Histochemical localization of calcium oxalate crystals in fruits of plantain and banana cultivars

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Abstract

Introduction. The most widespread ergastic substances found in flowering plants are crystals of calcium oxalate. Their varied forms of occurrence in organs and tissues of many plants have received great attention. Nevertheless, the description of the spectrum of occurrence of crystals of calcium oxalate in tissues and organs of the *Musa* genus has not been reported yet. This justified that a study was undertaken with banana and plantain fruits. **Materials and methods.** Green fruits of six plantain, five banana cultivars and one banana hybrid were collected fresh, immediately fixed for 48 h at least, then dehydrated and cut with a rotary microtome. To localize calcium oxalate, sections were examined, after treatment, using the light microscope. **Results.** In the peel of all cultivars studied, calcium oxalate occurred in the form of raphide bundles, but, in the pulp, it occurred as solitary, irregular or tri-radiate intra-amylar crystals. Plantain cultivars had larger and more tri-radiate crystals than banana cultivars, while the latter had smaller but more solitary crystals in the pulp. **Discussion.** The variation in the structure and size of the crystals in the peel and pulp of plantain and banana indicates that occurrence and distribution of structural forms of crystals in *Musa* could be tissue –or species– specific and can be exploited for *Musa* taxonomy.

Keywords

Musa, fruit, chemical composition, taxonomy.

Localisation histochimique de cristaux d'oxalate de calcium dans le fruit de plantains et de bananes dessert.

Résumé

Introduction. Les substances ergastiques les plus répandues parmi les plantes à fleurs sont les cristaux d'oxalate de calcium. Les diverses formes présentées par ces structures dans les organes et tissus ont été très étudiées chez de nombreuses plantes. Cependant, la description de la gamme de cristaux d'oxalate de calcium observable dans le genre *Musa* n'avait encore jamais été effectuée. Cela a motivé l'étude entreprise sur fruits de plantains et de bananes. **Matériel et méthodes.** Des fruits verts de six cultivars de plantain et de cinq variétés et d'un hybride de banane ont été récoltés, puis immédiatement fixés pendant 48 h au moins avant d'être déshydratés et découpés au microtome rotatif. Les préparations, traitées, ont été observées au microscope optique. **Résultats.** Dans la peau de chacun des cultivars étudiés, l'oxalate de calcium est apparu sous la forme de faisceaux de raphides, mais, dans la pulpe, cet élément a présenté des cristaux isolés, irréguliers ou trirayonnés, et intra-amylacés. Les cultivars de plantains ont eu de plus gros cristaux, plus souvent trirayonnés que ceux des variétés de banane, dans la pulpe desquelles ces structures apparaissaient plus petites et isolées. **Discussion.** Les variations de structure et de taille des cristaux, observées dans la peau et la pulpe de plantains et bananes, suggéreraient que la présence et la distribution des différentes formes de l'oxalate de calcium dans le fruit du genre *Musa* pourraient être des spécificités des tissus et des espèces ; cette particularité pourrait être exploitée pour des études de taxonomie du genre *Musa*.

Mots clés

Musa, fruit, composition chimique, taxonomie.

introduction

Plantain (*Musa* spp, AAB group) and banana (*Musa* spp, AAA or ABB groups) constitute sources of starchy and dessert forms of staple carbohydrate in sub-Saharan Africa. Secondary diversification of plantains led to the occurrence of four main bunch types: French, French Horn, False Horn and Horn (TÉZENAS DU MONTCEL et al, 1983; SWENNEN et al, 1995). Bananas are also known to have diversified genetically to give rise to various types including highland beer, cooking and dessert bananas (GOWEN, 1995).

The most widespread ergastic substances found in flowering plants are crystals of calcium oxalate (OKOLI and MCEUEN, 1986). Their varied forms of occurrence in organs and tissues of many plants have received great attention (BUSS and LERSTEN, 1972; STEBBINS et al, 1972; BUTTROSE and LOTT, 1978; OKOLI, 1988). Crystals of calcium oxalate occur neither uniformly nor in the same pattern of distribution (OKOLI, 1988) and are observed to occur in various apparently mutually exclusive forms.

To the authors' knowledge, the description of the spectrum of occurrence of crystals of calcium oxalate in tissues and organs of *Musa* genus has not been reported yet. The probable lack of literature on the occurrence of such crystals in this taxon does not imply their non-occurrence. Consequently, proper investigation of the taxon needs to be carried out in order to elucidate the form and pattern of occurrence and the systematic value of these crystals. Therefore, this paper describes the presence and forms of calcium oxalate in the fruits of plantain, banana and a banana hybrid.

Table I
Characteristics of calcium oxalate crystals in the fruits of some plantain, banana and hybrid cultivars.

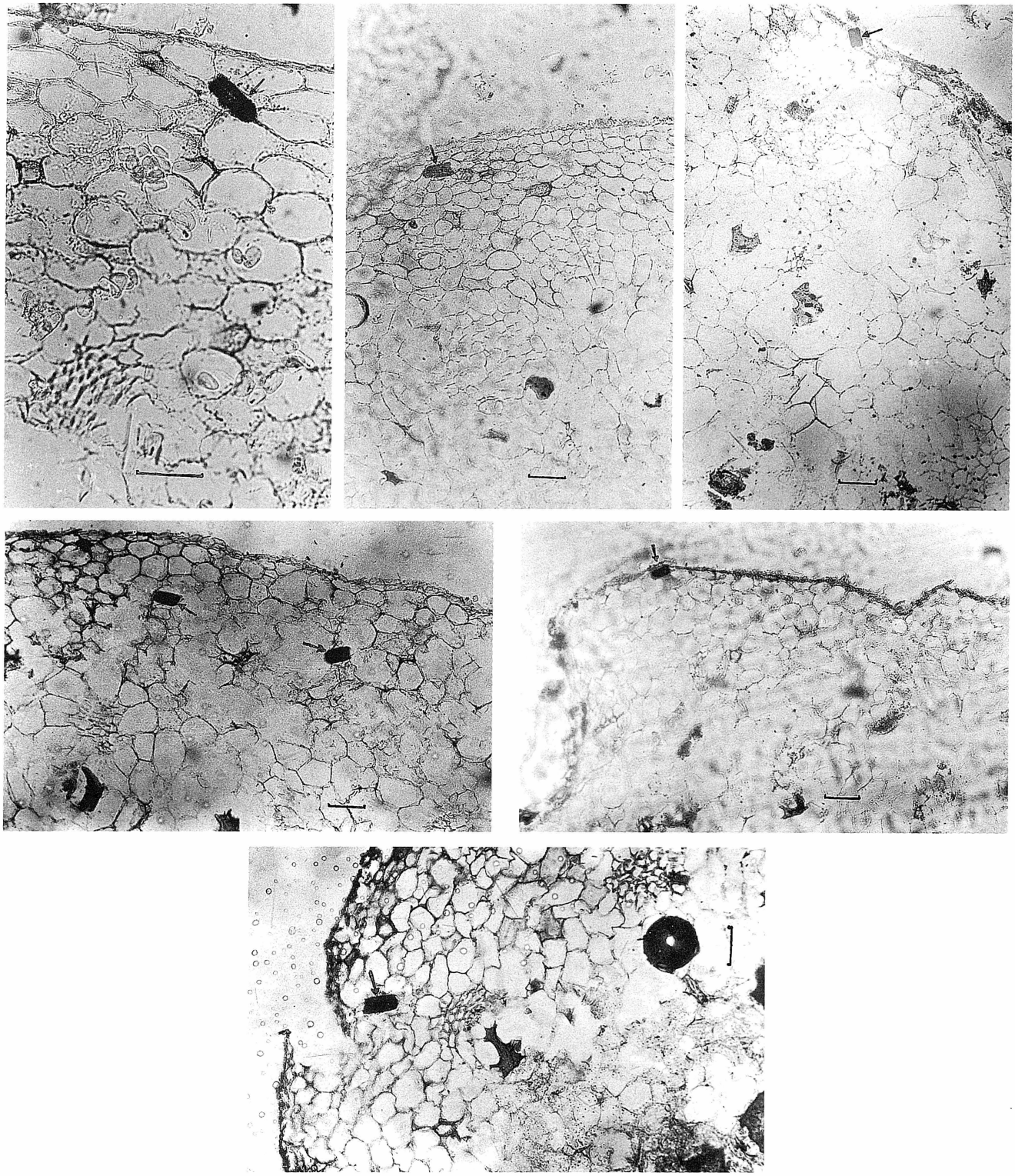
Cultivar (1)	*Sub-group*	*Types of crystal*	*Size (µm)*[2]	*Tissue*
Plantains				
Obino l'Ewai	French plantain	Br	61.4 ± 3.2	Peel
		Sol, Ir, Tr	7.6 ± 1.2	Pulp
Bobby Tannap	French plantain	Br	60.2 ± 2.9	Peel
		Sol, Ir, Tr	8.2 ± 1.2	Pulp
Big Ebanga	False Horn plantain	Br	59.5 ± 3.0	Peel
		Sol, Ir, Tr	8.2 ± 1.0	Pulp
Ntanga 2	French plantain	Br	63.6 ± 5.0	Peel
		Sol, Ir, Tr	5.6 ± 1.0	Pulp
Agbagba	False Horn plantain	Br	57.3 ± 3.2	Peel
		Sol, Ir, Tr	7.8 ± 0.9	Pulp
Ubok Iba	Horn plantain	Br	63.0 ± 4.0	Peel
		Sol, Ir, Tr	9.9 ± 1.2	Pulp
East African Highland bananas				
Igitsiri	Beer Banana	Br	62.1 ± 4.6	Peel
		Sol	3.5 ± 0.5	Pulp
Makara	Beer Banana	Br	49.3 ± 2.3	Peel
		Sol, Ir, Tr	3.9 ± 0.5	Pulp
Rugondo	Cooking banana	Br	48.6 ± 4.2	Peel
		Sol, Ir, Tr	4.1 ± 0.6	Pulp
Dessert bananas				
Yangambi Km 5	Dessert banana	Br	60.1 ± 2.7	Peel
		Sol, Ir, Tr	5.1 ± 0.6	Pulp
Valery	Dessert banana	Br	60.2 ± 3.3	Peel
		Sol, Ir	3.6 ± 0.5	Pulp
Tetraploid hybrid				
FHIA 3	Cooking banana	Br	56.0 ± 3.5	Peel
		Sol, Ir, Tr	6.0 ± 0.6	Pulp

(1) Names provided by INIBAP Transit Center at KUL, Belgium.
Br=bundle raphide; Sol=solitary intra-amylar crystal; Ir=irregular intra-amylar crystal; Tr=tri-radiate intra-amylar crystal; tr=smaller tri-radiate intra-amylar crystal; FHIA=Fundacion Hondurena de Investigacion Agricola.

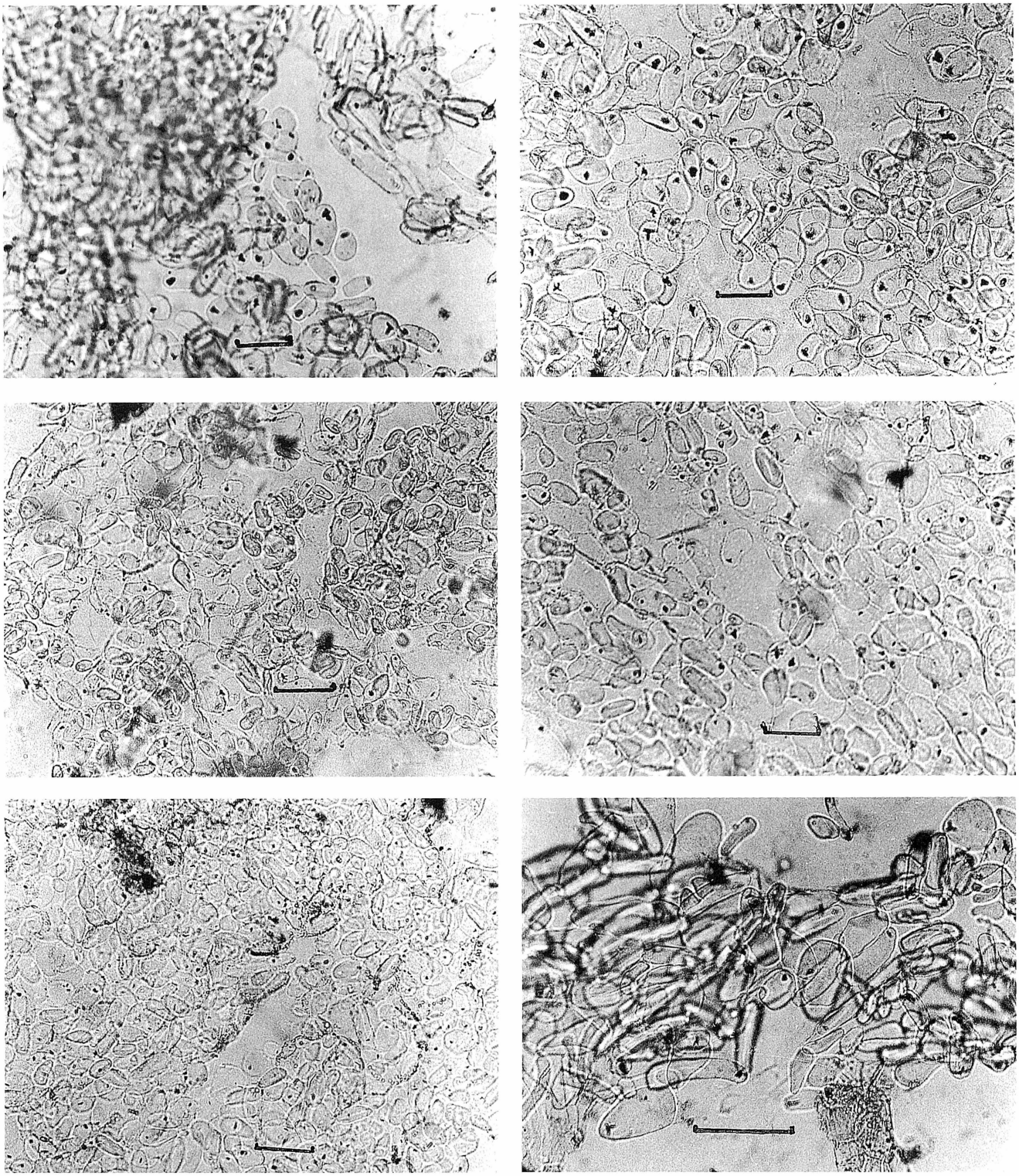
materials and methods

Mature fruits were obtained from six triploid plantains, four triploid bananas and one diploid banana and one tetraploid hybrid (table I) maintained, under standard cultural conditions (SWENNEN, 1990), in the field genebank of the International Institute of Tropical Agriculture (IITA), at Onne, near Port Harcourt (Nigeria). This location is in the lowland humid forest of Southeastern Nigeria.

Green fruits were collected fresh and immediately fixed in FAA (1 part formaldehyde, 1 part acetic acid, 18 parts 70% ethanol) for 48 h at least. Sections (15–20 µm thick) were cut with a rotary microtome from materials dehydrated through a graded series of ethanol, infiltrated and embedded in wax (60 °C melting point) following the method of



Figs 1–6
Transverse section of fruit peel tissue of various plantain and banana cultivars and hybrid showing the presence and location of bundle raphides (arrow) [Figs 1 and 2: Bobby Tannap and Agbagba (plantain), respectively. Figs 3–5: Igitsiri (highland beer banana), Valery (dessert banana) and Rugondo (highland cooking banana), respectively. Fig 6: FHIA 3 (cooking banana hybrid)]. Scale bar = 78 µm.



Figs 7–12
Transverse section of fruit pulp tissue of various plantain and banana cultivars and hybrid showing the location of intra-amylar crystals in starch grains [Figs 7, 8: Bobby Tannap and Agbagba (plantain), respectively. Figs 9–11: Igitsiri (highland beer banana), Valery (dessert banana), and Rugondo (highland cooking banana), respectively. Fig 12: FHIA 3 (cooking banana hybrid)]. Scale bar = 40 µm.

OKOLI and GREEN (1987). To localize calcium oxalate, sections were treated with 30% hydrogen peroxide (2 min) and 5% silver nitrate (4 min) in bright light following the method of SILVER and PRICE (1969).

results

Raphide bundles were present in the fruit peel of all cultivars (figs 1–6). These raphide bundles in the peel tend to be localized more below the epidermis. The raphide bundles measured 39.0 to 78.0 µm in length and 19.5 to 56.0 µm in thickness. These raphide bundles were rarely seen within the fruit pulp tissue.

The typical form of occurrence of calcium oxalate crystals in the pulp of banana and plantain cultivars studied is in the form of intra-amylar crystals. These intra-amylar crystals (figs 7–12) occur at the hilar regions of the starch grains. The intra-amylar crystals vary both in size and shape from solitary to irregular and tri-radiate (table I, fig 2). Intra-amylar crystals of the plantain cultivars were generally longer (3.0–14.0 µm long) than those of banana cultivars (2.0–6.0 µm long).

The intra-amylar crystals were smaller in size and by far more abundant than the raphide bundles in the *Musa* fruits. Most of the starch grains in the pulp showed presence of intra-amylar crystals. There were relatively more solitary intra-amylar crystals in banana fruit pulp and more tri-radiate forms in the plantain fruit pulp (figs 7–11).

discussion

The variation in the structure and size of the crystals in the peel and pulp of plantain and banana indicates that the structural form of crystals could be tissue–specific. Starch grains are known to serve storage functions in plants. Hence they are known to be identified within storage organs and tissues. For example, in *Ricinus*, druse crystals in stem, inflorescence and fruiting axis were degraded and replaced by starch grains (SCOTT, 1941). This implies that crystals and starch grains may occur together in diverse taxa (SCURFIELD et al, 1973; OKOLI and GREEN, 1987; OKOLI, 1988).

The actual reason for association between crystals and starch grains is not clear. However, OKOLI and GREEN (1987) reported that crystals in starch grains represent storage forms of calcium or merely provide 'nuclei' for deposition of starch. Furthermore, these authors doubted the later view since some starch grains did not contain crystals. This work showed that there is a high level of association between crystals and starch grains. Hence, plantain, which is known to contain more starch than banana (SWENNEN et al, 1995), has more intra-amylar crystals than banana.

The similarity in distribution pattern and structural variations of crystals in the fruits of different plantain types and the difference between plantain and banana types agree with morphological delimitation of plantain (*Musa* spp, AAB group) from banana (*Musa* spp, AAA group). Apparently, ploidy level did not affect crystal size, hence 'Rugondo', a diploid banana, did not show any reduction in crystal size compared with the other triploid banana cultivars. Also, FHIA 3, a tetraploid banana hybrid, did not show any increase in crystal size compared with the triploid plantains and bananas.

note

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Localización histoquímica de cristales de oxalato de calcio en el fruto de plátanos y de bananos.

RESUMEN

INTRODUCCIÓN. Las sustancias ergásticas más propagadas entre las plantas florales son los cristales de oxalato de calcio. Las diversas formas presentadas por estas estructuras en los organos y tejidos fueron muy estudiadas en numerosas plantas. Sin embargo, nunca se efectuó la descripción de la gama de cristales de oxalato de calcio observable en el género *Musa*. Esto motivó el estudio emprendido sobre frutos de plátanos y de bananos. **MATERIAL Y MÉTODOS**. Unos frutos verdes de seis cultivares de plátano y de cinco variedades y de un híbrido de banano fueron cosechados, y luego inmediatamente fijados durante 48 h por lo menos antes de ser deshidratados y cortados con el micrótomo rotativo. Las preparaciones, tratadas, fueron observadas con el micróscopo óptico. **RESULTADOS**. En la piel de cada uno de los cultivares estudiados, el oxalato de calcio pareció con forma de manojos de ráfidos pero en la pulpa este elemento presentó cristales aislados, irregulares triradiados, y intra-amilaceados. Los cultivares de plátanos tuvieron más gruesos cristales, más triradiados que los de las variedades de banano, en la pulpa de las cuales estas estructuras parecían más pequeñas y aisladas. **DISCUSIÓN.** Las variaciones de estructura y de talla de los cristales, observadas en la piel y la pulpa de plátanos y bananos, sugererían que la presencia y la distribución de las diferentes formas del oxalato de calcio en el fruto del género *Musa* podrían ser especificidades de los tejidos y de las especies ; esta particularidad podría ser explotada por estudios de taxonomía del género *Musa*.

PALABRAS CLAVES

Musa, fruta, composición química, taxonomía.