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Mitotic index studies on *Treculia africana* Decne. in Nigeria

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Abstract

Treculia africana Decne., (African bread fruit; wild jack fruit, or African boxwood), is a neglected and underexploited tropical tree crop belonging to the taxonomic family Moraceae. Mitotic index study was conducted on this species using fresh healthy root tips harvested from germinated seedlings. It was observed that cell division took place at all time in the day and night but took place more in the day time than at night. The most intense period of mitosis was between 2:00 and 6:00 pm with a peak at 4:00 pm (Nigerian Time). Mitotic counts showed that the number of prophase cells decreased when that of metaphase increased as the day progressed. The peak of anaphase was during the early hours of the night at 8:00 pm. The proportion of interphase cells reduced while that of dividing cells increased before the peak of metaphase. Information made available in this work henceforth pave the way for further progress in cytological and cytogenetic research towards improvement of this crop plant.

Introduction

Treculia africana Decne. (African breadfruit, African boxwood or wild jackfruit) belongs to the monospecific genus *Treculia* Decne. Ex Trec which is one of the fifteen genera of plants that belong to the tribe Artocarpeae in the Mulberry family, Moraceae. This species is believed to be native to a vast area extending from New Guinea through the indo-Malayan archipelago to western Micronesia. It is largely cultivated within the rainforest belt of West, Central and East Africa as well as Madagascar (around the West Indian Ocean). It is an evergreen forest fruit tree. The plant produces large, usually round, compound fruits covered with rough pointed outgrowths. The seeds are buried in spongy pulp of the fruits (keay, 1989).

There is increased interest in African breadfruit seed (Nwokolo, 1996), which is an important food item, popularly known as "Ukwa" by the Ibo tribal group of southeastern Nigeria. The seed is variously cooked as porridge alone or mixed with other food stuff such as sorghum (Onweluzo and Nnamuchi, 2009), or roasted and sold with palm kernel (*Elaeis guineensis*) as roadside snack. The flour has high potential usage for pastries (Onyekwelu and Fayose, 2007). The seeds are highly nutritious and constitute a cheap source of vitamins, minerals, proteins, carbo-hydrate and fats (Okafor and Okolo. 1974). Proximate analysis shows that the seeds contain 17-23 % crude protein, 11 % crude fat and other essential vitamins and minerals (Akubor *et al*., 2000). The seed kernel is used in preparing pudding, as a thickener in traditional soups and in the manufacture of food products such as flour for bread, beverages and weaning food for children (Onyekwelu and Fayose, 2007). African breadfruit is an important natural resource for the poor, contributing significantly to their income and dietary intake under poor heart conditions (Ogbonnia *et al*., 2008) and as animal food (Ejidike and Ajileye, 2007). African breadfruit is also useful in the ethnomedical management of diabetes mellitus.

The tree crop is widely cultivated in the southern states of Nigeria where it serves as low cost meat substitute for poor families in some communities (Badifu and Akubor, 2001; Ugwu *et al*., 2001). Despite the socio – economic importance of the plant to a very large population of the people of southern Nigeria, it is still a protected crop and a semi domes-

Table 1. Relative proportion of cells (in percentage) at the different stages of mitosis at the different periods of a 24-hour cycle of a calendar day.

Time	Prophase	Metaphase	Anaphase	Telophase	Interphase
6:00 am	41.2	8.8	7.4	6.6	36.0
8:00 am	35.0	11.9	11.3	13.0	28.8
10:00 am	28.0	18.7	14.5	15.4	22.4
12:00 pm	29.7	22.0	19.2	11.4	17.7
2:00 pm	27.8	24.8	17.9	10.8	18.7
4:00 pm	23.6	25.6	18.9	14.2	17.7
6:00 pm	17.2	23.4	18.8	20.1	20.5
8:00 pm	18.1	15.1	20.1	21.1	25.6
10:00 pm	27.2	13.6	14.7	20.4	30.2
12:00 am	17.5	12.5	12.5	14.5	43.0
2:00 am	22.5	11.6	12.2	6.9	46.8
4:00 am	28.9	11.7	9.1	8.0	42.3

ticated species. Increase in human population and agricultural practices have put pressure on forest reserves thereby depleting some genetic resources. To abate total loss or extinction of some of these important forest species, they must be cultivated more intensely. Significant to achieving this is the improved propagation of the concerned varieties of this species, either by seed or stem cutting. Few empirical studies (Baiyeri, 2003; Ugwunze, 2003) used fresh seeds for planting, and reported less than 80% seedling emergence. Besides, practical observations showed that viability decreases as the seed looses moisture during storage.

Four major research gaps have limited the widespread utilization of the African breadfruit as a major food crop and industrial feed stock. They are: limited agronomical information, lack of mechanical devices for large scale processing of the fruit and seed and lastly limited international public awareness regarding its use in regions other than some parts of West, Central and East Africa. The seeded breadfruit is always grown from seeds, which must be planted when fairly fresh as they lose viability in few weeks. The seedless breadfruit is often propagated by transplanting suckers, several of which spring up naturally from the roots. The seedless type is uncommon in Nigeria and most parts of West Africa.

One major handicap in the widespread propagation and improvement of the African breadfruit is the insufficient information on its genetics and breeding. Not much has been reported on the genetics of this species. Without good knowledge of its genetics especially chromosomal attributes, breeding of the species would hardly be optimized. Cytogenetic studies require knowledge of the most appropriate time for root tip collection for adequate observation of the chromosomes. This could best be determined through mitotic index studies.

The Mitotic index of a cell population has long been regarded as an important criterion of the growth and multiplication of the cells and tissues. It is commonly measured in fixed and stained specimens, and therefore represents the stage of the material at the time of fixing only. One of the reasons for the mitotic indexing of species is to generate data which is important for breeding purposes. Some of such data are the chromosomal attributes. Chromosome details are best studied on cells with optimal chromosome contraction or condensation, i.e. metaphase cells. These are best studied using root tips as well as floral buds. For forest tree crops such as *Trecilia africana*, the most convenient materials for such study are young root tips of germinating seeds. However, cells grown in tissue culture may be observed and photographed for a considerable period before any measurement is made; but such observations, which have been reported elsewhere (Walker and Yates, 1952), show that certain cytochemical results may require reinterpretation. Hence, the objective of this work was to confirm, through mitotic index studies, the best time for collection of root materials for chromosomal studies on *Treculia africana*.

Materials and methods

The materials used for this research work were gathered from local farmers in Imo, Anambra and Rivers States, Nigeria. Mature fruits of *Treculia africana* were bought. The seeds were washed and air dried for about 7 days before planting in plastic pots and black polyethylene bags containing Sawdust to generate healthy roots. After 2 weeks of planting, fresh healthy root tips were collected at two–hourly intervals for 24 hours (starting from 6.00 am to 4.00 am the following day) from the seedlings. The root tips were transferred into a specimen bottle contain-

ing 2 ml of 3:1 ethanol acetic acid to fix for 24 hrs. After fixing, the root tips were transferred into another specimen bottle containing 2 ml 70 % ethanol solution for storage. Prior to squashing, the roots were hydrolyzed in 0.5 % aqueous HCl (for 4 to 5 minutes) in a watch glass. About 1 mm tip of each hydrolyzed root was excised and squashed in FLP–orcein (Osuji, 2003; Osuji *et al*., 1996) under No. 0 cover slip on a clean glass slide. The chromosome spreads were then observed microscopically. The numbers of dividing cells at the different stages of mitosis were recorded for the various root tip collections (in the day and at night). The mitotic data were subjected to statistical analysis using SPSS version 11.

Results and discussion

Cytoplasmic as well as nuclear staining was observed in meristematic cells but the level of cytoplasmic staining was very low compared with the nuclear staining. Cytoplasmic staining had earlier been reported for *Musa* spp (Osuji *et al*., 1996) and *Xanthosoma* and *Colocasia* (Ekanem and Osuji, 2006). The common occurrence of the cytoplasmic staining is apparently due to the diffusion of some of the nuclear contents into the cytoplasm due to the disassembling of the nuclear membrane at the commencement of the mitotic prophase.

At all times around the clock, there was cell division (Table 1; Figure 1). The rate of division varied widely between the night and the day. The percentage of dividing cells is higher in the day time than at night. During the period between 4.00 am and 6.00 am, the number of cells involved in mitotic division radically starts to increase. At about 6:00 am, a high percentage of prophase cells was recorded. The percentage of prophase cells remained relatively high till about 6.00 pm when the percentage of dividing cells started decreasing. Late into the night between 12:00 and 2:00 am, relatively very low level of mitosis took place.

The cells that enter mitotic process start from the prophase stage as corroborated by the rise in the number of cells at this stage (Table 1; Figure 2). Metaphase increased after prophase and continued mostly between 8.00 am and 6.0 pm but had its optimum at about 12.00 noon to 4.00 pm. The number of cells at anaphase started to rise at about 10.00 am and continued till sunset. At sunset, the number of cells in the mitotic process reduced very obviously (Figure 1). Consequently, the number of cells at interphase became higher while the number of dividing cells reduced and remained relatively low in the night (i.e. as from after sunset till sunrise) compared with the day.

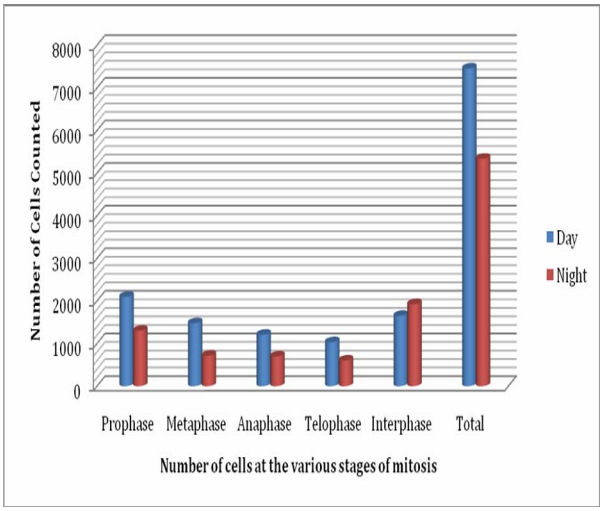

Fig 1. Diurnal variation in the level of mitosis expressed as variation in the proportion of cells at the various stages of mitosis.

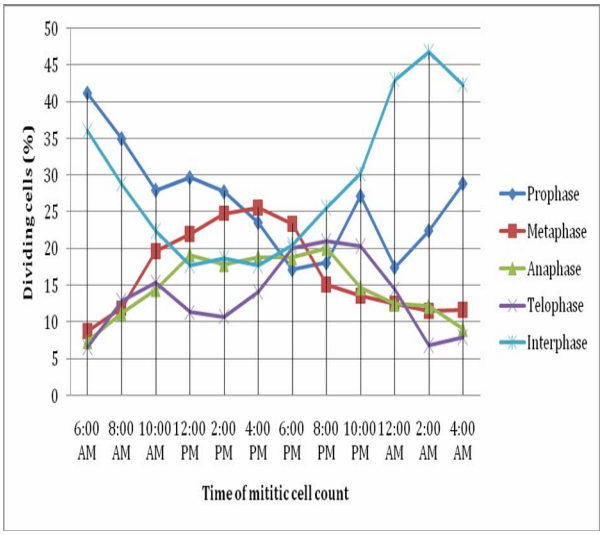

Fig 2. The proportion of cells at the various stages of division and the dynamics of division showing that the highest number of metaphase cells was obtainable at 4:00 pm while the highest number of resting cells is at 2:00 am.

At night however, most of the meristematic cells rested. For other cells that proceeded on mitosis late in the day, the process draged and got late into the night time. Hence there were always cells at the various stages of the mitotic division. The observation of the highest number of metaphase cells at the period between 2:00 and 4:00 pm, a period when most plants record optimum photosynthetic level showed that it is within this same period that most active mitosis take place in plants, especially *Treculia africana* just as in

Xanthosoma and *Colocasia* spp. (Ekanem and Osuji, 2006). It is important to note here that there was slight difference between the peaks of mitosis between *Treculia africana* and those reported for *Xanthosoma* and *Colocasia* by Ekanem and Osuji (2006). This implies that the peaks of photosynthesis as well as mitosis may vary between species and could be exploited taxonomically.

The number of mitotic cells that commenced mitosis in a day was quite high but the pace was not uniform. Hence some cells were faster than others in the mitotic process. As the day progressed, the number of cells at the metaphase stage increased being highest at about 2:00 to 6:00 pm with its peak at 4:00 pm. Though the number of dividing cells increased during the day, the number of interphase or resting cells increased during the night (Figure 2). The rise in the number of cells in anaphase was after the rise in the number of those at metaphase and reached its peak at 8:00 pm. The number of cells at anaphase diminished sharply after reaching its peak and remained very low between 2:00 am and 6:00 am.

Though less of the mitotic division process took place during the night time, it is very probable that during the night time DNA synthesis and resting phases of the cell cycle mostly commence. Information and data presented in this work is expected to set the pace for invigorated cytogenetic research and improvement by breeding of this novel, invaluable but neglected and underexploited economic crop.

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