

SHORT COMMUNICATION

## Morphological, cytogenetic and genotypic differences between *spicata* and ordinary tall coconut (*Cocos nucifera* L.)

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**Abstract:** *Cocos nucifera* L. coconut is an economically important crop throughout the humid tropics for food, oleochemicals and as a source of uncontaminated sweet beverage. Palms that contain morphologically different inflorescences are referred to as *spicata* coconut and are considered as a special form of the variety *Typica*. A number of differences exists in the *spicata* inflorescence, when compared to that of the ordinary tall variety. The main difference in the *spicata* inflorescence is the suppression of male flowers on the inflorescence and the presence of a large number of female flowers compromising the former. Based on the type of branching of the inflorescence and the structure of the male and female flowers, different *spicata* types can be identified. The objective of the present study is to document the different *spicata* types of coconut and to investigate whether there exists karyotypic differences between the different types of *spicata* and the ordinary tall coconut. The genomic composition of the root tip cells of *spicata* seedlings was studied using the chromosome squash technique. The squash method performed on root tip cells obtained from both *in vivo* as well as *in vitro* grown seedlings using aceto-carmine showed that *spicata* consists of cells of the normal chromosome complement of coconut  $2n=32$  and cells with an aneuploid chromosome complement varying from 18-24. Length measurements of the chromosome complement in diploid *spicata* cells and ordinary tall coconut showed similar length categories. Further studies are necessary to differentiate the lengths accurately. However, an accurate measurement of the DNA content of the diploid and aneuploid cells obtained using a Flow Cytometer indicated that the ratio between the relative DNA contents of diploid to aneuploid cells in *spicata* ranged from 1:0.5 to 1:0.6. Thus, *spicata* can be considered as a mutation of ordinary tall coconut.

**Keywords:** Chromosome, coconut (*Cocos nucifera* L.), cytology, flow cytometry, *spicata*

### INTRODUCTION

*Cocos nucifera*, (coconut) is the most widely grown plantation crop in Sri Lanka. It is also grown in about 85 other countries around the world. Due to the numerous uses of the coconut palm it is commonly known as the 'tree of life'. The occurrence of coconut palms with unbranched inflorescences appears to be rare in all coconut growing regions of the world<sup>1</sup> and this type of palms is referred as *spicata*<sup>2,3</sup>. The major classification of *Cocos nucifera* L. that is based on the stature of the palm, recognizes two types; *Typica* (Tall type) and *Nana* (dwarf type)<sup>4</sup>. Apart from the above classification there are various other classifications based on local studies. Liyanage<sup>5</sup> has classified the coconut varieties and forms occurring in Sri Lanka into three varieties namely *Typica*, *Nana* and *Aurantiaca*. Narayana and John<sup>6</sup> have developed a synoptic classification of varieties and forms of coconut into *Typica*, *Nana*, *Androgyne*, *Javanica* and *spicata*.

There are different ideas about the taxonomy of *spicata* in the literature. While it is described as a variety<sup>6</sup>, it is also indicated as a form of the variety *Typica* rather than a distinct botanical variety<sup>7,8</sup>. Ohler<sup>1</sup> has stated that as the palm had no male flowers or only a few, it can hardly reproduce itself true to type and as such it cannot be called a variety. He further states that *spicata* palms occur in different coconut varieties.

*Spicata* palms represent a distinct form of ordinary tall coconut with a characteristic unbranched inflorescence or rarely with one or two spikelets, in which there is a considerable suppression in the number of male flowers and a preponderance of female flowers. This character

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of a high number of female flowers in the *spicata* inflorescence is a desirable character which could be introduced in a breeding programme in order to increase the nut production. However, a complete documentation on the morphology, taxonomy and genetic control of the *spicata* character in coconut is lacking and this in turn limits the potential of exploiting this character in a breeding programme. The present study was, therefore, planned with the objectives of documenting different types of *spicata* in coconut and investigating the karyotypic characteristics of the *spicata* and ordinary tall coconut.

## METHODS AND MATERIALS

The plant materials (inflorescences, root tips and leaves) used in this study were collected from healthy palms and seedlings of the ordinary tall coconut (as control) and *spicata*, available at the Bandirippuwa Estate, Coconut Research Institute (CRI), Lunuwila, the Isolated Seed Garden (ISG), Ambakelle and a home garden in Chilaw.

Identification of different types of *spicata* inflorescences, the characteristic features of each inflorescence type and their pollination behaviour (the duration of male and female phases of inflorescence) were studied. Furthermore, the male and female flowers of the inflorescences were studied by observing seven *spicata* palms from the Bandirippuwa Estate, Lunuwila, two *spicata* palms each from ISG and a home garden.

Cytological observations were conducted using actively growing root tips<sup>9</sup> obtained from inbreeding type *spicata* mother palms and seedlings of ordinary tall coconut (control). The observations were made between 9.00-9.30 a.m, in order to access the highest number of actively dividing cells from *in vitro* cultured *spicata* plants. The root tip was cut 1 cm from the apex. After removing the root cap, it was pre-treated with colchicine<sup>10</sup> and washed thoroughly. Then, the tissue was subjected to fixation and hydrolysis. Slides were prepared using the simple acetocarmine squash technique<sup>11</sup> and observations were made through the light microscope using the oil immersion technique. The number of chromosomes and the relative lengths of each chromosome within the actively growing cells were recorded.

The DNA content of cells was analyzed using the flow cytometer<sup>12,13</sup>. Mature leaf material and root tips (obtained from *in vivo* as well as *in vitro* seedlings) were used and 1-2 g of material was chopped with the PARTEC (Partec, GmbH) buffer and filtered with a 50 µm nylon mesh. The filtrate was stained with the PARTEC stain, and was kept in the dark on ice (14). The maximum signal amplitudes, which causes the sharpness of the peaks, were obtained with a Flow Cytometer (Partec CA II, GmbH). The flow speed of analyzed cells was in the range of 50-100 per second.

## RESULTS AND DISCUSSION

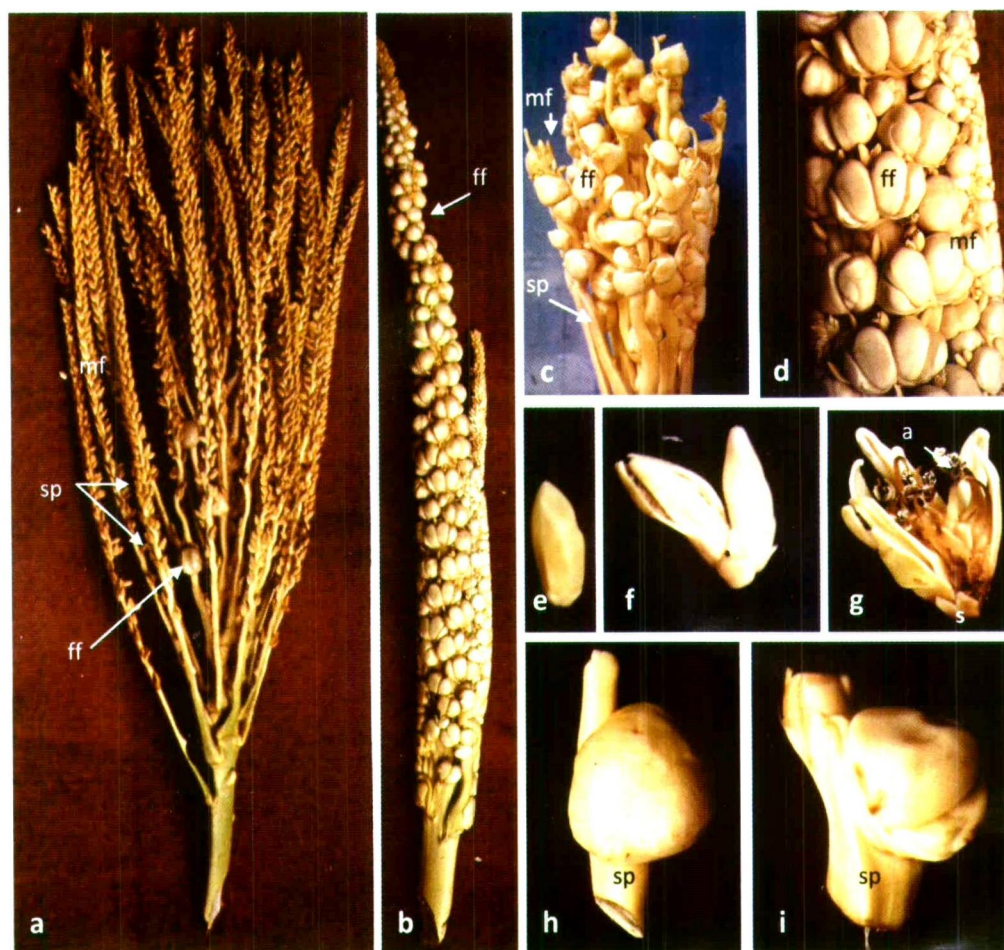
*Spicata* palms, have inflorescences which are different from those of the ordinary tall palms (Figure 1a). There are some specific characters in the *spicata* inflorescences (Figures 1b, 1c). The spathe that covers the inflorescence of *spicata* is much thicker than that which covers the inflorescence in the ordinary tall variety. This may cause difficulty in splitting of the spathe, resulting in delayed pollination. The most interesting characteristic features of the *spicata* inflorescence is the presence of a large number of female flowers and reduction of the number of male flowers. The number of female flowers in *spicata* varies from 50-500 whereas in ordinary inflorescences it is in the range of 15-50. The number of male flowers in *spicata* is in the range of 100-1000 compared to 5000-8000 found in ordinary inflorescences.

There is a wide variation in *spicata* based on morphology as well as the pollination behaviour. *Spicata* in coconut, exhibits a range from the characteristic unbranched inflorescences (Figure 1b) to branched inflorescences (Figure 1c). The spikelets (branches) of the branched inflorescences are shorter than those of the ordinary inflorescences. Both male and female flowers can be found throughout the spikelet in *spicata* whereas the female flowers are concentrated at the basal part of the spikelet in an ordinary inflorescence. The characteristic feature of the unbranched type of *spicata* is that female flowers are attached to the main rachis from top to bottom, and male flowers are interspersed among the closely set female flowers (Figures 1b, 1d).

**Table 1:** The relative length categories of the chromosomes of *spicata* and ordinary tall coconut

Spicata	4.3	3.5	3.5	3.1	3.0	2.9	2.8	2.7	2.6	2.5	2.4	2.3	2.2	2.1	2.0	1.8
Standard I	3.0	2.7	2.7	2.5	2.4	2.4	2.3	2.1	2.1	2.0	1.9	1.8	1.8	1.7	1.4	1.3
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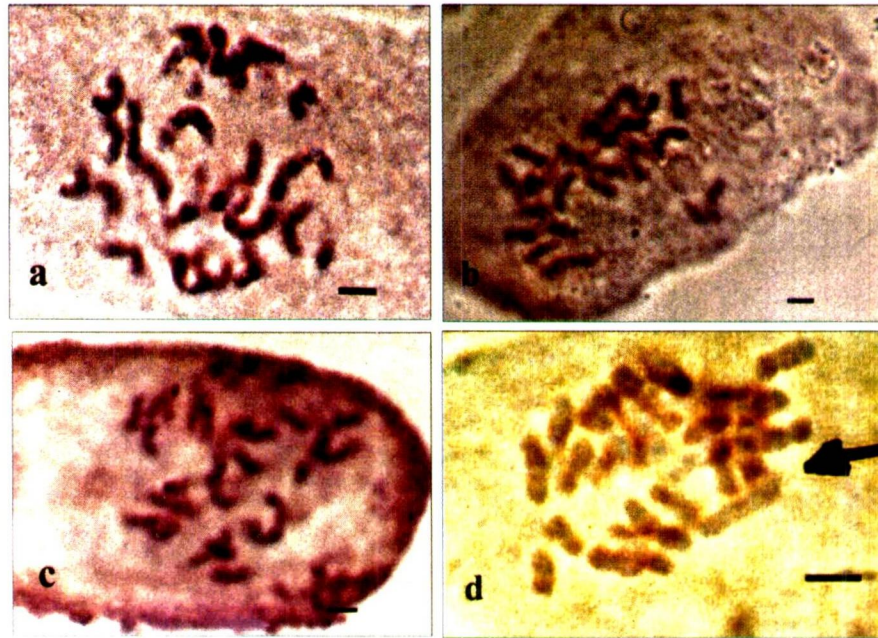
\* Recorded by Ninan and Satyabalan<sup>7</sup>



**Figure 1:** *Spicata* inflorescence in coconut (*Cocos nucifera* L.). **a.** Inflorescence of ordinary tall coconut. **b.** Inflorescence of ideal *spicata* (unbranched type) in which both male flowers (mf) and female flowers (ff) attached to the main rachis (r) of the inflorescence. **c.** Branched type inflorescence. Both male and female flowers attached throughout the spikelets **d.** Close view of ideal *spicata* type **e.** An ordinary male flower. **f.** Peculiar form of male flower of *spicata*; two flowers attached together at the base. **g.** Peculiar characters exist in *spicata* male flowers with varying numbers of sepals (s) and petals (p) and anthers (a). **h.** Female flower of ordinary tall coconut. **i.** Female flower of *spicata*. Note that two flowers are attached together at the base.

The close observation of structural characteristics of the male flowers indicated that *spicata* inflorescences contain single male flowers, like the ordinary male flowers (Figure 1e) as well as those where two or three flowers united at the base (Figure 1f). The number of segments in the male flowers is highly variable. Perianth segments vary from 3-15 and stamens vary from 6-18 (Figure 1g). Maturing of the male flowers does not show a distinct pattern as in those from the inflorescences of ordinary tall type in which the maturing of male flowers begins at the top of each spikelet and proceeds towards the base. *Spicata* inflorescences consist of single female flowers (Figure 1h) as well as ones which are attached to one or two other flowers (Figure 1i). Hermaphrodite flowers were also observed on *spicata* inflorescences. However, it is not normally found in *spicata*.

There is a variation in the pollination behaviour of different *spicata* inflorescences. Ohler<sup>1</sup> has indicated that there are no reports of autogamous *spicata* palms. In the present study, both overlapping and non-overlapping of male and female phases in inflorescences were observed in different *spicata* palms leading to inbreeding and out breeding types. Overlapping of male and female phases could be observed in inbreeding type of *spicata* in which self pollination occurs before the spathe is split opened. The inflorescences of the unbranched type (with characteristic *spicata* type) showed inbreeding type. However, in the out breeding type, male phase is distinctly different from the female phase resulting in cross-pollination, which is similar to the pollination behavior of tall coconut. This could be identified in the branched type *spicata*.



**Figure 2:** Cytological views of the cells of root tips obtained from ordinary tall coconut (*Cocos nucifera* L.) and *spicata*. **a.** Chromosome complement of ordinary tall coconut **b.** Normal diploid cell of *spicata*. **c.** Normal diploid cell of embryo cultured *spicata*. Note that only part of the number of chromosomes could be observed in a single plane of the squash in each slide and other chromosomes could be observed after fine focusing. **d.** An aneuploid cell of *spicata* (Bar=10 $\mu$ m).

Even though *spicata* consists of a large number of female flowers, the seed setting percentage is very low. Normally, only very few (0-4) nuts could be seen in a bunch. This may be due to several reasons such as the inflorescence consisting of a comparatively low number of male flowers; the pollen of these flowers may not be sufficient for selfing of all the female flowers. The studies revealed the viability of the pollen in these inflorescences is also comparatively lower than the ordinary type (data not shown). Furthermore, abnormalities occurred in the female flowers (as described earlier) may lead to failure of pollination. The space between the female flowers may not be sufficient for them to be developed into complete fruits. With a high setting percentage, shedding of immature nuts will be inevitable as there is not enough space for so many nuts on the spadix, unless the palm bears very small nuts<sup>1</sup>.

The best slide preparations were obtained in the squash method by adopting the following conditions; pre-treatment in 0.1% colchicine for 2 hours, fixation in glacial acetic acid/ absolute alcohol (1:3) fixative for 1 1/2 hours and hydrolysis in 1N HCl at 60 °C incubation for 20 minutes. Comparable results were obtained with the *in vitro* grown root tip cells.

The cytological study showed that there are two types of cells in the *spicata*, diploid cells (Figures 2a,

2b,2c) with a chromosome complement of  $2n=32$ , and aneuploid cells (Figure 2d) with a varying chromosome complement from 18-24. However, majority of the cells were diploid and about 35% cells contained an aneuploid chromosome complement. Previous Studies<sup>15,16</sup> reported that the chromosome complement of *spicata* is  $2n=32$ . The relative length measurements of chromosomes were recorded and the length categories were similar for diploid cells of the two types (Table 1). The relative length measurements were compared with the standard chromosome lengths recorded previously<sup>7</sup>. According to these results, the relative length categories were similar for diploid cells of both *spicata* and ordinary tall coconut. The same study<sup>7</sup> suggested that the *spicata* character is incompletely dominant or pleiotropic. Hunger<sup>17</sup> described *spicata* character as a mutation and heritable.

The relative DNA content of *spicata* was compared by using leaf and root tip samples of ordinary tall coconut as the internal reference standard. The  $G_0$  peak of this standard recorded a channel number of 59.93 (Figure 3a). A large number of signals occurring in the lower channel region resulted from the disrupted nuclei and non specific staining of other cell constituents. The channel numbers of the peaks of the mixture of *spicata* cells and standard (1:1) recorded 38.4 and 22.4 (Figure 3b) with percentage of cells in each category at 72% and 45% respectively, indicating a difference in DNA content in the reference

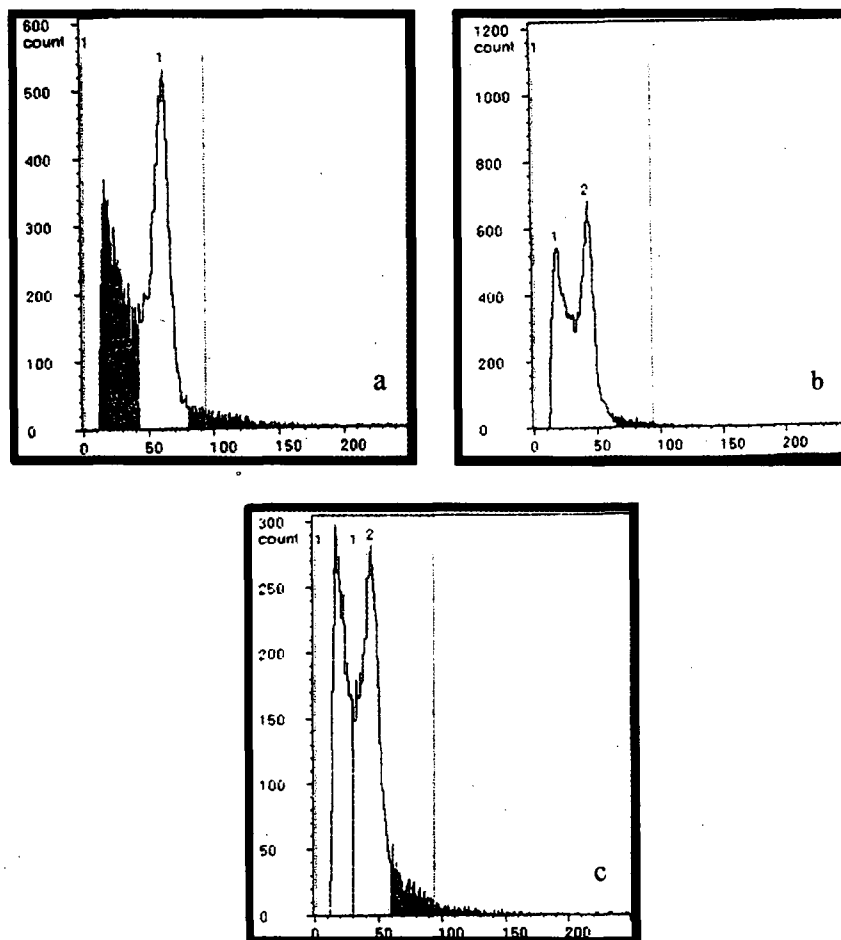


Figure 3: DNA histograms of fluorescence intensity of nuclei isolated from tall and *spicata* leaf samples after staining with PARTEC stain. a. Standard diploid coconut that was used as the internal reference. b. Histogram obtained from the mixture of leaves from *spicata* and tall coconut in the proportion of 1:1. c. Histogram of *spicata*.

and target samples. The DNA histogram resulting from *spicata* cells recorded two peaks indicating channel numbers 43.3 and 20.7 (Figure 3c) with percentages of cells in each category 50% and 36% respectively, indicating that *spicata* consists of cells of two different DNA contents. The coefficient of variability for all peaks was between 9% - 27%, which implies the accuracy of the results.

## CONCLUSION

Under Sri Lankan conditions, classification of *spicata* can be done based on either pollination behaviour or the branching habit of the inflorescence. Based on pollination behaviour, selfing (in-breeding type) and outcrossing (out-breeding type) *spicata* forms can be identified. *Spicata* can also be categorized into two types namely branched and unbranched type (ideal *spicata* type), based on the

branching habit of the inflorescence. The chromosome squash studies revealed that the *spicata* type consists of two types of cells; diploid cells with 32 chromosomes and aneuploid cells with chromosomes varying from 18-24. The relative length categories of chromosomes of the diploid *spicata* are parallel with ordinary tall type cells. Flow Cytometer measurements indicate that the *spicata* cells has two types of cells, normal diploid cells and aneuploid cells. The relative DNA contents of these diploid to aneuploid cells of *spicata*, range from 1:0.5 to 1:0.6. Thus, *spicata* can be considered as a mutation of ordinary tall coconut.

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