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Full Length Research Paper

Mitotic index studies on edible cocoyams (*Xanthosoma* and *Colocasia* spp.)

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Mitotic index studies were carried out on three cultivars of *Xanthosoma* and four cultivars of *Colocasia*. Young healthy roots (about 15 mm) were collected at 2 hourly intervals from 6:00 am to 8:00 pm. Root tips were fixed in 1:3 ethanol : acetic acid for 24 h and stored in 70% ethanol prior to squashing in FLP orcein. Microscopic counts showed that the dynamics of mitosis varied slightly between the two groups of cultivars. The peak of metaphase remained between 12:00 noon and 2:00 pm for most of cultivars but one (NCY 00Sa), which had its metaphase rising to a peak between 2:00 and 4:00 pm. This suggests that the best time to harvest root samples for optimum metaphase is immediately before 12:00 noon.

Key words: Chromosomes, cocoyam, mitotic division, metaphase, young healthy root tips.

INTRODUCTION

Cocoyam, a member of the Araceae family is an ancient crop grown throughout the humid tropics for its edible corms, cormels and leaves, as well as for other traditional uses (Pinto, 1990; Onwueme, 1994). The Araceae family is made up of some hundred genera and more than fifteen hundred species. They are mostly tropical and subtropical. The aroids, as they are very often called, grow mainly in moist or shady habitats. Some are terrestrial while others are vines, creepers or climbers. Many species of Araceae are also epiphytes.

The major edible aroids are classified into two tribes and five genera: Lasioideae (*Cyrtosperma* and *Amorphophallus*) and Colocasioideae (*Alocasia, Colocasia* and *Xanthosoma*; Purseglove, 1972). In Nigeria, *Colocasia* (taro) and *Xanthosoma* (tannia) are the most important of the edible genera. According to the National Root Crops Research Institute, Umudike, *Xanthosoma saggittifolium* has three cultivars in Nigeria namely: Ede Ocha (NCY 001), Ede Uhie (NCY 002) and Ede Okorokoro (NCY 003). *Colocasia esculenta* has four cultivars in Nigeria namely: Coco India (NCY 004), Ede .Ukpong (NCY 006), Ede Ghana (NCY 008) and Ede Ofe (NCY 00Sa). The edible aroids are very nutritious.

Cocoyam research is still at the primary stage. There was an opinion that all *Xanthosoma* in Nigeria belong to the species *X. maffafa* and not *X. saggittifolium* (Okeke, S.E., personal comm.) thereby implying an error in the taxonomy of the Nigerian species of *Xanthosoma*. This situation calls for more indebt and careful studies of these crops, especially to produce more empirical data for the improvement of cocoyams, which has been difficult to date. Hence, new, improved novel cultivars are unavailable. No indebt information is available on the cytology and cytogenetics of this very important source of staple food.

The only cytological data on cocoyam genomes are counts of 2n = 42 for *Xanthosoma* and 2n = 24 for *Colocasia*. To date, information is lacking on the mitotic index, chromosome features, breeding behaviour, phylogenetics and molecular genetics of the cultivars. Recently, increasing knowledge in molecular genetics has allowed the characterization of a number of molecular events that influence cell division or cell expansion. Studies involving plants have yielded much evidence of the molecular and genetic control of cell division. The rate of cell division has been depicted to reflect rate of increase in size and weight. This increase in size and weight are influenced by special genes,

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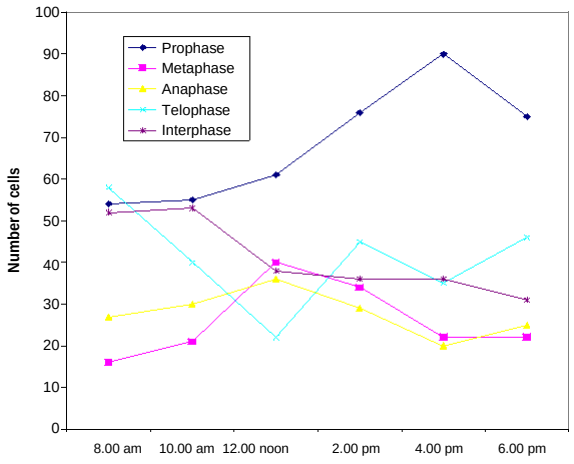

Figure 1a. NCY 001

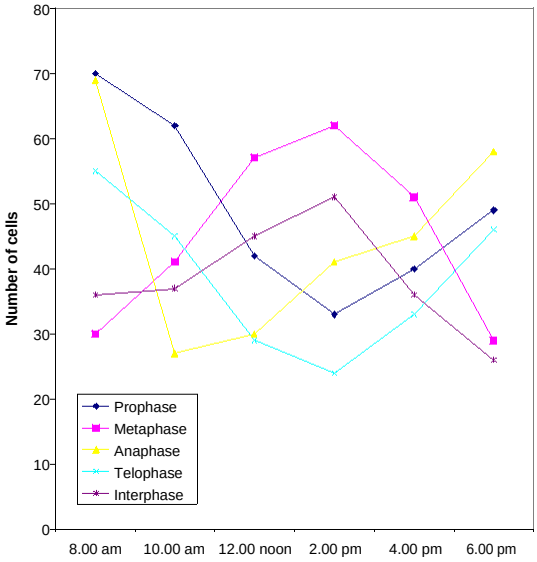

Figure 1b. NCY 002

Which may translate to varied expression in related cultivars (Guo et al., 2004; Tanksley, 2004). Expression of the genes that control size and weight through cell division in cocoyams could be monitored through mitotic indexing.

In addition, good mitotic indexing of cocoyam cultivars would generate information required for proper chromosomal characterization of the cocoyam germplasm. Good knowledge of the cocoyam genomes is very necessary in order to establish a sound approach to its improvement. Consequently, the aims of this work were: 1) to index the mitotic cycle in order to establish the most ideal time for collection of root stock for chromosome studies in these aroid genera, species and cultivars and 2) to find out any possible cultivar-related differences in the general pattern of the cell division

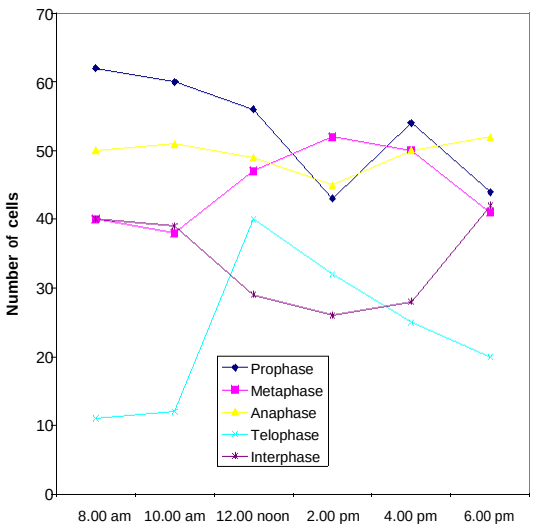

Figure 1c. NCY 003

cycle.

MATERIALS AND METHODS

The cocoyam cultivars used for this study were obtained from the field germplasm of the National Root Crops Research Institute, Umudike, Umuahia. The plant materials include three assessions of *Xanthosoma* namely: 'Ede Ocha' (NCY 001), 'Ede Uhie' (NCY 002) and 'Ede Okorokoro' (NCY 003) and four cultivars of *Colocasia esculenta* namely: Coco India (NCY 004), 'Ede Ukpong' (NCY 006), 'Ede Ghana' (NCY 008) and 'Ede Ofe' (NCY 00Sa) . Corms of *Xanthosoma* and cormels of *Colocasia* were planted in plastic pots and in ridges in the Demonstration plot at the University of Port Harcourt Botanic Garden and moderately watered till rooting.

Fresh roots were harvested at two-hourly intervals starting from 8.00 am to 6.00 pm in 3:1 ethanol : acetic acid for 24 h. Storage of root tips pending squashing was in 70 % ethanol solution. The roots were hydrolyzed in 0.5 M aqueous HCl (for 4-5 min) in a watch glass. About 1 mm tips of hydrolyzed roots were excised and squashed in FLP-orcein (Osuji, 2003) on a clean glass slide. Chromosome slides were then observed microscopically. Numbers of dividing cells at different levels of mitosis were recorded. Mitotic data were subjected to statistical analysis using SPSS version 11.

RESULTS AND DISCUSSION

Chromosome count of 2n = 42 was confirmed for all the *Xanthosoma* cultivars while 2n = 24 was confirmed for the *Colocasia* cultivars investigated. The chromosomes were relatively large (the largest of which was 7 µm long) and showed good stainability. There was slight cytoplasmic staining, though this did not constitute a significant problem on microscopical observations.

There was evidence that mitotic divisions in cocoyam were continuous during the time covered by the investigation, though at varied levels (Figure 1). The level of mitosis remained low before mid day as is reflected in the relative high numbers of interphase cells before 12.00

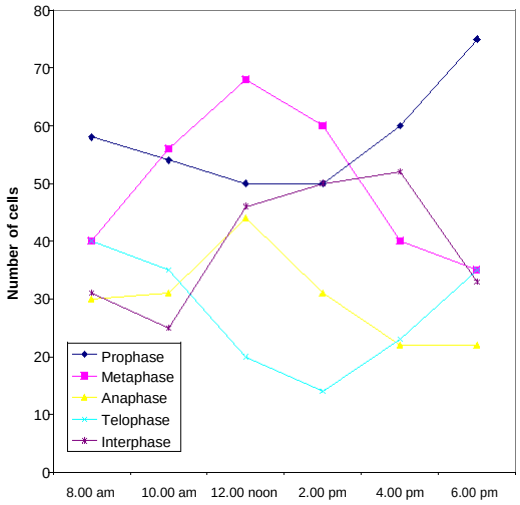

Figure 1d. NCY 004

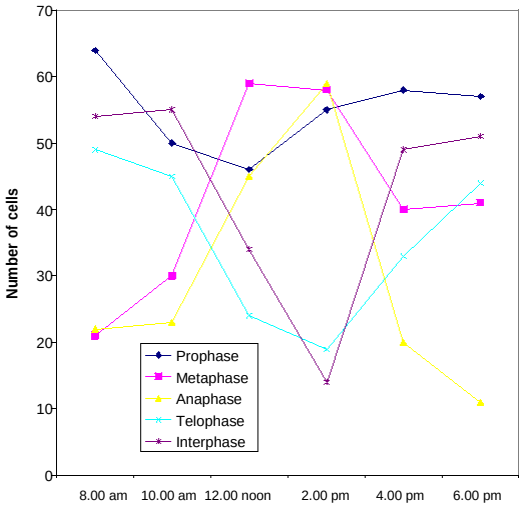

Figure 1e. NCY 006

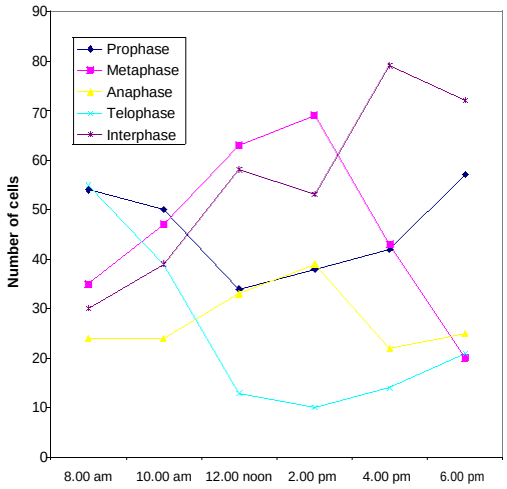

Figure 1f. NCY 008

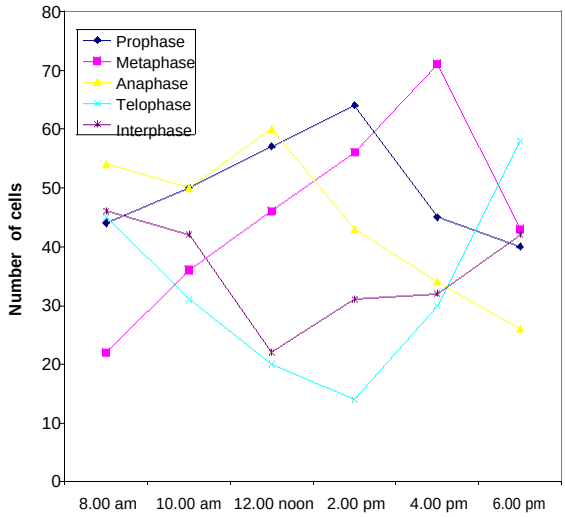

Figure 1g. NCY 00Sa

Figure 1. Dynamics of mitosis showing relative percentage of cells at the different stages of mitosis at different hours of the day. *Xanthosoma* a) NCY 001, b) NCY 002, c) NCY 003 and *Colocasia* d) NCY 004, e) NCY 006, f) NCY 008, and g) NCY 00Sa.

noon. The mitotic process increased as from noon but the peak of metaphase for most of the cultivars was between 12.00 noon and 4.00 pm.

The level of prophase cells remained high due to a relapse to prophase and interphase by dividing cells owing to disturbance caused by harvesting and fixing of root tips. However, for most cultivars, the number of metaphase cells rose from 12.00 noon and peaked between 12.00 noon and 2.00 pm.

In most cases, the pattern of metaphase and anaphase showed slight relationship. Thus they had slight synchrony in their cycles as depicted in the peaks of their curves. On the contrary, prophase and telophase had slight synchrony in their rhythms, but prophase increases were matched with drops in interphase in most cases. Generally, the number of metaphase cells increased with decrease in interphase cells except in NCY 002 which recorded rise in metaphase with interphase cells.

The chromosome counts recorded in this work agrees with earlier counts and suggests that evolution of these two related species may have arisen from common progenitor, which has a basic somatic chromosome number of n = x = 6. Obviously therefore, the *Colocasia* may have arisen through polyploidization whereas *Xanthosoma saggittifolium* may have evolved through one-step chromosome doubling.

Cytoplasmic staining was reported for *Musa* spp. when

chromosomes were stained with FLP orcein (Osuji, 1998; Osuji et al., 1996). The observation of slight cytoplasmic staining in this work indicates that the cytoplasm does not respond to the stain equally in all plants. This stain is chromatin-specific and the staining of cytoplasm of different plants to varying degrees by this stain corroborates variation in the level of chromatin-associated structures in the cytoplasm. In the case of these genera, species or cultivars hereby worked on, the level of such structures is low.

As observed, the level of mitosis rose with time and peaked at a time when maximum anabolism is expected due to the availability of maximum sunlight. This implies that the much energy required for this process. Therefore, natural adaptation for this was necessary to allow for optimum use of light energy and at the same time enabling optimum conservation of energy through catabolism.

The processes of metaphase and anaphase, no doubt requires most energy, hence the timing of the two stages of cell division to correspond to the time of optimum energy availability. This therefore implies that for optimum metaphase harvest, collection of root stock should be conducted at about 12.00 noon. If root materials are properly harvested with caution to avoid upsetting a relapse of dividing cells to interphase, the number of metaphase harvest would be optimized, especially if the root stocks are first pretreated for up to three hours before fixing.

It is obvious from this work that there is not much difference between the *Xanthosoma* and *Colocasia* cultivars investigated with regard to the pattern of mitotic cycles and the peak time for optimum number of metaphase cells. Similarly, there was not much difference between cultivars within each of the two species investigated in terms of metaphase yield. Though NCY 002 showed relative number of interphase cells rising with metaphase, the situation does not affect the timing for collection of root material for chromosomal studies.

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