Colchicine-Enabled Genomic Doubling In Oil Palm (*Elaeis Guineensis* Jacq.)

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Abstract

The African oil palm (*Elaeis guineensis.* Jacq.*)* is an oleaginous tree belonging to the family *Arecaceae*. The crop is naturally a diploid species (2n = 2x = 32) and the most important oil bearing perennial tree crop cultivated in tropics. In spite of its huge economic importance, it has a very narrow genetic base making its improvement using conventional techniques very difficult. All commercial planting materials are hybrids derived from heterozygous pisifera and dura parents. The imperativeness of generating adequate novel materials with sufficient genetic diversity in order to increase progress in oil palm improvement is therefore, very essential. Genomic manipulations through chromosome doubling may present opportunities to broaden the genetic base and increase the diversity within the commercial oil palm species. Developing an efficient genome doubling protocols using anti-mitotic agents is very important in this respect. It will also complement haploid/doubled haploid production efforts by enabling successful deployment of doubled haploids in oil palm breeding programmes. The objectives of this study were, therefore, to optimize genomic doubling in oil palm by determining optimum colchicine concentrations and also outline procedures for screening for putative polyploids. Oil palm seedlings were subjected to colchicine treatment at concentrations of 0, 1.26, 3.76, 6.26 and 8.76 mM for 0, 3, 6, 12 or 24 hours. The colchicine treatment produced variable physio-morphologies in the treated seedlings. Principal component analysis and agglomerative hierarchical clustering of genotype dependent treatments were carried out to provide an ordination guide. The response of oil palm seedlings to colchicine treatment showed genotypic, concentration of colchicine applied and treatment duration dependency. Ploidy level analysis of the colchicine treated seedlings revealed that polyploidization in oil palm increased significantly ($p \leq 0.05$) with increasing colchicine concentration. Seedlings of the genotype G-131 responded more favourably to the colchicine treatment than seedlings of G-132. Optimal colchicine concentration for genome doubling in G-131 (28.9 %) and G-132 (17.8 %) was 8.76 mM applied for a period of 24 h. A population of 154 tetraploids out of 1800 seedlings of the two genotypes mutagenized has been identified and being raised for further evaluation and genetic exploitation.

Background

The African oil palm (*Elaeis guineensis.* Jacq.*)* is an oleaginous, erect and single-stemmed monoecious tree belonging to the family *Arecaceae*. The oil palm trunk is cylindrical with dense adventitious roots at the base occurring in the upper 40–60 cm depth of the soil and spread out to a radius of 3–5 m wide. The crop is naturally a diploid species (2n = 2x = 32) with a relatively small genome size of approximately 1.8 Gb (Singh et al, 2013). It is the most important oil bearing perennial tree crop cultivated in most tropical regions worldwide. In spite of its huge economic importance, it has a very narrow genetic base making its improvement using conventional techniques very difficult. All the commercial planting materials are hybrids from predominantly heterozygous pisifera and dura parents selected through reciprocal recurrent and family-individual selection approaches. (Barcelos et al, 2015; Corley and Tinker, 2003). The breeding populations used by breeders in developing these parental lines often referred to as breeding populations of restricted origins (BPROs) could be identified by specific traits traceable to

usually unique and small clusters of wild or unimproved ancestral palms (Barcelos et al., 2015). The genetic implications of this dependence on such breeding materials for oil palm improvement are limited available genetic diversity and a reduced rate of progress in terms of overall genetic improvement of the crop. The imperativeness of generating adequate novel materials with sufficient genetic diversity in order to increase progress in oil palm improvement is therefore, very essential.. Genomic manipulations through chromosome doubling may present opportunities to broaden the genetic base and increase the diversity within the commercial oil palm species.

Though chromosome doubling can occur spontaneously, there are a number of bottle necks to its wide spread application. Firstly, the event is rare in nature and its frequencies are often extremely low. For instance, in citrus, spontaneous autotetraploids occur at a frequency of between 1–7% in seedlings of haploid apomictic seedlings (Saleh, et al., 2008). The process of chromosome doubling can come about as a result of nuclear fusion following cell division and therefore culminating in genome doubling by somatic diploidization and meiotic or gametic un-reduction leading to the formation of 2n gametes and polyploid individuals (Castillo et al., 2009). Secondly, to date in oil palm, the use of naturally occurring polyploids for the breeding of the crop has not been reported. Thus the use of induced genome doubling may be an essential and unavoidable process in the exploitation of haploids, doubled haploids or polyploids with higher ploidy levels for improvement of the crop. Efficient genome doubling protocols using anti-mitotic agents are therefore, pre-requisite to successful deployment of doubled haploids and other genomic variants or polyploids in oil palm breeding programmes. Antimitotic agents are chemicals that inhibit the formation of microtubules and spindle fibres during cell division at early anaphase. By their action, though duplication of chromosomes may occur during mitosis further cell division is inhibited due to the absence of spindle fibres. Thus the chromosomes, after doubling are retained in the original cell resulting in direct numerical increase in the ploidy level of the cell. Individual plants with such variations in cytosolic contents can be exploited in mutation breeding programmes for useful mutations in designing and development of novel cultivars with desirable traits that meet producer and end-user needs. In breeding programmes, polyploids have exhibited special traits such as tolerance to plants stress factors such as drought (Xu *et al*., 2018; Rao *et al*., 2020), salinity (Liu and Sun, 2017) and heat (Zhang *et al*., 2010) stresses. In most cases, polyploids develop large fruit or petal sizes and increased content of secondary plant metabolites than their diploid species. Besides their deployment in mutation breeding, doubled haploids are also very important in genomic studies.

Of the known antimitotic agents (trifluralin, oryzalin and amiprophos-methyl) which are used to terminate further division of a cell containing duplicated chromosomes, colchicine by far remains the most effective and widely applied chemical for doubling of ploidy level in most plant species (Mustafa et al., 2017; Castillo et al., 2009; Stanys et al., 2004). Conventionally, colchicine extracted from the seeds and bulbs of Autumn crocus (*Colchicum autumnale* L*)* has been used in genomic doubling in many crop species including maize (Obert and Barnabas, 2004; Antoine-Michard and Beckert, 1997), wheat Barnabas et al., 1991; Soriano et al., 2007; Zamani et al., 2000), rice (Alemanno and Guiderdoni, 1994) and onion (Grzebelus and Adamus, 2004).

Due to the difficulty in obtaining haploids in such sufficient quantity to enable practical replicated trials genome doubling protocols established using their diploids counterparts may also be useful in doubled haploid development programmes. In this manner valuable haploid materials which are difficult regenerate are not wasted and therefore preserved for subsequent doubling to produce doubled haploids. In chemical mutagenic treatment the concentration, as well as the duration of exposure or treatment are very essential in determining optimum survival and mutation frequency (Jancowicz-Cieslak and Till (2016). Developing an efficient protocol for genome doubling using colchicine is therefore essential and will complement haploid production efforts and help raise large populations of doubled haploids and also allow for development, manipulation and exploitation of polyploid genotypes for genetic improvement of oil palm. The objectives of this study were, therefore, to optimize genomic doubling in oil palm by determining optimum colchicine concentration for chromosome doubling and also outline procedures for screening for putative polyploids developed.

Results

Variables distribution and clustering of the genotype-colchicine treatment associations

Normal probability plot correlation coefficients of the variables used to carry out ordination and cluster analysis indicates that the data showed normal distribution (Fig. 2a) with very high correlation coefficients ranging from 0.863 (Surv-16) to 0.9963 (CC) (Fig. 2b). Cluster analysis performed on the genotype-colchicine treatment associations also revealed five distinct clusters with a cophenetic correlation coefficient of 0.7341 (Fig. 3.) based on the physio-morphological traits in response to the mutagenic treatment. The first cluster (cluster I) consisted of 3 colchicine-induced morphotypes (CIMs) which are morpho-variants of G-132 resulting from colchicine treatment of G-132 at low concentrations of 1.26 and 3.76 mM and for long and short durations of 12, 24 and 3 hours respectively. Individuals in this cluster included V2C1-12, V2C1-24 and V2C2-3.

The second cluster (cluster II), however, was made up of 9 CIMs which included mixed genotypes of G-131 and G-132. CIMs of G-131 were seedlings treated with colchicine concentrations of between 3.76–8.76 mM at variable treatment durations ranging from 6–24 hours. CIMs of G-132 included seedlings treated with 3.6 and 8.76 mM colchicine for 24 hours. Individuals in this cluster included V1C2-6, V1C2-12, V1C2-24 V1C3-6, V1C3-12, V1C3-24, V1C4-24, and V2C2-24, V2C4-12. This second cluster was further sub-clustered into 2 groups. The first sub-cluster consisted individual morphotypes derived solely from oil palm genotype G-131 treated with 3.76 mM colchicine for 6 and 12 hours and 6.26 mM colchicine for 6 hours (V1C2-6, V1C2-12 and V1C3-6). The second sub-cluster was made up of G-131 and G-132 treated with between 3.76–8.76 mM colchicine for variable durations ranging from 12–24 hours (V1C2-24, V1C3-12, V1C3-24, V1C4-24, V2C2-24 and V2C4-12).

The third cluster (cluster III) consisted of 12 CIMs derived from the two genotypes G-131 and G-132 as a result of exhibition of trans-morphologies of G-131 and G-132. Individuals in this group were made up

predominantly of G-132 genotype treated with between 3.76–8.76 mM colchicine at variable durations 3–24 hours. CIMs of G-131 were seedlings treated with 8.76 mM colchicine for 3, 6 and 12 hours. Members in this third cluster were thus, V1C4-3, V1C4-6, V1C4-12, V2C2-6, V2C2-12, V2C3-3, V2C3-6, V2C3-12, V2C3-24, V2C4-3, V2C4-6 and V2C4-24. Furthermore, 2 sub-clusters were identified within the third cluster and this included V1C4- 3 and V1C4-12 in one sub-group and V1C4-6, V2C2-6, V2C2-12, V2C3-3, V2C3-6, V2C3-12, V2C3-24, V2C4-3, V2C4-6 and V2C4-24 in the other sub-cluster.

The fourth cluster (cluster IV) comprised solely of individuals derived from the genotype G-132 treated with only distilled water as controls for durations ranging from 3–24 hours. Individuals in the fourth cluster were V2C0-3, V2C0-6, V2C0-12 and V2C0-24.

In the fifth cluster (cluster V), 12 CIMs predominantly derived from G-131 were observed. It also included some morphotypes derived from the genotype G-132 treated at very low colchicine concentrations for shorter durations of 3 and 6 hours. Individuals derived from G-131 were mainly seedlings treated with only distilled water for variable durations of 3–24 hours and also seedlings treated with low to relatively high colchicine concentrations (1.26–6.26 mM) for variable treatment durations (3, 6, 12 and 24 hours). Individuals in this cluster were, V1C0-3, V1C0-6, V1C0-12, V1C0-24, V1C1-3, VICI-6, V1C1-12, V1C1-24, V1C2-3, V1C3-3, V2C1-3 and V2C1-6.

Two sub-clusters were further identified under cluster V which included individuals of G-132 treated at very low colchicine concentrations (1.26 mM) for 3 and 6 hours in one sub-group (V2C1-3 and V2C1-6) and those of G-131 treated with only distilled water for between 3–24 hours and seedling treated at between 1.26–6.26 mM for variable durations of between 3–24 hours in the other sub-group (V1C0-3, V1C0-6, V1C0-12, V1C0-24, V1C1-3, VICI-6, V1C1-12, V1C1-24, V1C2-3, V1C3-3).

Variables with the widest range and variability and therefore contributed significantly to variations observed among the colchicine-mutagenized oil palm populations in order of decreasing strength include stomatal conductance (SC), chlorophyll content (CC), leaf area (LA) and plant height (PH) (Fig. 4).

Frequency of aberrancy in the colchicine-mutagenized oil palm population.

Seedlings with aberrant (off-type) phenotypes or morphologies such as thick and reduced leaf sizes and plant height of approximately 50% of the control were also studied and statistically significant, ($p \leq 0.05$) variation was found between the oil palm genotypes (Fig. 5). Genotype x concentration, concentration x treatment duration and genotype x treatment duration interactions were also statistically significant ($p \leq 0.05$). At higher colchicine concentrations (6.26 and 8.76 mM) the genotype G-131 produced significantly higher proportions of aberrant seedling types than G-132. No aberrant seedlings were recorded among the control seedlings and seedlings treated with 1.26 mM colchicine for 3, 6, 12 and 24 hours respectively in both genotypes. Furthermore, G-132 did not record any aberrant seedling at a lower concentration of 3.76 mM applied to the seedlings for 3 hours.

Seedlings with aberrant morphologies increased significantly ($p \leq 0.05$) with increasing level of colchicine and treatment durations. In G-131, seedlings with aberrant phenotypes ranged from 0–22.23, 0–40.0, 0 -57.80 and 0–66.67% for treatment durations of 3, 6, 12 and 24 hours, respectively. G-132, however, recorded between 0–20.0, 0–28.90, 0–37.80 and 0–35.57% for colchicine treatment durations of 3, 6, 12 and 24 hours, respectively. In G-131, the highest proportion of seedlings with aberrant phenotypes (66.7%) was recorded among seedlings treated with 6.26 mM for 24 hours. In G-132, nevertheless, the highest proportion of seedlings with aberrant phenotypes was observed among seedlings treated with 8.76 mM for 12 hours. The off-type seedlings in G-132 ranged from 0–20.0, 0–28.90, 0–37.80, 0–37.80 and 0–35.60% in seedlings treated with colchicine for 3, 6, 12 and 24 hours respectively.

Principal component analysis

The principal components determined on the 12 physio-morphological traits in the oil palm genotypes subjected to the colchicine mutagenic treatment together with the eigen values and the proportion of the variance explained by each of the principal components are presented in Table 1. The analysis revealed 12 principal axes but only 4 which had proportion of the eigen values greater than 1% were retained. The scree plot (Fig. 6a) also showed that after the first 4 principal components the percentage contribution of the remaining axes were negligible. Together the 4 principal components explained 99.95% of the total variation observed in the colchicine-induced response variables. The principal component 1 (PC1) explained 65.89% of the total variation while the second, third and fourth principal components, PC2, PC3 and PC4 accounted for 27.40% 5.59% and 1.09% of the total variation observed, respectively. The most variable parameters on the first two principal axes PC1 and PC2 were SC, LA, CC and PH (Fig. 6b).

The loadings derived to determine the most relevant traits, which influenced the 4 principal component axes are shown in Table 2. Principal axis 1 (PC1) correlated positively with stomatal conductance and chlorophyll content and negatively with leaf area. On PC2, the most relevant traits were, number of leaves per plant, leaf emission rate, plant height, proportion of aberrant seedlings, leaf area ratio, and plant height ratio. PC3 was defined mainly by chlorophyll content, leaf area, stem girth, plant height and leaf area ratio. PC4 correlated with leaf thickness, survival of seedlings/ and frequency of seedlings with aberrant morphologies

Table 1
Vector loadings of the principal components of the colchicine-induced response variables determined on the colchicine treated oil palm genotypes

Trait	PC1	PC2	PC3	PC4
SUV	-0.010	0.0057	0.0114	**0.0233**
SC	**0.7123**	0.6806	-0.1087	0.1325
LT	0.0001	-0.0002	0.0003	**0.0004**
CC	**0.4531**	-0.2768	**0.7438**	-0.4036
LA	**-0.4666**	0.5430	**0.6216**	0.2255
SG	-0.0022	**0.0030**	**0.0035**	0.0007
NL	-0.0148	**0.0184**	0.0172	-0.0064
LER	-0.0037	**0.0046**	0.0041	-0.0016
PH	-0.1118	**0**.1166	**0.1181**	0.1166
LAR	-0.0041	0.0045	**0.0047**	0.0014
PHR	-0.0028	**0.0028**	0.0027	0.0012
PAB	0.2381	**-0.3890**	0.1848	**0.8687**
Eigen value	**3596.88**	**1495.64**	**304.918**	**59.3642**
% Variation	**65.885**	**27.396**	**5.5853**	**1.0874**
Cumulative %	**65.885**	**93.281**	**98.8663**	**99.9537**

SUV = seedling survival rate; SC = stomatal conductance; LT = leaf thickness; CC = chlorophyll content; LA = leaf area; SG = seedling stem girth; LER = leaf emission rate; NL = number of leaves; LAR = Leaf area ratio; PH = plant height; PHR = plant height ratio; PAB = proportion (frequency) of aberrant individuals. Most relevant traits that contributed to most of the variations on each principal component axis are printed in bold.

Ploidy level determination by flow cytometry in the colchicine-mutagenized population.

Variations in chromosome doubling frequency in the oil palm seedlings treated with colchicine are presented in Fig. 7. In determining the ploidy level of the genotypes, the commercial tenera (control, un-treated) with a diploid ploidy status (Figs. 8 and 9) was used as an internal reference standard. Variation observed in the chromosome doubling frequency between the genotypes was found to be statistically significant ($p \leq 0.05$). Concentration, treatment duration as well as genotype x concentration, genotype x duration and concentration x duration interactions were also statistically significant ($p \leq 0.05$). Genotype x concentration x duration interaction was, however, not statistically significant. The untreated seedlings

of both genotypes, G-131 and G-132 did not record any doubling of chromosome at all durations of soaking in sterile distilled water. Nevertheless, in both genotypes, the colchicine treatment induced several morphological variations (Fig. 10) and genome doubling frequency generally increased significantly ($p \leq 0.05$) with increasing colchicine concentration and duration of treatment. In G-131, doubling frequency for treated seedlings ranged from 0–6.7, 6.7–22.2, 8.9–28.3 and 13.6–28.9% for treatment durations of 3, 6, 12 and 24 hours respectively. In G-132 however, between 0–6.7, 0–8.9, 0–13.3 and 6.7–17.8% chromosome doubling frequency were observed for treatment durations of 3, 6, 12 and 24 hours respectively. At a relatively mild colchicine concentration of 1.26 mM, G-131 and G-132 began to experience doubling in ploidy level but at a lower frequency of 6.7% at treatment durations of 6 and 24 hours respectively. Seedlings treated with 8.76 m M colchicine for 24 hours recorded the highest doubling frequency in ploidy level in G-131 (28.9%) and G-132 (17.8%).

Discussion

The oil palm genotypes exhibited differential responses to the colchicine treatment. In both genotypes seedling survival decreased significantly ($p \leq 0.05$) with increasing colchicine concentration. However, higher colchicine concentrations applied for longer durations exceeding 12 hours had favourable effects on chromosome doubling in the oil palm genotypes. Genome doubling is very critical for deployment of haploids in breeding programmes. Though chromosome doubling can occur spontaneously, its occurrence is rare. This makes induced genome doubling very essential in doubled haploid breeding programmes (Nelson et al., 2010). Haploids in most regeneration systems are obtained in scanty numbers and this precludes their practical use in replicated trials for establishment of optimal genome doubling rates. However, protocols developed using their diploid counter parts may have relevance in application to doubled haploid production.

The significant ($p \leq 0.05$) variation observed in the morphology of colchicine-treated oil palm seedlings provides an indication of the effectiveness of the colchicine treatment in inducing genetic variations in oil palm. Colchicine is an anti-mitotic agent used in the induction of polyploidy in plants (Stanys et al., 2004). It acts by preventing formation of microtubules and spindle fibres during cell division at anaphase (Petersen et al., 2003). Chromosomes at this stage of cell division get duplicated but because further cell division is inhibited the cell content becomes doubled resulting in polyploidy. These polyploids generally have larger cells, tissues and organs than their diploid relatives (Vainola, 2000). Contrarily, in the present study, the oil palm polyploids identified among the aberrant seedling types had smaller tissues and organs than their diploid counterparts (Fig. 3). Though preliminary visual selection of aberrant types based on previous description of oil palm polyploid morphology, (Madon et al., 2005) resulted in large number of off-type individuals actual polyploid frequencies determined by flow cytometry were lower. Ploidy level and genome size determinations using flow cytometry provides a more reliable and accurate estimates than any other conventional technique. This suggests that the use of morphological attributes is only useful in pre-screening and in assigning tentative polyploid status in oil palm. In a similar investigation, Nelson et al. (2010) suggested use of ordination approaches to improve the efficiency of using morphological characteristics in classifying off-types and normal genotypes of oil palm. Cluster analysis performed on the colchicine treatments-, durations- and genotype-associations revealed 5

classes with a cophenetic coefficient 0.7341 indicating high quality and reliability of using colchicine-induced morpo-variables in pre-screening.

In the present study, not only did the colchicine treatment of the oil palm genotypes provide a guide to ploidy level and genome doubling in oil palm but also produced an array of tenera tetraploids (104 from G-131 and 50 from G-132) which in themselves can be evaluated for genetic variations in important agronomic characteristics or represent a valuable genetic stock for oil palm improvement. For instance, selfing of the tenera tetraploids will lead to generation of homozygous duras (ShShShSh) and pisiferas (shshshsh) and wide range of teneras (ShShShsh, ShShshsh and Shshshsh) with variable shell-thickness characteristics which can be exploited in breeding programmes for oil palm genetic improvement.

Conclusions

The colchicine treatment produced variable physio-morphologies in the treated seedlings. Polyploidization was dependent upon genotype, concentration of colchicine applied and treatment duration. Consequently, 28.9 5% polyploidy was observed in G-131 which was higher than 17.8% recorded for G-132. Polyploidization in oil palm increased with increasing colchicine concentration. Autopolyploidy in oil palm can therefore, be induced with colchicine treatment at optimum concentration (8.76 µM) for longer durations (≥ 24 hrs).

Methods

At the end of the treatment period, the mini sacks containing the seedlings were carefully removed from the colchicine solution using an attached metal rod into a plastic basket placed in a plastic basin. The basket and basin were immediately placed under running tap for 30 min to wash off excess colchicine from the seedlings. After washing, the mini sacks were removed, air-dried on a concrete platform at 27–30 ^{o}C for 15 min and were nursed in polyethylene nursery bags (17.8 cm x 12.7 cm) containing 1:3 top soil: saw dust-market waste compost. The top soil consisted of clayey loam obtained from the Nuclear Agriculture Research Center, Ghana Atomic Energy Commission. The nursery bags were arranged in a split-split plot design with 3 replications. Controlled hand weeding and watering were done as and when required. Insects were controlled by regular spraying the seedlings with the Hercule® insecticide (IPROCHEM, China).

Data collection and statistical analysis

All morphometric data were taken on seedlings surviving at the nursery up to or at sixteen (16) weeks after treatment (Surv-16) with the colchicine mutagen. Ploidy level analysis was carried out at fifty-four (54) weeks after treatment. Chlorophyll content (CC) was measured using the chlorophyll content meter, CCM 300 plus (Opti-Sciences, USA) and expressed in mg/g. Stomatal conductance (SC) was was determined using the SC-1 leaf porometer (Decagon Devices, Inc. USA). Leaf thickness (LT) was determined on first fully expanded leaves using Vernier calipers. Stem girth (SG) was measured at the

widest portion close to the base of seedlings using a pair of Vernier callipers and expressed in centimetres. Plant height (PH) was determined as the total length of seedling as measured from the base of the seedling to the longest leaf and expressed in centimetres. Plant height ratio was computed as ratio of seedling height of the colchicine treated seedlings to that of the control seedlings. Number of leaves (NL) referred to the total number of leaves developed by the seedling over the nursery period. Leaf emission rate (LER) referred to the number of leaves produce per unit time interval expressed in months. For each leaf, the length and the broadest width were measured. Leaf area (LA) was calculated as;

$$A = (L \times B) \times Cf$$

where; A = leaf area (cm^2); L = length of leaf (cm); B = broadest width of leaf (cm); Cf = leaf area correction factor of 0.57 for single undivided leaves and 0.5 for divided leaves .

Leaf area ratio was calculated as the ratio of leave area of colchicine treated seedlings to that of the control seedlings. Seedlings with aberrant morphology were determined by visual observation of the colchicine treated seedlings and comparing them to the control seedlings. Seedling survival rate referred to the total number of surviving seedlings at 16 weeks expressed as percentage of the control. The Paleontological Statistics (PAST3) software (version 3.21) was used to carry out principal component analysis and also perform agglomerative hierarchical clustering of genotype dependent treatment units by Unweighted Paired Group Method with Arithmetic mean (UPGMA). Euclidean distance was used to estimate the similarity index.

Ploidy Level Analysis

Ploidy level of colchicine treated seedlings were analysed using the CyStain UV Precise P DNA test kit, 05-5002 (Sysmex-Partec, Germany) containing 4',6-diamidino-2-phenylindole (DAPI) fluorescent stain. Approximately 5 mg fresh leaf samples were taken from the first fully expanded leaves and placed in a plastic petri dish (15 mm x 60 mm) containing 0.5 ml of nuclei extraction buffer and chopped with a sharp razer blade to release the DNA of samples into the solution. A 1.5 ml of staining buffer was added and mixed thoroughly with a 3.5 ml plastic dropper. The mixture was sieved through a 30 µm sieve into a 3.5 ml sample tube and incubated for 30 min in the dark. At the end of the incubation period, the C-DNA contents of the samples were analysed on a CyflowSpace flow cytometer (Partec, Germany) operating with the FloMax Software (version 2.4) and fitted with UV light source with an internal band pass (IBP) of 455 nm. The gain value of the instrument was set at 505. Lower and upper limits were set at 80 and 999.9 respectively. The samples were run on channel FL3 UV at a speed of 0.5 μls^{-1} and an acquisition time of 60 seconds (s). The commercial oil palm variety, tenera was used as an internal reference standard.

Abbreviations

CC chlorophyll content

Cf leaf area correction factor

DAPI 4',6-diamidino-2-phenylindole fluorescent stain

FL3 Fluorescence channel 3

IBP Internal band pass

LA leaf area

LAR Leaf area ratio

LER leaf emission rate

LT leaf thickness

NL number of leaves

PC principal component

PH plant height

SC stomatal conductance

SUV seedling survival rate

SG seedling stem girth

PHR plant height ratio

PAB proportion (frequency) of aberrant individuals.

Declarations

Ethics approval and consent to participate

Not Applicable

Consent for publication

Not applicable

Availability of data and materials

Datasets during and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interest.

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Authors' contributions

Authors WN, HMA, DA-D, and KED were involved in the conception and design of the study.

Authors WN, DA-D and KMO were involved in data acquisition.

Authors WN and DN analysed and interpreted the data.

Authors WN, RA, HMA, AWK and AMA-G were involved in drafting and revision of the work.

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Figures

Figure 1

Colchicine treatment of oil palm seedlings: Seedlings ready to be bagged (A); Bagged seedlings in plastic bowls containing colchicine solution (B, C); Plastic bowls containing seedlings placed on rotary shaker (D).

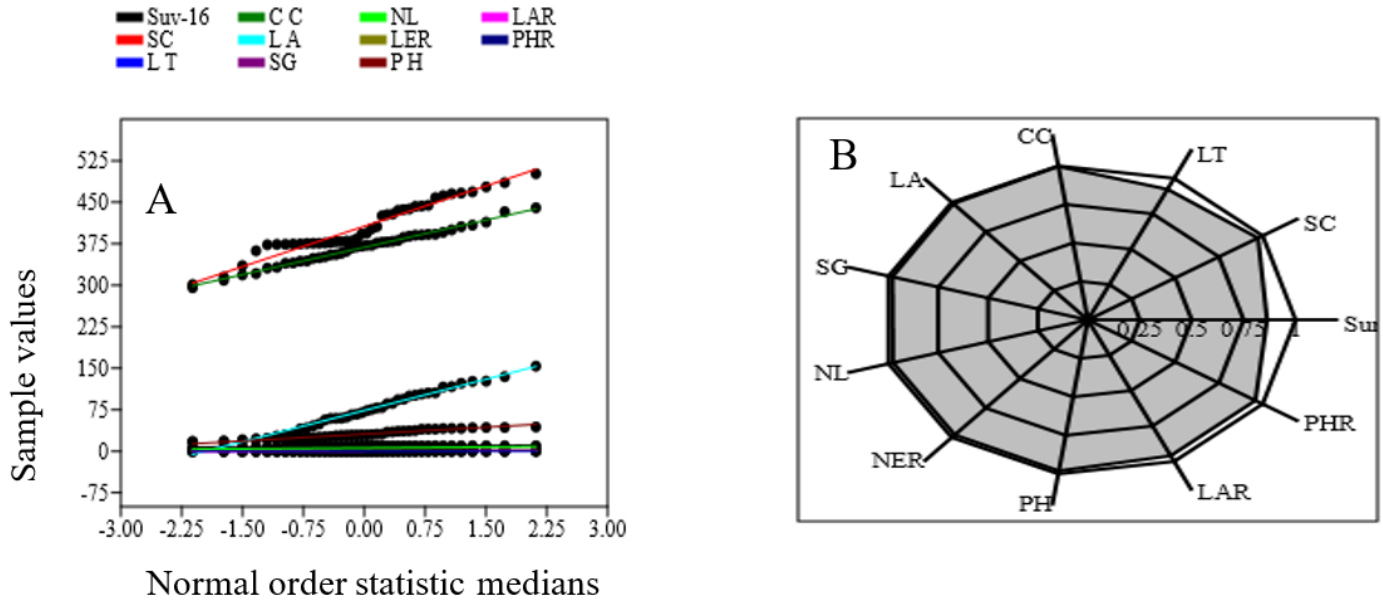

Figure 2

Normal probability plot (A) and related correlation coefficients (B) of variables determined in the colchicine-mutagenized population of two oil palm genotypes.

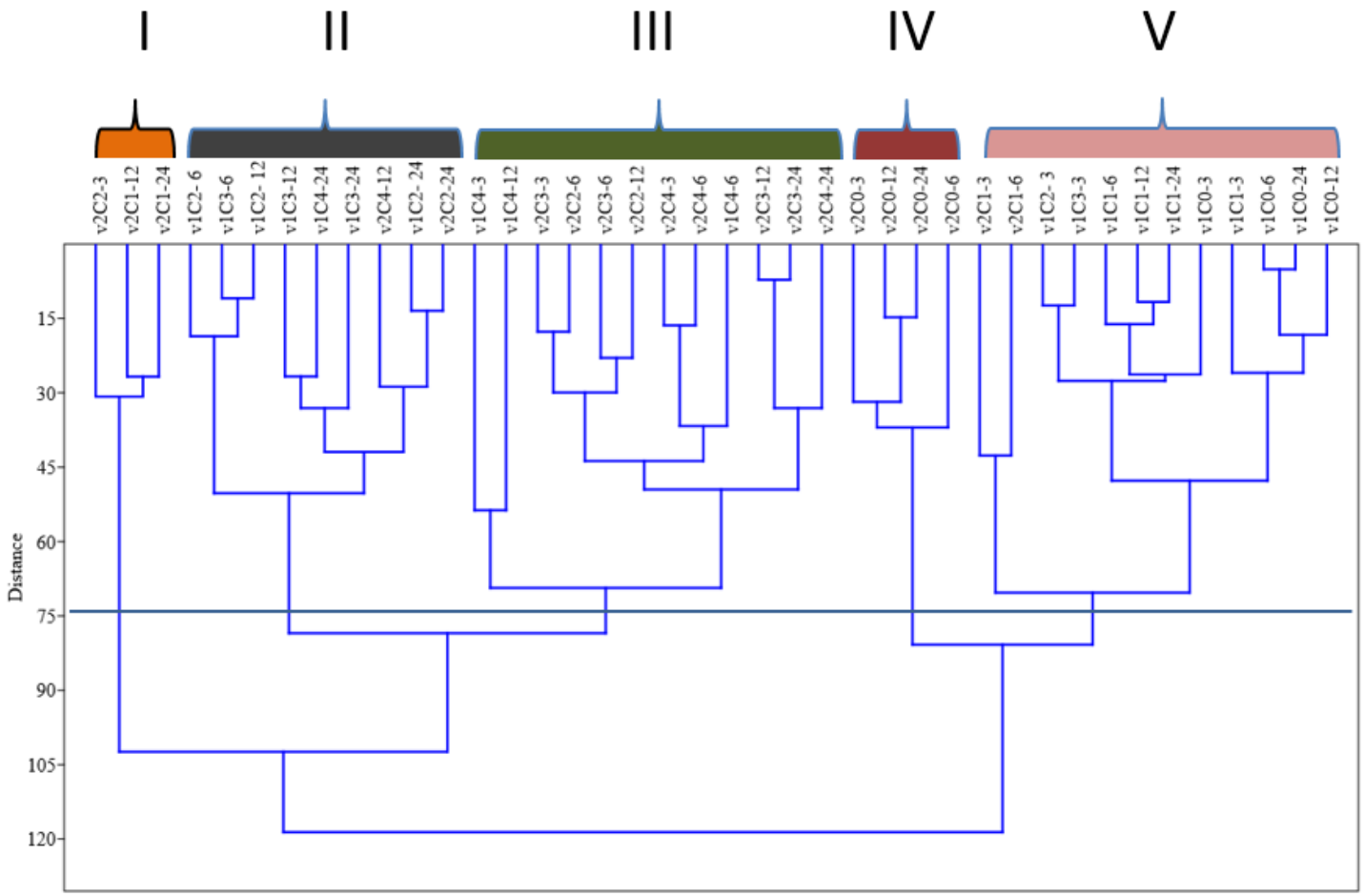

Figure 3

Dendrogram showing cluster analysis of colchicine-genotype treatment associations of the colchicine treated oil palm genotypes. V1= genotype G-131; V2 = genotype G-132; Co, C1, C2, C3 and C4 are concentrations of colchicine at levels of 0, 1.25, 3.76, 6.26 and 8.76 mM. -3, -6, -12 and -24 are duration of colchicine treatment application expressed in hours.

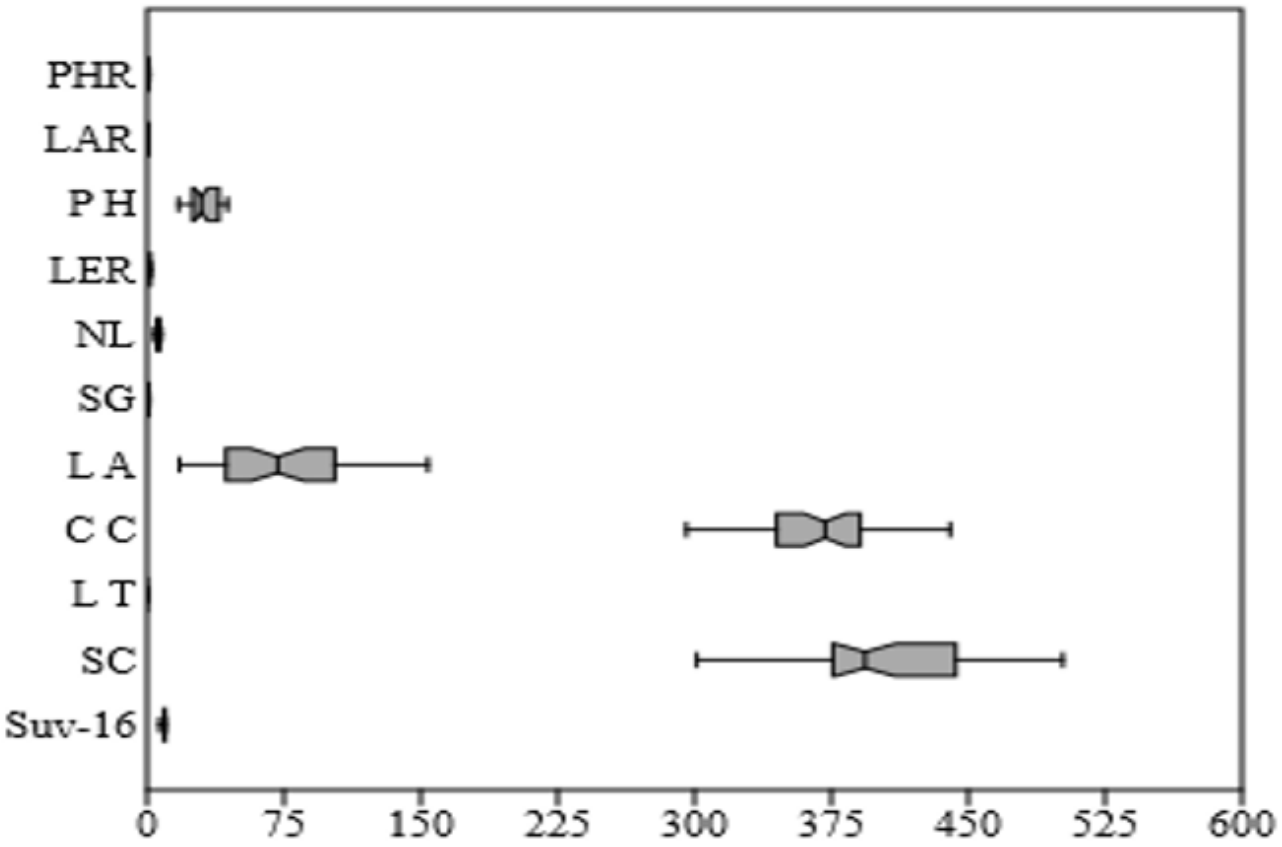

Figure 4

Box plot of variables used in phenotyping the colchicine-induced oil palm population of G-131 and G-132.

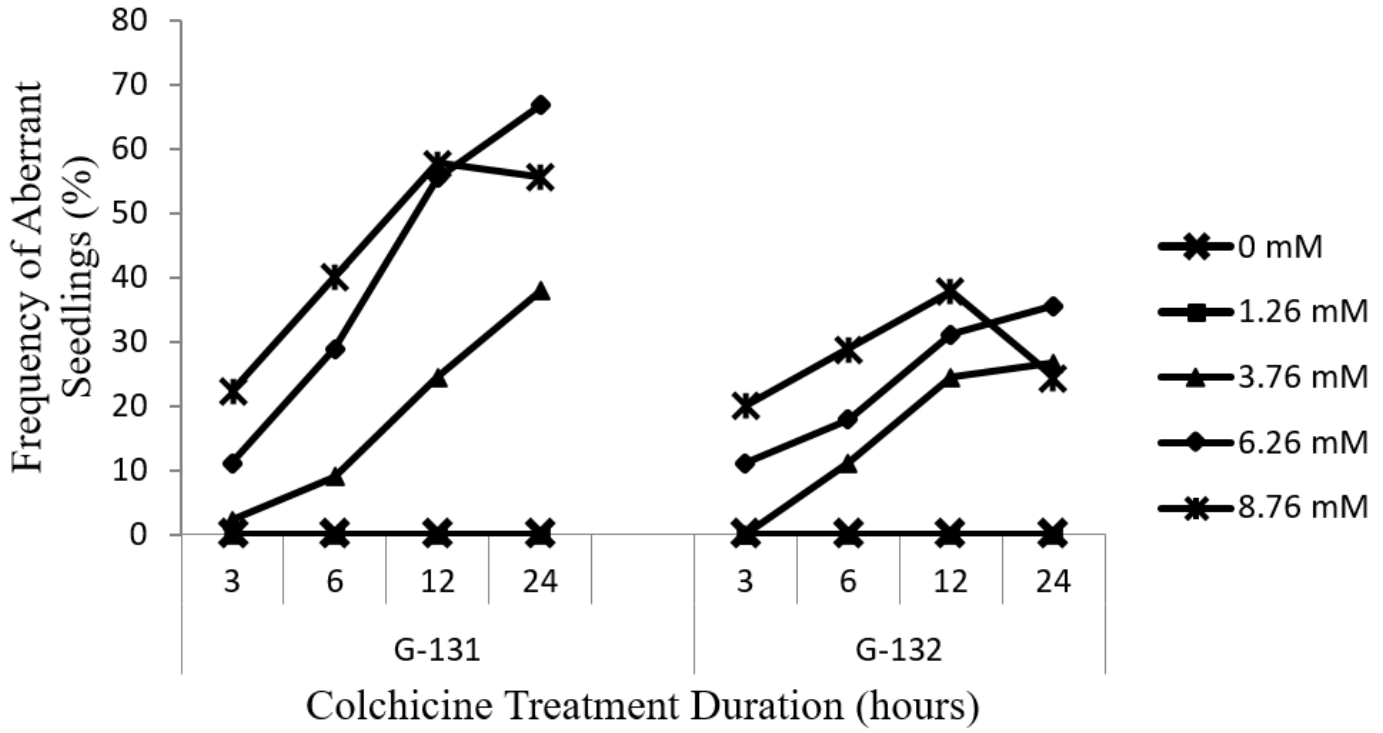

Figure 5

Effect of colchicine on induction of aberrant (off-type) seedlings in two oil palm genotypes G-131 and G-132.

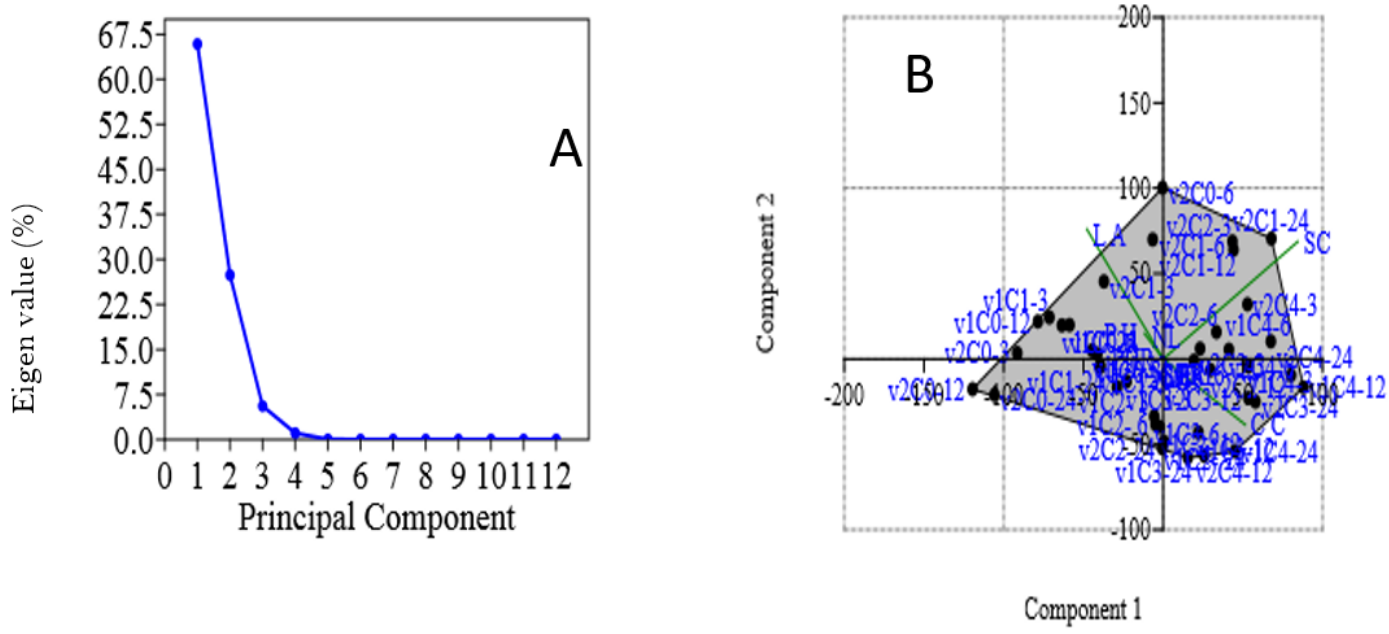

Figure 6

Eigen values of variables used to determine the oil palm traits that influenced the major principal components (A), and biplot of the variables the determined the first two principal components (B).

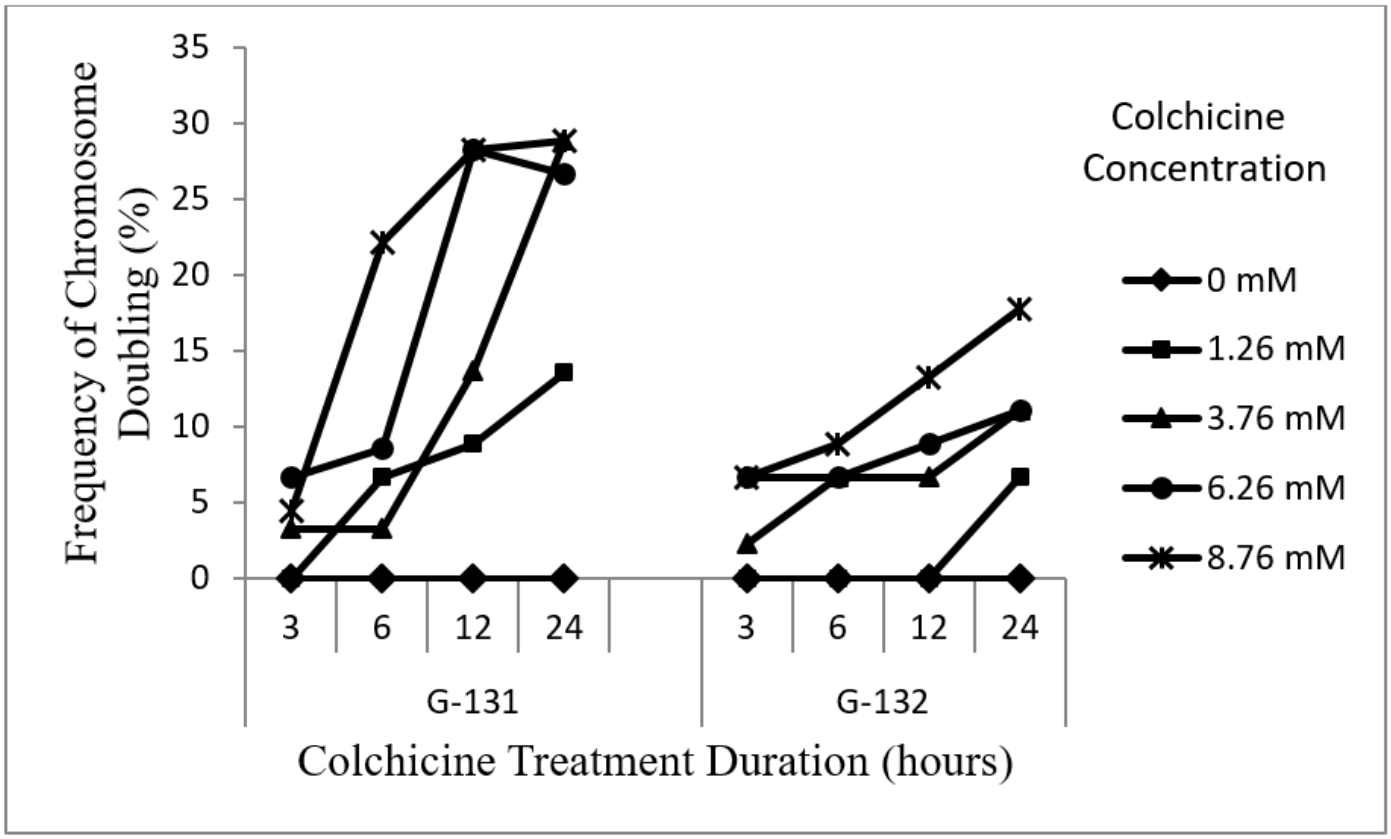

Figure 7

Variation in frequency of chromosome doubling in two colchicine-treated oil palm genotypes.

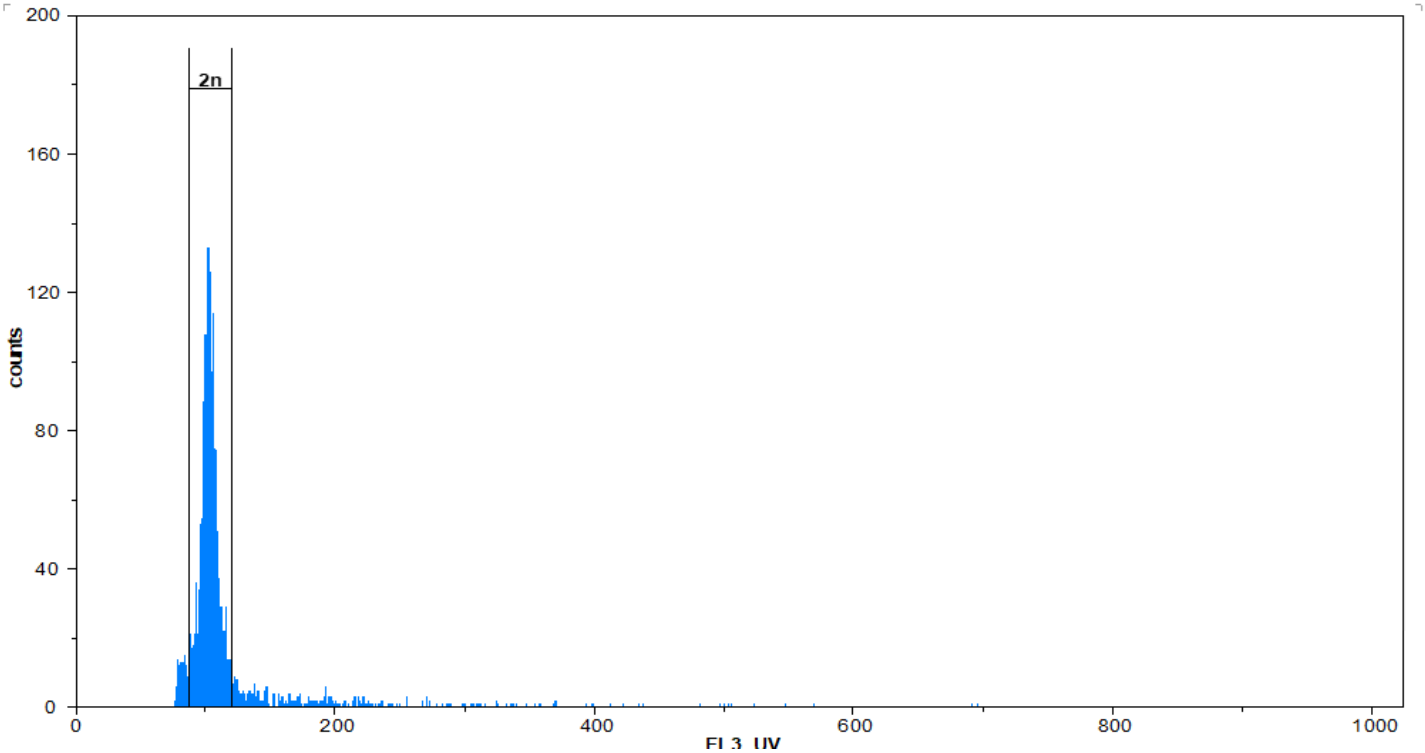

Figure 8

Commercial oil palm variety ‘tenera’ (diploid internal reference) showing 2C- DNA content at channel 100

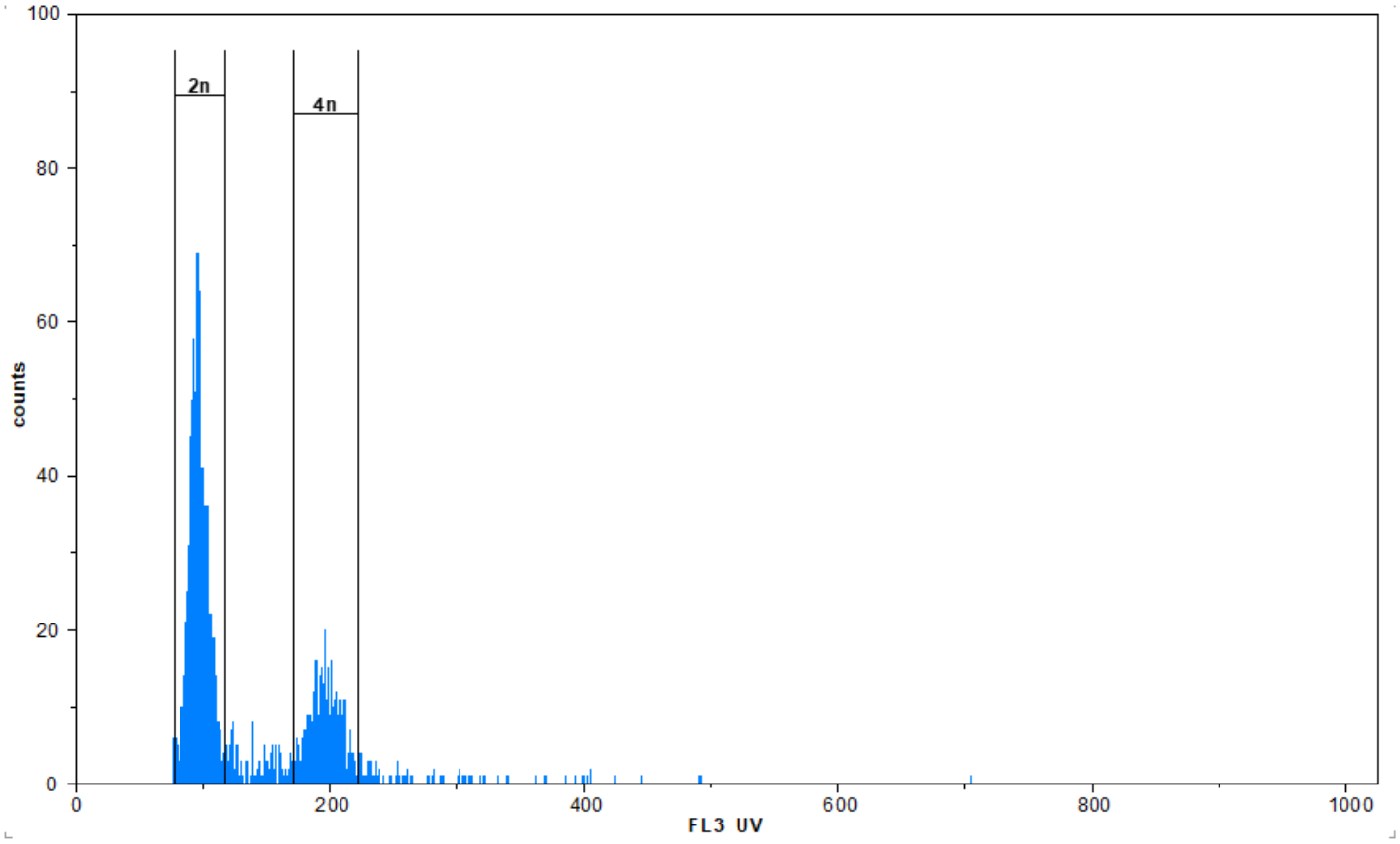

Figure 9

Variations in 2C –DNA contents of diploid internal reference (2n) and colchicine-induced tetraploid (4n).

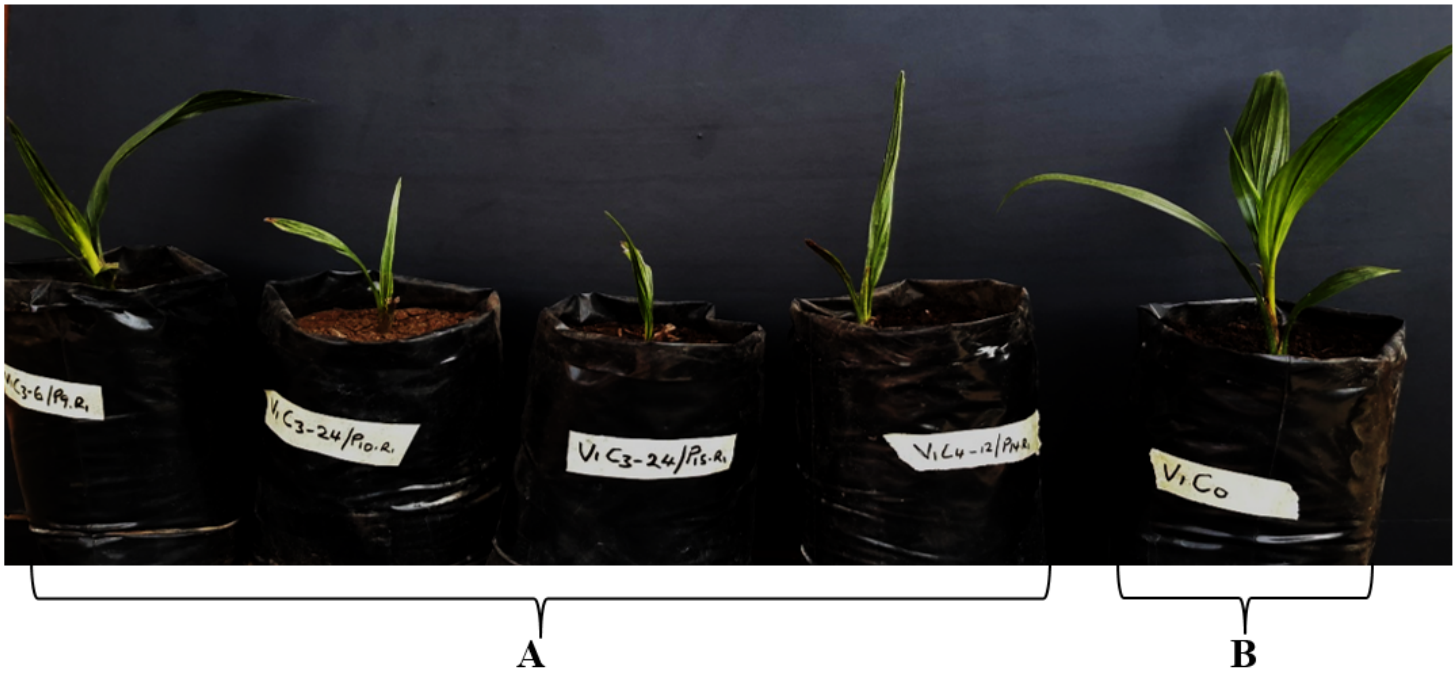

Figure 10

Variation in oil palm seedling morphology induced by colchicine treatment: (A)- Seedlings with doubled genome after colchicine treatments; (B)- Control, un-treated seedling.