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Full Length Research Paper

Probable functions and remobilisation of calcium oxalates in *Musa* L.

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The occurrence and distribution of calcium oxalate crystals were investigated in the young and ageing structures of *Musa* L. cultivars. The calcium oxalate crystals were tested histochemically and examined using light microscopy. The studies revealed the presence of varying forms of the crystals including intra-amylar, solitary and raphide bundles in the different tissues and organs. Remobilization and redistribution of the crystals were observed following senescence in all the cultivars investigated. The usefulness of these observations in understanding the functions and fate of calcium oxalate crystals in plants are highlighted.

Key words: Calcium oxalate, *Musa* cultivars, remobilization, senescence.

INTRODUCT ION

Crystals of calcium are among various ergastic chemical substances found in plants. These crystals have been variously reported present in storage, vegetative and reproductive organs of a number of plants. For instance, they have been found to occur in the lumina of the cells and tissues of the epidermal layer of leaves (Edeoga and Ugbo, 1997), bark and secondary xylem (Wattendorff, 1978), wood (Scurfield et al., 1973), flowers (Buss and Lersten, 1972; Tilton, 1978), seed protein bodies (Buttrose and Lott, 1978) and fruits and floral buds (Stebbins et al., 1972), and ovaries (Dormer, 1961).

The presence of calcium oxalate crystals in the leaves of *Dioscorea* L. (Edeoga and Okoli, 1992, 1995) and in the starch grains of yam (Okoli and Green, 1987) have been reported. Osuji et al. (1997) investigated the presence and distribution of calcium oxalate crystals in *Musa* L. and reported widespread occurrence of these crystals in leaves, pseudostem, bracts, fruit peel and pulp. Okoli and Green (1987) and Osuji et al. (1997) exploited the occurrence, structure and distribution of calcium crystals for taxonomic purposes. The authors showed that the crystals vary in distribution, frequency of occurrence, size and shape between plant taxa (especially at species and generic levels). Obviously, the reason and mechanism for the bioaccumulation of these crystals is yet to be fully understood. Apparently, a genetic factor may be involved since there is obvious similarity in their frequency, structure and distribution in materials from related taxa. The report of occurrence of calcium oxalates in fruits of *Musa* species in the form of solitary and bundle raphides as well as intra-amylar crystals suggest that the crystals are storage products (Osuji et al., 1997).

The objective of this work was to determine how the crystals accumulate in the cells, tissues and organs and their probable fate following maturity and senescence in the various cultivars of *Musa*.

MATERIALS AND METHODS

The plant materials used for this work were obtained from the *Musa* field germplasm of the International Institute of Tropical Agriculture (IITA) at Onne, near Port Harcourt, Nigeria. The field gene bank is maintained under conditions typical of the low land, low attitude agro-ecology of the humid forest region where plantains and bananas are cultivated in Nigeria. Fruits of three plantain 'AAB' and

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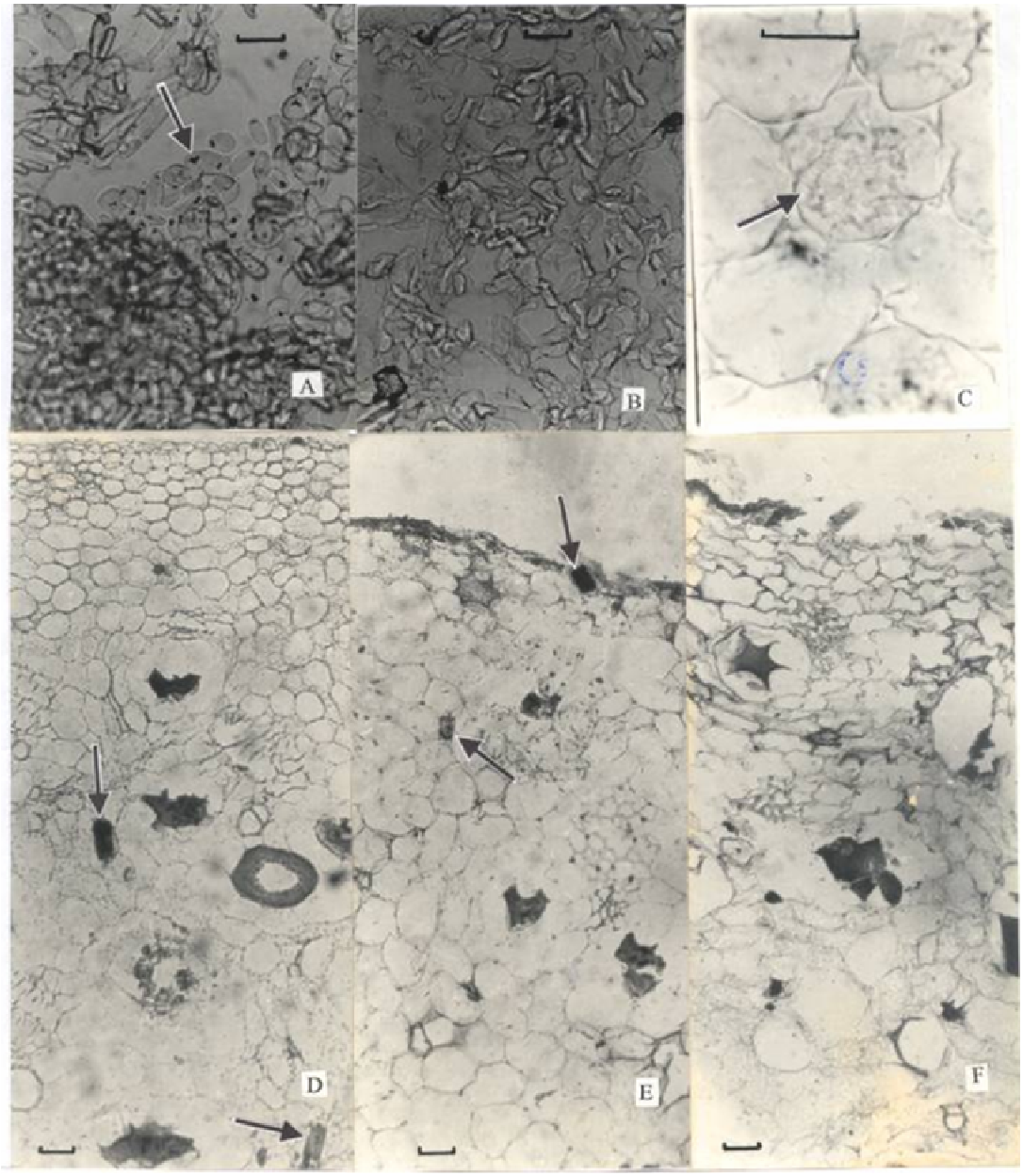

Figure 1. Tissues of the pulp and peel of *Musa* cultivars showing levels of calcium oxalate crystals. (A) Unripe plantain fruit pulp with distinct intra-amylar crystals; (B) ripened fruit pulp with little or no crystals; (C) over-ripened fruit pulp with disintegrated starch granules; (D) unripe fruit peel with heavy raphide bundles; (E) ripened peels showing reduction in size of the raphide bundles; and (F) over-ripened peel showing apparent lack of raphide bundles. Scale Bar = 40µM.

three banana 'AAA' cultivars, at different stages of age and ripening were used. The samples were histochemically processed following the methods of Silver and Price (1969) and Osuji et al. (1997). The samples were dehydrated using ethanol series, clarified in chloroform, embedded in wax at 60°C, sectioned at 15 µm and exposed to 3% H_2O_2 (3 min) and 5% $AgNO_3$ (5 min) under intense light from a 100 m electric bulb. The sections were permanently mounted with DPX and examined under a light microscope. Photographs were taken from good preparations using a Leitz Laborlux-12 photomicroscope fitted with a Wild MPS-camera.

RESULTS

In the fruits of the banana and plantain investigated, calcium oxalate exists in two main forms namely bundle raphides and intra-amylar crystals. In young fruits, the crystals were generally abundant (Figure 1A). A similar observation was made in the mature but unripe fruits.

In the fruit pulp, the crystals exist inside starch grains, forming the skeletal hileum upon which layers of starch

are deposited. The size of the intra-amylar crystals does not necessarily vary with the age of the fruits but more closely with the age of the individual starch grains. Thus fully enlarged starch grains have well developed hileums marked by intra-amylar crystals (Figure 1A). However, as ripening progresses, the starch grains lose integrity and shape (Figure 1B). The progressive reduction in the size, shape and presence of intra-amylar crystals become obvious. Hence very ripe fruits of plantain and banana lack intra-amylar crystals (Figure 1C). This situation is uniform among all cultivars of plantain and banana studied.

In the peel, the crystals exist mostly as bundle raphides (Figure 1D). In the peel of both the young and mature unripe fruits the frequency of raphide bundles was identical. However, as the fruits ripen, the size of the bundles reduced (Figure 1E). Consequently, very ripe fruits have little or no raphide bundles (Figure 1F). The rate of reduction in number of raphides as ripening progresses was similar in both plantain and banana materials.

DISCUSSION

The wide occurrence of calcium oxalate crystals in plants indicates a well-developed system of genetic events. These events are targeted at provision of variable functions depending on the tissue and organ where they are translocated. Therefore occurrence of calcium crystals could serve a protective function when they exist in peripheral cells and tissues. For instance, the occurrence of epidermal raphide idioblasts (Osuji et al., 1997) makes banana bracts unpalatable to browsing animals. Where solitary and bundle raphides are numerous, they often give itching property to the species or an organ of it. In this case, the involved plant is protected from herbivores.

The occurrence of calcium crystals in starch grains (Okoli and Green, 1987; Osuji et al., 1997) implies a storage purpose for this ergastic substance. In the starch grains, the calcium oxalate is used as a structural framework around which the starch is deposited. The crystals in this case give the starch grains shape, consistency and firmness. As the fruit matures and begins to ripe, the architectural and protective need for intra-amylar calcium oxalate crystals becomes unnecessary. Therefore, the intra-amylar calcium is translocated to younger tissues where it assumes a protective function. For this same reason, calcium oxalate crystals have been observed to be more abundant in young tissues of *Telfairia occidentalis* Hooker f. than the older senescing tissues (Okoli, 1988).

The process of recycling and/or remobilization of calcium in plants, though seems well developed, has not been investigated in detail. It is necessary to find out whether this translocation uses existing stellar channels or is performed through intercellular passive or active mechanisms. Intra-cellular transport mechanisms though may serve to conduct the calcium compounds out of their cellular domains; stellar transport would be necessary for organellar or trans-organ transport.

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