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Full Length Research Paper

Targeted mutagenesis in *Vigna unguiculata* (L.) Walp. and *Cucumeropsis mannii* (NAUD) in Nigeria

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The mutagenic effects of 0.2% aqueous solution of colchicine on gross and micromophological features of seedlings of *Vigna unguiculata* (L) Walp. and *Cucumeropsis mannii* (Naud.) were investigated. Terminal buds of two-week old seedlings were assaulted with 0.2% colchicine by the cotton plug and micro syringe methods and performances were monitored until maturity. Colchicine-treated *V. unguicu-lata* seedlings were observed to grow slower, had malformed leaves, flowered late and produced less number of seeds per pod than the control. Mean values of features like stomatal indices on both the abaxial and adaxial surfaces, terminal leaflet dimensions and trichome distribution between the treated and control were found to be significantly different. In contrast, treated seedlings of *C. mannii* flowered and fruited earlier than the case of control material. However, growth was also slowed down by the treatment while features like stomatal indices and trichome distribution were not significantly different. The results here have shown that apart from doubling of chromosomes, colchicine can also be used to induce other mutagenic changes which may be of agronomic utility.

Key words: Chromosomes, colchiploidisation, mutagenesis, agronomy.

INTRODUCTION

Mutation increases genetic diversity which leads to subsequent phenotypic plasticity upon which natural selection acts. Many genera and families of plants include naturally occurring polyploidy species ranging from diploids to extreme polyploids. Since natural polyploidy has played a role in the evolution of many cultivated crops (Davis and Heywood, 1967) plant breeders have utilized experimentally produced polyploids to obtain an even wider range than is naturally available. Polyploidy can be induced artificially in plants with colchicine, a highly potent c-mitotic agent, from the meadow saffron, *Colchicum autumnale* that inhibits the process of mitosis (Blakeslee and Avery, 1937; Eigsti and Dustin, 1955). It hampers the development of nuclear spindle so that during anaphase the chromatids are not distributed to the poles of the dividing cell. Colchicine also induces polyploidy in plant cells during cell division by inhibiting chromosome segregation during meiosis. Half the resulting gametes therefore, contain no chromosomes, while the other half contain double the usual chromosome number (i.e. diploid instead of haploid as gamete usually are) and lead to embryos with double the usual chromosome number (tetraploid instead of diploid). It has been argued that chromosomes determine the characters whereas the characters do not determine the chromosome. Obviously differences in chromosome number within a species complex are sometimes, but certainly not always correlated with differences in morphology thus there are cases where chromosomal differences are not accompanied by breaks in morphology (Davis and Heywood, 1967).

Genetic manipulations with colchicine have led to the production and release of a number of new cultivars of important crops. Chromosome manipulation is one of these methods used by plant breeders to produce improved varieties (plants with hybrid vigour) of economically important plants. The elucidation of chromosome structure and behaviour is unarguably relevant in unravelling genome organization and interaction (Kurata et al., 2002) with a view to utilizing the information amassed in this regard with higher technological approaches for crop improvement. Apart from chromosomal changes, various morphological changes have been effected by colchicine in different plants in the past years. Blakeslee and Avery (1937) worked on *Datura stramonium* and reported retar-

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ded growth with colchicine. Similarly, such results have been obtained by other workers such as Ugborogho and Sodipo (1985) with *Lycopersicum esculentum*, Hewawasam et al. (2004) with *Crossandra infurdibuliformis,* and Ndukwu and Obute (2006) with *Solanum nigrum*. Ugborogho and Obute (1994) got the same result while working with *Vigna unguiculata* but their result varied from the observations of others in that the treated *V. unguiculata* later overcame the induced suppression and grew taller than the controls at maturity. While Franzke and Ross (1952) reported that their treated *Sorghum vulgare* grew very much taller than the control.

Furthermore, colchicine induced mutations affected agronomic characters in sorghum including production of homozygous genotypes, morphological changes of inflorescences and chimeral panicle (Franzke and Ross, 1952). Additionally, leaf abnormalities ranging from changes in shape (unequal development of lamina), size, margin, and apex of leaves to development of forked and double leaves have been reported by (Blakeslee and Avery, 1937; Hewawasam et al., 2004; Obute et al., 2005).

Changes in the floral behaviours from delayed flowering, bigger flower production to malformed flowers are also a common feature of colchicine treated plants with reports of different colours of floral parts on the same branch (Blakeslee and Avery; 1939; Dirk et al., 1956). Increased branching (bushy habit) has been reported with colchicine treatment (Blakeslee and Avery, 1937; Hewawasam et al., 2004; Ndukwu and Obute, 2006). At the micro morphological level, Ndukwu and Obute (2006) also reported higher stomatal indices accompanied by reduced number of epidermal cells, larger and fewer stomata as consequences of colchicine treatment. Meanwhile, colchicine has also been reported not to have effected chromosome doubling in *flax* by Dirk et al. (1956), *Sorghum vulgare* by Franzke and Ross (1952), *V. unguiculata* by Ugborogho and Obute (1994).

It is against this background that we undertook to investigate the effects of colchicine treatments on the life history and genetic make up of *V. unguiculata* and *Cucumeropsis mannii* which are crops of agronomic importance in sub-Saharan Africa. The study is with a view to ascertain areas where targeted mutation can enhance the utility of these crops and improve food security in this region.

MATERIALS AND METHODS

Seeds

Dry seeds of *V. unguiculata* were bought from ADP (Agricultural Development Programme) Rivers State, Port Harcourt while dry seeds of *C. mannii* were bought from New Market in Aba, Abia State. These were planted in separate nursery bags at the Botanical Gardens of University of Port Harcourt. They were allowed to grow for 2 weeks before treating with colchicine.

Colchiploidisation

The methods of Obute et al. (2006) were used to apply colchicine on the plants. The first involved assaulting growing buds with cotton wool soaked with 0.2% (w/v) solution of colchicine. The cotton wool was left on the bud for 2 h then removed and the process was repeated again for three consecutive days. The second entailed sucking the 0.2% (w/v) solution of colchicine into a micro syringe and released in drops on the growing buds. The process was repeated for three consecutive days. Then, the treated seedlings were properly label-led and left to grow to maturity.

Morphological studies

The gross morphological features of the treated and the control plant were visually examined and records were taken of any change due to colchiploidisation in aspects of the plant growth habit, leaf type shape, leaf area, number of shoots per plant, flower behaviour and fruit size. Measurements of these morphometric features were taken from five plants of each cytotype.

Micro morphological studies

Rectangular cuttings were obtained from the equatorial regions of mature terminal leaflets using a razor blade from both the treated and control. The epidermal preparations were made by bleaching the cut pieces in 3.5% m/v sodium hypochlorite solution (NaOCl). The transparent epidermal peels were properly rinsed off with water, the strips were then stained with safranin and the excess stain was still rinsed of with water. Forceps were used to pick the stained strips which were temporary mounted with the near mesophyll surface on a drop of glycerine on a clean glass slide. Cover slips were placed over these and the preparations were examined microscopically. The number of epidermal cells, stomata and trichomes were scored at X 40 field view on a camera Lucida microscope. Stomatal indices were computed with the technique of Stace (1965) while good plates were photographed with a research microscope fitted with a Model RCA video monitor to reveal foliar epidermal features of interest. Images were photographed from the screen of the monitor with an Olympus digital Camera.

RESULTS

Treatment with 0.2% aqueous solution of colchicine produced morphological variations in both *V. unguiculata* and *C. mannii*. The seedlings of treated *V. unguiculata* were slower in growth; the first foliage leaves were malformed or irregular in shape and darker green colour than the control. In contrast, those of treated *C. mannii* were faster in growth than the control.

Qualitative morphological characters

Treatment with colchicine produced no noticeable effect on the pattern of pigmentation of the stem and perianth of *V. unguiculata* and *C. mannii*. Bases of the lateral leaflets, bases of the branches and those of the petioles were pigmented in both treated and control of *V. unguiculata* and *C. mannii*. Moreover, in the *V. unguiculata,* some of the treated and control seedlings had indeterminate and spreading growth habit while others had determinate and erect habit. For the *C. mannii*, both the treated and control had indeterminate and spreading growth

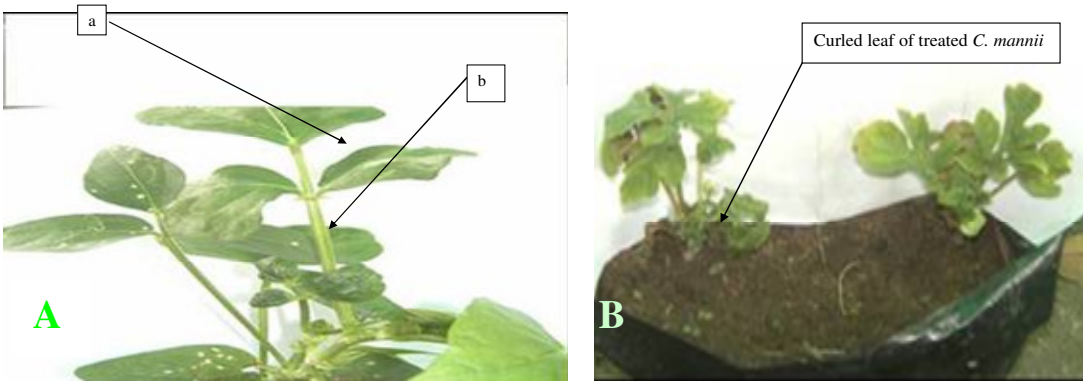

Figure 1. (A) The effect of colchicine on leaf of *Vigna unguiculata*. a). normal leaf b). curled leaf. **(B)** Treatment of seedlings with colchicine produced curled (arrowed) leaf in *C. mannii*.

Table 1. Mean linear dimensions of leaves of treated and control plants of *V. unguiculata*..

Leaf dimension	Length (cm)			Breadth (cm)		
	Min	X ± s.d.	Max	Min	X ± s.d.	Max
Treated	8.2	8.50 ± 0.41[a]	9.2	5.3	6.56 ± 0.94[b]	7.3
Control	8.9	9.52 ±0.66[b]	10.5	6.1	6.90± 0.55[b]	7.4

Values with similar superscripts are not significantly different.

habit. However, most of the treated *V. unguiculata* did not grow above the heights they were when they were treated and subsequently died off. This was not noticed in *C. mannii* for both the treated and control grew to maturity.

Quantitative morphological characters

The treated seedlings of both *V. unguiculata* and *C. mannii* were observed to have both smooth and curled leaves as evident in Figures 1a and 1b, respectively. Moreover, *V. unguiculata* had shorter branches, late flowering (about 3 weeks after the control had flowered), fewer flowers, smaller leaf dimension than the control as shown in Table 1. However, statistical evidence revealed that while the difference in means between the treated and control, *V. unguiculata* was highly significant for the leaf length; it was not significant for the leaf breadth ($P \leq 0.05$). Contrary to the results in *V. unguiculata*, *C. mannii* flowered earlier (about 1 week before the control) and produced more flowers than the control. However, much more information is needed here because the fruits rotted before harvest and we could not analyse this seedlings further. Differences occurred in the linear dimensions of the leaflets arising from colchicine treatment. Those of the treated material were shorter on the average than those of the control and these differences were highly significant for the length but not for the breadth (Table 1).

Number of seeds per pod

Treated *C. mannii* had early fruiting, *V. unguiculata* fruited late (production of pods), had fewer pods, shorter pod length and subsequently, less number of seeds per pod than the control (Table 2). The tests for difference in the means of treated and control *V. unguiculata* showed that it was significant at ($P \leq 0.05$).

Micromophological characters

Colchicine treatment led to variations in the number of stomata, epidermal cells and trichomes on the different leaf surfaces. In the adaxial surface of the epidermis of the treated *V. unguiculata*, shorter but many unicellular trichomes, fewer epidermal cells and many smaller sized stomata (Figure 2) were observed when compared with the control. However, the shape of the epidermal cells ranged from pentagonal to hexagonal. When compared with the control the stomata and epidermal cells on the treated material were larger. However, the adaxial surface of the epidermis of the treated *C. mannii* had rounded stomatal pore and fewer stomata, small number and size of epidermal cells and also fewer and shorter trichomes when compared with the control. The control had cylindrical shaped stomatal pore, well spaced stomata and more regular shaped epidermal cells than the treated as evident in Figure 3. Moreover, in the abaxial surface of the treated *C. mannii*, the trichomes were observed to be more in number and longer and the epidermal cells were also larger and more in number than that of the control. The epidermal cells appeared to be more irregular in shape than the control. On the whole, the nature of trichomes was unicellular and adaxial trichomes of the treated pla-

Table 2. Mean trichome distribution of treated and control *V. unguiculata* and *C. mannii.*

Plant species	N	Adaxial Epidermis (X ± s.d.)	Abaxial Epidermis (X ± s.d.)
V_T	30	1.4 ± 0.62^a	0.8 ± 0.66^e
V_C	30	0.3 ± 0.45^b	0.4 ± 0.51^e
C_T	30	1.8 ± 0.40^c	0.4 ± 0.79^f
C_C	30	1.7 ± 1.37^d	0.8 ± 0.90^f

Mean values with similar superscripts are not significantly different. T = Treated, and C = control.

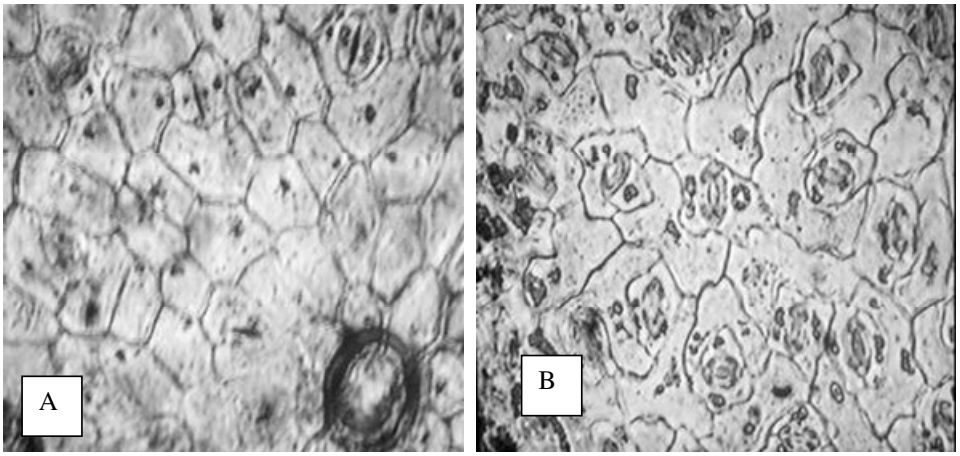

Figure 2. The leaf epidermes of treated *V. unguiculata* showing: A). Few stomata on the adaxial surface. B). More stomata on the abaxial surface.

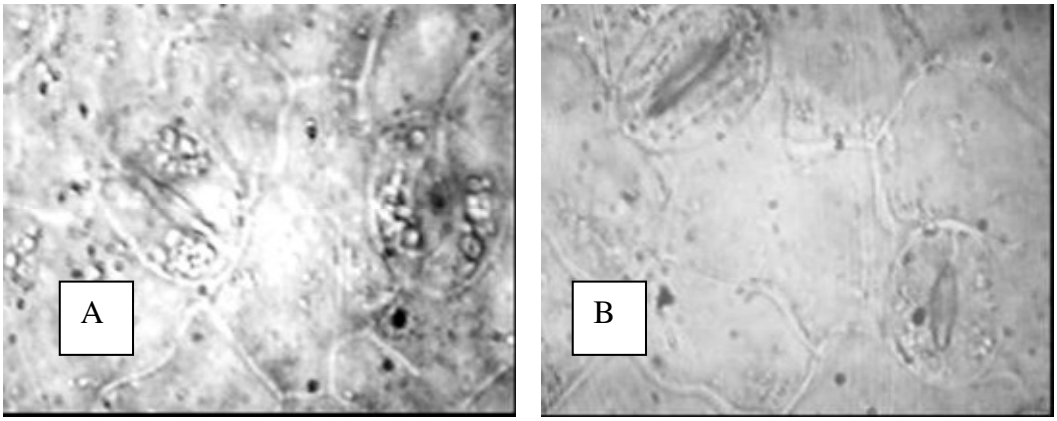

Figure 3. The abaxial leaf epidermes of control and colchicine-treated plants of *Cucurmeropsis mannii*. A. Control. B. Colchicine-treated.

nts were shorter than the controls.

However, the treated abaxial epidermal cells and larger trichomes than the controls. Moreso, the epidermal cells of both the treated and control cultivars had irregular shaped epidermis except the control *V. unguiculata* and *C. mannii* adaxial surfaces that ranged from pentagonal to hexagonal and more regular shaped than the treated, respectively. Furthermore, the only difference observed in the stomata is the shape of the stomata pores of the adaxial surfaces of both treated and control *C. mannii*. While the treated had rounded shaped stomatal pore, the control had cylindrical shaped stomatal pore. Table 3 shows the mean number of stomata and stomatal indices of both treated and control cultivars. Statistical evidence reveals that observed differences of mean between the treated and the control were highly significant ($P \leq 0.05$) for *V.*

Table 3. Mean value of stomata and stomatal indices of treated and control *V. unguiculata* and *C. mannii*.

Lower Epidermis				Upper Epidermis			Stomatal Indices (%)	
Plants	**Min**	**X ± s.d.**	**Max**	**Min**	**X ± s.d.**	**Max**	**Abaxial**	**Adaxial**
V_T	21	27.8 ± 3.91^a	38	18	24.2 ± 3.59^e	30	41.8	40.5
V_C	22	39.1 ± 7.61^b	57	12	15.4 ± 3.27^f	23	42.8	21.7
C_T	28	39.0 ± 5.21^c	49	11	19.8 ± 5.17^g	34	20.4	13.3
C_C	30	28.3 ± 5.06^d	38	19	30.1 ± 8.31^h	56	19.2	18.5

Mean values with similar superscripts are not significantly different. T = treated; C = control.

unguiculata.

DISCUSSION

Doubling of the number of chromosomes in plants does not yield the expected results in all cases as pointed out by Davis and Heywood (1967) and the general target is to yield diverse features. The suppression of growth observed is not strange since it has been reported by other researchers (Blakeslee and Avery, 1937; Ndukwu and Obute, 2006; Hewawasam et al., 2004; Ugborogho and Sodipo, 1985; Ugborogho and Obute, 1994). Moreover, the slow growth may be explained by the logic that the treatment was an assault to the genomic integrity of the plant at the apical region. It would usually lead to the disturbance of the natural rate of cell cycle thereby affecting overall growth (Ndukwu and Obute, 2006) as manifested in curled leaves (Figures 1a and b). Ugborogho and Sodipo (1985) adduced the reason for the death of seedlings of *L. esculentum* as a result of clumping of chromosomes in the meristems. Furthermore, the seedlings of *C. mannii* had very rapid growth; this is in line with the findings of Franzke ands Ross (1952) who attributed this to enhanced assemblage of materials to resume growth leading to an increased rate of cell cycle that subsequently affected the overall plant growth.

The reason for these smooth and curled leaves on the same branch is due probably to the mixture of both diploid and tetraploid tissues. Moreso, the smaller leaf size and abnormalities of the treated *V. unguiculata* is in accordance with previous reports (Blakeslee and Avery, 1937; Hewawasam et al., 2004). However, other reasons adduced by other authors (Franzke and Ross 1952) suggest that chromosome breakage, reduction in the auxin level, change in enzyme activity and variation in ascorbic acid concentration are some factors, which lead to the development of abnormal leaves. Delayed flowering observed in the treated V. *unguiculata* is not novel. Stebbins (1950) has observed this phenomenon in other species. This may be due to the reduction in the rate of various physiological processes. However, early flowering and fruiting observed in the treated *C. mannii* is similar to the report of Franzke and Ross (1952). This may be due differing levels and pathways adapted by plants of different genetic backgrounds in response to assault on their chromosomal integrity. Shorter pod length and fewer seeds per pod observed in the treated *V. unguiculata* is at variance with Ugborogho and Obute (1994). The longer trichomes observed in the abaxial surfaces of both treated *V. unguiculata* and *C. mannii* are supported by Ndukwu and Obute (2006) who adduced that it is reflective of higher chromosome numbers. Some authors have also reached similar conclusions when diploid and polyploids of the same species were compared (Matthew and Matthew, 1980). Fewer and larger stomatal sizes observed in the treated abaxial surface of *V. unguiculata* were evidence of autopolyploid according to Schulz-Schaeffer (1985). In fact, the works of other authors Allard (1960), Stebbins (1950), Davis and Heywood (1967), Ugborogho and Sodipo (1985), Ugborogho and Obute (1994) and Ndukwu and Obute (2006) are in agreement with these results. Ugborogho (1982) noted that micro-morphological features are rarely affected by the environment; thus the changes here are due to alteration of chromosome numbers in the treated plants. The control of *C. mannii* had fewer and larger stomata as well. The argument that these features are determined by the nature of polyploid in question should be strongly considered as an evolutionary phenomenon worth further investigation.

In conclusion, although there were enhanced morphological characters induced by colchicine in both plants, the agronomic properties are the most important targets. Colchicine appeared not have enhanced the fruits of these plants. However, *V. unguiculata* leaves are used as food in some places and this need to be further researched for better utility. Similarly the early flowering and fruiting induced in *C. mannii* need to be further investigated to find its application in the production of this crop.

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