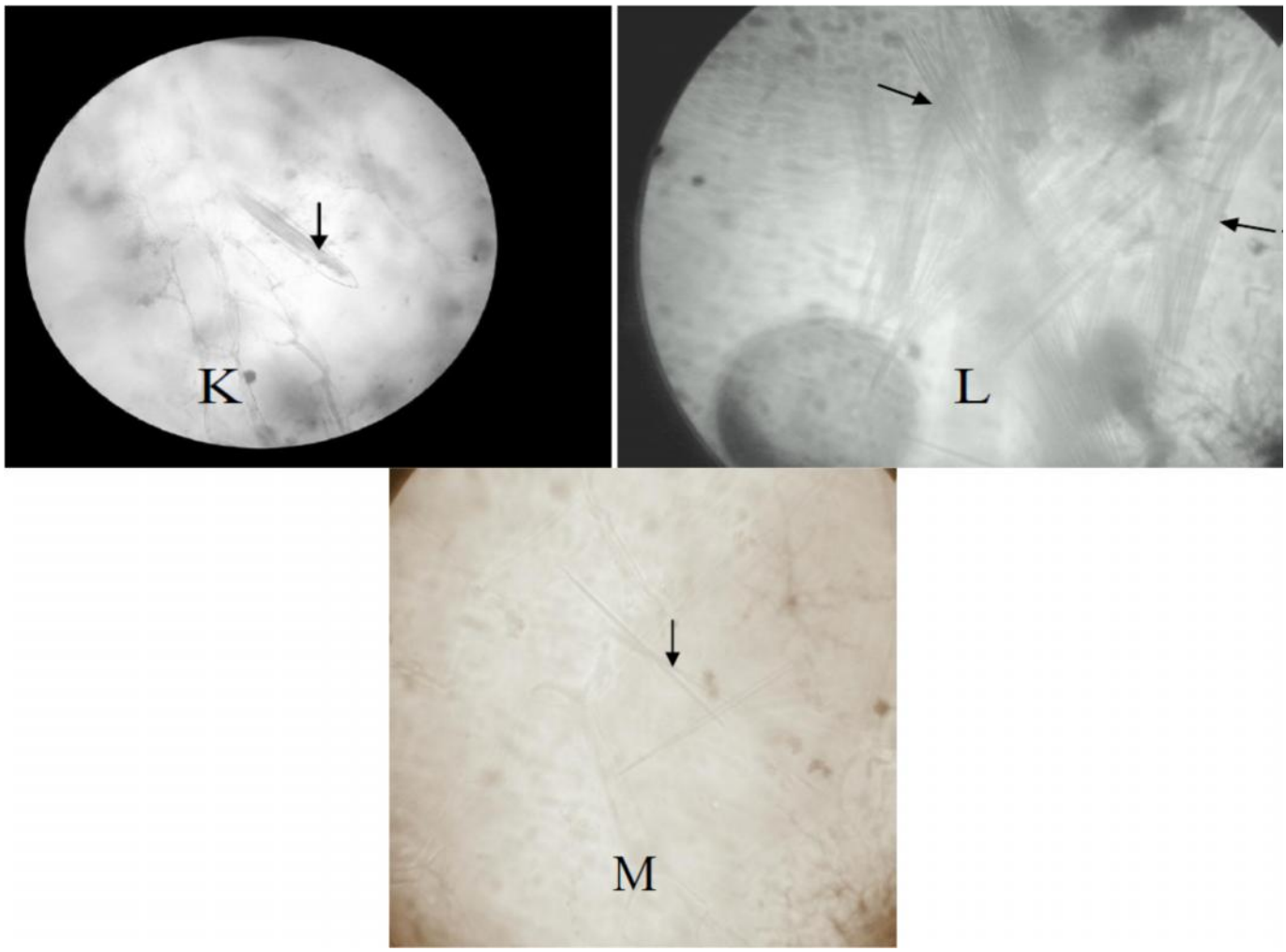

FIGURE 2: Calcium oxalate crystals in petioles of varieties of *Colocasia esculenta* X1000. (A). Crystal sand in *Colocasia esculenta* var. *esculenta*. (B).Crystal sand in 'nwine'. (C). Druses in 'kochuo'. (D). Rosettes in 'kochuo'. (E-Ji). Rhombohedral in *Colocasia esculenta*. (E). *Colocasia esculenta* var. *antiquorum*. (Fii). *Colocasia esculenta* var. *esculenta.* (G). 'kochuo'. (H). 'nwine'. (I). 'ogeriobosi'. (Ji). *Colocasia esculenta* var. *antiquorum.* (Jiii-M). Raphides in *Colocasia esculenta*. (Jiii). *Colocasia esculenta* var. *antiquorum.* (K). 'kochuo'. (L and Fi). *Colocasia esculenta* var. *esculenta*. (M). 'ogeriobosi'. (Jii). Styloid in *Colocasia esculenta* var. *antiquorum*.

Moreover, a bar of styloid was only found in petiole of *C. esculenta* var. *antiquorum* (Fig. 2Jii), which distinguished it from other varieties, and therefore could be referred to as a diagnostic feature. Although the morphology of raphides and styloids look alike, yet they can be differentiated. Raphides are bundles of narrow, elongated needle-shaped crystals, usually of similar orientation, with pointed ends at maturity, whereas styloids also known as 'pseudoraphides', are thicker than raphides and usually solitary within a cell (Prychid and Rudall, 1999). In addition, styloids may have pointed or squared ends, and may be elongated or cuboidal.

Raphides were present in the petioles of all the varieties (Fig. 2Jiii-M), with the exception of 'nwine', while raphide bundle was found in 'kochuo' (Fig. 2K). The raphide bundle found in 'kochuo' is probably a defensive one. Raphide bundles with needle like calcium oxalate crystals, aligning parallel with the long axis of the idioblast and fill up nearly the entire cell, are said to be defensive raphide idioblasts while the crystals which may be situated at oblique angles within the cell or the ends might be interdigitate, are referred to as the non defensive raphide idioblasts (Saadi and Mondal, 2012ab). Although there was raphide bundle in petiole of 'kochuo', a variety which does not require prolong cooking; the concentration might be low. Presence of raphides in the petioles of *C. esculenta* var. *esculenta*, *C. esculenta* var. *antiquorum* and 'ogeriobosi' indicated that they would have irritating activity. In addition, this implied that corms and cormels of these varieties of *C. esculenta* would be irritating to the mouth and oesophagus when eaten, especially when not properly cooked; which is probably the reason behind prolong cooking of their corms and cormels before consumption. Greatest quantity of raphides was detected in *C. esculenta* var. *esculenta* (Fig. 2L). This implied that the irritating action of *C. esculenta* var. *esculenta* might be highest in relation to other irritating varieties. Absence of raphide in petioles of 'nwine' suggested that it would not have irritating quality, and as a result may not have to be boiled for a long time before

ingestion. Acridity in plants has been reported to be associated with the occurrence of needle-like raphides of calcium oxalate (Bradbury and Nixon, 1998). Moreover, Saadi and Mondal (2011) stated that the defensive raphide idioblasts which eject their calcium oxalate needles have been implicated in the irritation produced when aroids are eaten or handled fresh.

CONCLUSION

The variation in sizes and appearances of pores present in petioles of all the varieties could be used as a delimiting parameter among the varieties of *C. esculenta*. In addition, the size and arrangement of vessels of 'ogeriobosi' could be employed as diagnostic characters, while the shape of the vessels of all the varieties might serve as differential features.

Secondly, this study established that forms of calcium oxalate crystals can differ within varieties of a species; hence, a range of forms exhibited could be of taxonomic significance. Presence of druses and rosettes in petioles of 'kochuo' only, and possession of very large vessels; occurrence of styloid in *C. esculenta* var. *antiquorum*, as well as presence of crystal sand in *esculenta* var. *esculenta* and 'nwine' could serve as diagnostic characters. 'Nwine' might not have an irritating quality, as a result of absence of raphides. Presence or absence of different oxalate crystal types in various parts of plants could therefore, be regarded as significant taxonomic characters. Hence, evidence from histochemistry might aid plant taxonomists in delimitation of *C. esculenta*.

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