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Mitotic chromosomal variation in *Dioscorea* spp. from Parents to F₂ progenies



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ABSTRACT

Yam (*Dioscorea* spp.) is an important staple crop in West Africa, and genetic diversity plays a crucial role in its improvement. The complexity of ploidy levels and chromosomal instability in yam species presents a significant challenge to breeding efforts. Therefore, this study aimed to investigate chromosomal variation in *Dioscorea* spp. from parental lines to F₂ progenies to provide crucial information for optimising yam breeding programs and improving hybrid selection processes. A total of 67 accessions, including parental lines (*Dioscorea praehensilis*, *D. abyssinica*, and *D. rotundata*), their F₁ and F₂ progenies, were assessed. Chromosome counts were conducted on mitotic cells using the squash technique and FLP orcein staining. The number of chromosomes and abnormalities were recorded, and data were analysed using descriptive statistics. Among the 15 parental lines (*D. praehensilis*, *D. abyssinica*, and *D. rotundata*) assessed, 12 had diploid chromosome counts of $2n = 2x = 40$, indicating chromosomal stability. Chromosomal abnormalities, including aneuploidy ($2n = 34$, $2n = 37$, and $2n = 35$), were observed in three parental accessions, accounting for a 19.05% abnormality. Interestingly, of the 30 F₁ hybrids, 10 exhibited $2n = 20$ (a putative haploid chromosome number), while the others showed high chromosomal variation, with chromosome counts ranging from $2n = 18$ to $2n = 33$, resulting in a 66.67% abnormality rate. In contrast, 14 of 22 F₂ progenies exhibited increased chromosomal stability, with a higher proportion of diploid counts ($2n = 2x = 40$) aligning with those of the parental lines. The abnormality rate dropped to 33.52% in F₂ progenies, indicating that chromosomal balance was restored through selfing and backcrossing. This study demonstrates that while interspecific hybridisation induces chromosomal instability in F₁ yam hybrids, subsequent selfing and backcrossing with *D. rotundata* can restore diploid stability in F₂ progenies. The findings offer cytogenetic insights vital for optimising hybrid selection and developing genetically stable yam cultivars for breeding programmes.

Keywords: Hybridisation; Ploidy diversity; Yam breeding; Cytogenetics; Chromosomal stability

INTRODUCTION

Yams (*Dioscorea* spp.) are essential food crops in tropical and subtropical regions, playing a vital role in food security and cultural traditions (Padhan & Panda, 2020; Morse, 2022).

Among the cultivated species, *Dioscorea rotundata* (Guinea white yam) is widely grown in West Africa due to its high nutritional value (Matsumoto *et al.*, 2021). Wild yam species possess significant genetic diversity, offering valuable

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resources for crop improvement in the face of climate change and food security challenges (Zhang *et al.*, 2017; Li, 2024; Oyedoyin *et al.*, 2025c). Studies have revealed substantial variations in morphological and molecular traits among wild yam species, with some showing high genetic similarity to cultivated varieties (Padhan *et al.*, 2019; Adewunmi *et al.*, 2023; Oyedoyin *et al.*, 2025a). While earlier research suggested that cultivated guinea yam (*D. rotundata*) originated from *D. praehensilis* (Magwé-Tindo *et al.*, 2018), more recent analyses have indicated a hybrid origin involving both *D. praehensilis* and *D. abyssinica* (Sugihara *et al.*, 2020; Oyedoyin *et al.*, 2025a). These studies found a greater genomic contribution from *D. abyssinica* to the sex chromosomes of *D. rotundata*. The evolutionary history of *Dioscorea* is complex, characterised by hybridisation and polyploidisation, which complicates the understanding of their domestication (Sugihara *et al.*, 2021). Yams exhibit diverse ploidy levels across species, with chromosome numbers ranging from $2n = 20$ to $2n = 120$ across species (Arnau *et al.*, 2009; Nemorin *et al.*, 2013; Lebot *et al.*, 2017; Darkwa *et al.*, 2020). Chromosome counting by the squash technique, flow cytometry, and genotyping-based methods has revealed that most cultivated yams are diploid, with some accessions being triploid and tetraploid (Siadjeu *et al.*, 2018; Gatarira *et al.*, 2021). For instance, the cultivated water yam *D. alata* is known to exist in diploid ($2n = 2x = 40$), triploid ($2n = 3x = 60$), and tetraploid ($2n = 4x = 80$) forms (Arnau *et al.*, 2009; Nemorin *et al.*, 2013), and the Guinea yam *D. rotundata* varies from $2n = 2x = 40$ to $2n = 3x = 60$. Furthermore, wild yam species also display these chromosomal variations. Reports have also shown that some wild species, such as *D. abyssinica*, *D. praehensilis*, *D. hamiltonii*, *D. glabra*, and *D. esculenta*, display chromosomal variations between $2n = 30$ and $2n = 80$ (Lauzer *et al.*, 1992; Paul & Debnath, 2019; Gatarira *et al.*, 2021). These findings suggest that wild yams possess diverse cytogenetic profiles, which may contribute to their adaptability and resilience in various ecological niches. Yam breeding efforts face challenges due to the crop's complex genetic makeup, which includes both cultivated and wild species exhibiting varying ploidy levels (Darkwa *et al.*, 2020). While previous studies have confirmed the diploid nature of this important crop, little information is available on its chromosome variations in hybrids, particularly from the parental generation to their interspecific progenies. Without a clear understanding of cytogenetic variation and chromosomal stability, breeding programs struggle to develop improved yam varieties with predictable and desirable traits. This study aimed to investigate mitotic chromosomal variation in *Dioscorea* spp. from parental lines to F₂ progenies to provide crucial information for optimising yam breeding programs, improving hybrid selection processes, and enhancing the genetic resilience of cultivated yam species.

Materials and Methods

Sample Source, Collection, and Experimental Location

Fifteen accessions comprising *D. praehensilis*, *D. abyssinica*, and *D. rotundata* were selected from the parental crosslines.

After the production of F₁ progenies, 30 were selected from the total hybrids produced from *D. abyssinica* × *D. praehensilis* based on their phenotypic profiles and agro-morphological traits (Oyedoyin *et al.*, 2025b). Twenty-two accessions of F₂ progenies from the self-cross of F₁ and crossing of F₁ progenies and *D. rotundata* were examined. A total of sixty-seven plant accessions were collected from IITA, and chromosomal studies were carried out at the Department of Botany, Genetic and Molecular Laboratory, University of Ibadan, Nigeria.

Mitotic chromosome counting

The squash technique described by Olorode *et al.* (1974) and modified by Olawuyi and Oladele (2020) was used to count the chromosomes. Mitotic root tips were collected from plant samples at 10 AM. The root tip samples were immediately fixed in a solution of 3:1 ethanol (96%) and acetic acid for 24 h to arrest the cells in metaphase without any pre-treatment. The fixed root tips were then hydrolysed in 18% HCl for five to ten minutes to degrade the cell walls. The root tip meristem from two to three roots was cut and placed on a glass slide. A drop of the fixing solution was added to the root meristem, which was then squashed.

Staining and microscopic examination

The samples were stained with freshly prepared FLP orcein solution for a minimum of 30 minutes or up to one hour using previously prepared staining solutions (Olawuyi & Oladele, 2020). The prepared slides containing chromosome samples were examined under a 40X optical microscope. Chromosomes were observed, counted, and captured at 100X magnification using oil immersion to ensure clarity and detail.

Data collection and analysis

Chromosomes were counted in multiple trials of mitotic stages and recorded on an Excel sheet. For each accession, chromosome numbers were determined from at least 8–10 well-spread metaphase cells obtained from two to three root tips. Only clearly resolved metaphase plates with minimal chromosome overlap were used for counting, and the modal chromosome number was recorded for each genotype. Photographs of the chromosomes were taken under a microscope. Data were analysed using descriptive statistics. Chromosomal percentage abnormalities were calculated based on the number of cells showing deviations from the expected chromosome counts at the metaphase stage. This was computed using the following formula:

$$\text{Percentage abnormality (\%)} = \frac{\text{Total No of Abnormal Cells}}{\text{Total No of examined cells}} \times 100$$

Results

Parent lines chromosome count

The results in Table 1 show the number of chromosomes counted in individual metaphase cells for various wild yam accessions, along with their modal chromosome number and ploidy levels at the metaphase phase. Twelve accessions, TDpr 1002, TDpr 1003, TDpr 1009, TDpr 1011, TDpr 1018, TDpr

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1020, TDpr 1035, TDpr 1037, TDpr 1044, TDab 1042, TDabWild, and TDr9518544, revealed a stable diploid chromosome number of $2n = 2x = 40$ across all cells analysed, confirming their diploid status. Conversely, anomalies were observed in TDpr 1004, TDpr 1019, and TDpr 1022 with $2n = 34$, $2n = 37$, and $2n = 35$ chromosome numbers, respectively

(Table 1 and Figure 1). This accounted for a percentage abnormality of 19.05% of the total population examined from the parental lines (*D. praehensilis*, *D. abyssinica*, and *D. rotundata*). Some metaphase plates showed overlapping chromosomes, a known limitation of squash techniques (Figure 1).

Table 1: Chromosome counts in accessions of *D. praehensilis*, *D. abyssinica*, and *D. rotundata*

Accession	The number of chromosomes counted in the individual metaphase							Modal	Ploidy status
	1	2	3	4	5	6	7		
TDpr 1002	40	40	40	40	40	40	40	40	$2n = 40$
TDpr 1003	40	40	40	40	40	40	40	40	$2n = 40$
*TDpr 1004	34	32	32	36	36	34	34	34	$2n = 34$
TDpr 1009	40	40	40	40	40	40	40	40	$2n = 40$
TDpr 1011	40	40	40	40	40	40	40	40	$2n = 40$
TDpr 1018	40	40	40	40	40	40	40	40	$2n = 40$
*TDpr 1019	37	35	40	36	39	36	36	37	$2n = 37$
TDpr 1020	40	40	40	40	40	40	40	40	$2n = 40$
*TDpr 1022	35	34	35	35	36	35	35	35	$2n = 35$
TDpr 1035	40	40	40	40	40	40	40	40	$2n = 40$
TDpr 1037	40	40	40	40	40	40	40	40	$2n = 40$
TDpr 1044	40	40	40	40	40	40	40	40	$2n = 40$
TDab 1042	40	40	40	40	40	40	40	40	$2n = 40$
TDabWild	40	40	40	40	40	40	40	40	$2n = 40$
TDr9518544	40	40	40	40	40	40	40	40	$2n = 40$

Asterisk (*) accessions showed variation (suspected aneuploidy). The basic chromosome number in *Dioscorea* spp. is $x = 20$; therefore, $2n = 40$ represents euploid diploid, while deviations from

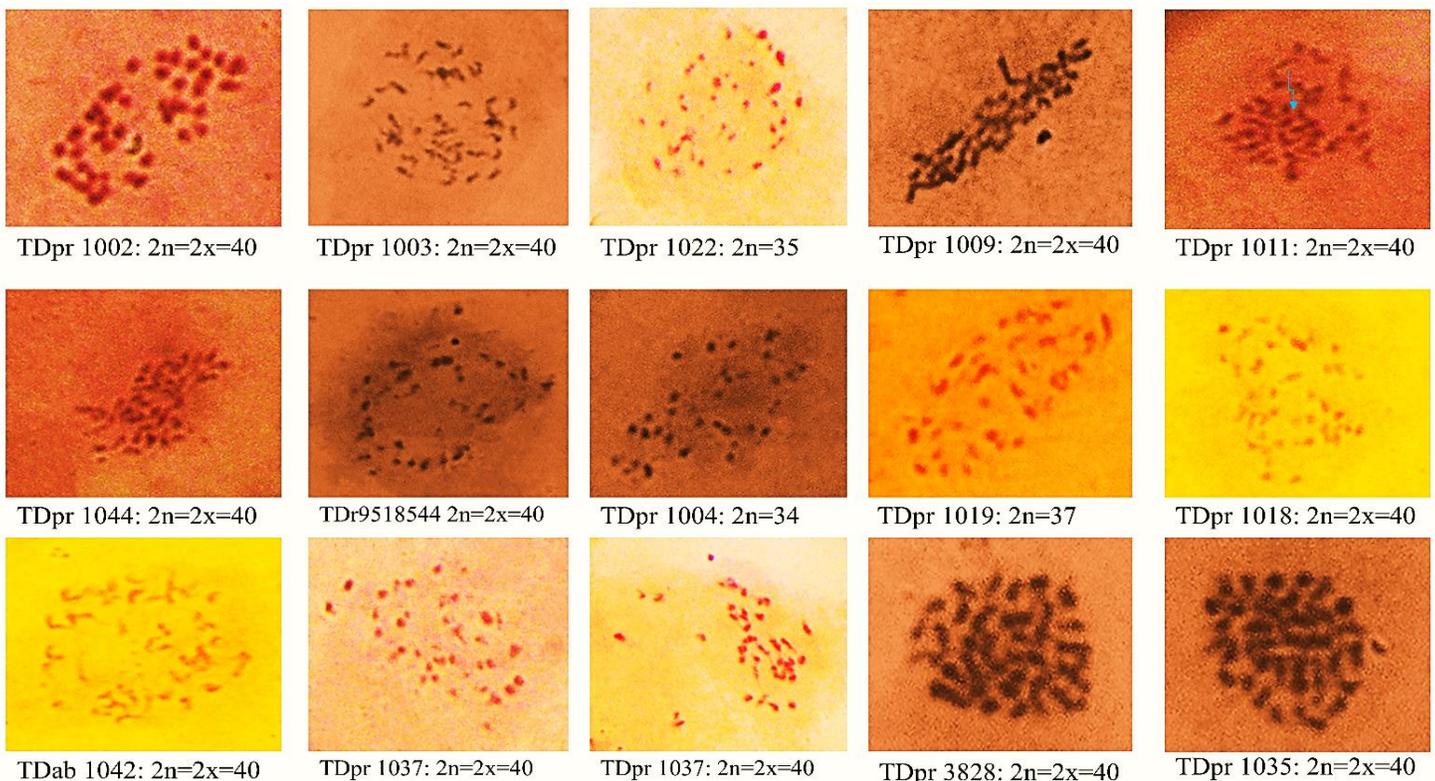


Figure 1: Photographs of chromosomes of *Dioscorea* spp. Accessions at the metaphase stage.

Chromosome counting of F₁ Progenies from the cross between *Dioscorea praehensilis* and *Dioscorea abyssinica*.

Chromosome counting of the F₁ progenies, as shown in Table 2, revealed variations in ploidy levels among the progenies and parental lines. The maternal (TDabwild) and paternal (TDPr 1018) parents consistently exhibited a chromosome count of 40 (2n = 2x = 40), confirming their diploid status. This result was corroborated by the control accession *D. rotundata* (TDr9518544) with the same chromosome count of 2n = 2x = 40 (Table 2). Among the 30 F₁ progenies examined, 10 accessions (TDabp2101002, TDabp2101004, TDabp2101006, TDabp2101007, TDabp2101009, TDabp2101012, TDabp2101013, TDabp2101046, TDabp2101015 and TDabp2101016) showed a chromosome count of 2n = 20, corresponding to the haploid chromosome complement (x = 20). Some accessions exhibited chromosomal abnormalities,

such as TDabp2101017, which displayed a chromosome count of 2n = 31. Accessions TDabp2101018, TDabp2101019, TDabp2101020, TDabp2101021, and TDabp2101023 consistently exhibited a count of 2n = 25. TDabp2101026 and TDabp2101028 showed chromosomes 2n = 22, indicating minor deviations from haploidy (Table 2). Accessions TDabp2101035 and TDabp2101036 exhibited a count of 2n = 18, representing the lowest ploidy level observed. Whereas higher counts were recorded in TDabp2101054 and TDabp2101062 (chromosomes, 2n = 33), TDabp2101058 and TDabp2101060 had 2n = 32. In this study, a cell was considered normal if it had either 20 chromosomes (haploid) or 40 chromosomes (diploid). Of the 231 cells analysed, 91 met this criterion, while 140 cells displayed abnormal counts (other than 20 or 40). Hence, the percentage of abnormality was approximately 66.67% in the F₁ generation. This high percentage suggests significant chromosomal instability in the F₁ progenies. (Table 2).

Table 2: Ploidy status by chromosome counts of the F₁ progenies

S/N	Genotypes	Chromosome count in individual metaphases								modal	ploidy
1	TDabp2101002	20	20	20	20	20	20	20	20	20	2n = 20
2	TDabp2101004	20	20	20	20	20	20	20	20	20	2n = 20
3	TDabp2101006	20	20	20	20	20	20	20	20	20	2n = 20
4	TDabp2101007	20	20	20	20	20	20	20	20	20	2n = 20
5	TDabp2101009	20	20	20	20	20	20	20	20	20	2n = 20
6	TDabp2101012	20	20	20	20	20	20	20	20	20	2n = 20
7	TDabp2101013	20	20	20	20	20	20	20	20	20	2n = 20
8	TDabp2101046	20	20	20	20	20	20	20	20	20	2n = 20
9	TDabp2101015	20	20	20	20	20	20	20	20	20	2n = 20
10	TDabp2101016	20	20	20	20	20	20	20	20	20	2n = 20
11	TDabp2101017	31	31	31	31	31	31	31	31	31	2n = 31
12	TDabp2101018	25	25	25	25	25	25	25	25	25	2n = 25
13	TDabp2101019	25	25	25	25	25	25	25	25	25	2n = 25
14	TDabp2101020	25	25	25	25	25	25	25	25	25	2n = 25
15	TDabp2101021	25	25	25	25	25	25	25	25	25	2n = 25
16	TDabp2101023	25	25	25	25	25	25	25	25	25	2n = 25
17	TDabp2101026	22	22	22	22	22	22	22	22	22	2n = 22
18	TDabp2101028	22	22	22	22	22	22	22	22	22	2n = 22
19	TDabp2101032	26	26	26	26	26	26	26	26	26	2n = 26
20	TDabp2101033	26	26	26	26	26	26	26	26	26	2n = 26
21	TDabp2101035	18	18	18	19	18	17	18	18	18	2n = 18
22	TDabp2101036	18	18	19	18	18	18	17	18	18	2n = 18
23	TDabp2101040	30	30	30	30	30	30	30	30	30	2n = 30
24	TDabp2101043	30	30	30	30	30	30	30	30	30	2n = 30
25	TDabp2101047	30	30	30	30	30	30	30	30	30	2n = 30
26	TDabp2101050	30	30	30	30	30	30	30	30	30	2n = 30
28	TDabp2101052	31	31	31	31	31	31	31	31	31	2n = 31
27	TDabp2101054	33	33	33	33	33	33	33	33	33	2n = 33
28	TDabp2101058	32	32	32	32	32	32	32	32	32	2n = 32
29	TDabp2101060	32	32	32	32	32	32	32	32	32	2n = 32
30	TDabp2101062	33	33	33	33	33	33	33	33	33	2n = 33
31	*TDabwild female parent	40	40	40	40	40	40	40	40	40	2n = 40
32	*TDpr 1018 Male Parent	40	40	40	40	40	40	40	40	40	2n = 40
33	*TDr9518544	40	40	40	40	40	40	40	40	40	2n = 40

Chromosome numbers are reported as somatic metaphase counts (2n). The basic chromosome number of *Dioscorea* spp. is $x = 20$; therefore, $2n = 40$ represents the euploid diploid condition, while reduced or non-multiple chromosome numbers indicate haploid or aneuploid complements. Asterisks (*) indicate parental lines.

Chromosome counting of F₂ Progenies from crosses of F₁ self and F₁ x *D. rotundata*

The results of the chromosome count presented in Table 3 for the F₂ progenies highlighted significant variation in ploidy levels, reflecting the genetic consequences of selfing and hybridisation with *D. rotundata*. The single surviving progeny from the self-cross (TDX 23009003) exhibited a stable diploid chromosome count of $2n = 2x = 40$ across all metaphase cells analysed (Table 3). Among the F₂ progenies derived from F₁ x *D. rotundata* crosses, different chromosome counts were observed, ranging from $2n = 25$ to $2n = 40$, reflecting variability and instability. Several progenies, including TDX 23010-6, TDX 23010-68, TDX 23010-15, TDr 23019-1, TDr 23019-8, TDr 23019-20, TDr 23020-30, TDr 23021-1, TDr 23009-10, TDr 23005-35, TDr 23018-25, TDr 23032-9, and TDX 23011-

6, maintained a consistent chromosome count of $2n = 40$ across all examined cells (Table 3, Figure 2). Other progenies showed moderate variation (Figure 3). TDr 23002-24 and TDr 23002-43 exhibited some variation, with a count of $2n = 35$, indicating potential structural rearrangements. TDr 23003-1 and TDr 23019-59 had counts ranging between $2n = 28$ to $2n = 32$, suggesting aneuploidy (Table 3). Reduced chromosome counts were observed in three other progenies (TDr 23007-16; TDr 23000-68, and TDr 23001-68) with consistently 25 chromosomes ($2n = 25$), indicating significant meiotic instability (Table 3). The parental accessions (TDabp2101006, TDabp2101046, and TDabp2101016) exhibited a stable haploid chromosome count of $2n = 20$, and TDabp2101060 with $2n = 32$, aligning with the previous year's F₁ count (Table 3, Table 2, Figure 2). The overall percentage of abnormalities (PA) was 33.52% (Figure 3).

Table 3: Ploidy status of chromosome counts of F₂ Progenies from F₁ Selfed and F₁ x *D. rotundata*

S/N	Progenies	Chromosome count in individual metaphases								Modal	ploidy
1	TDX 23010-6	40	40	40	40	40	40	40	40	40	2n = 40
2	TDX 23010-68	40	40	40	40	40	40	40	40	40	2n = 40
3	TDX 23010-15	40	40	40	40	40	40	40	40	40	2n = 40
4	TDr 23002-24	35	35	35	35	35	35	35	35	35	2n = 35
5	TDr 23002-43	35	34	35	35	36	34	36	35	35	2n = 35
6	TDr 23003-1	30	30	32	30	30	28	30	30	30	2n = 30
7	TDr 23005-35	20	20	20	20	20	20	20	20	20	2n = 20
8	TDr 23005-19	40	40	40	40	40	40	40	40	40	2n = 40
9	TDr 23009-10	39	40	39	30	40	40	40	40	40	2n = 40
10	TDr 23018-25	40	40	40	40	40	40	40	40	40	2n = 40
11	TDr 23019-1	40	40	40	40	40	40	40	40	40	2n = 40
12	TDr 23019-8	40	40	40	40	40	40	40	40	40	2n = 40
13	TDr 23019-20	40	40	40	40	40	40	40	40	40	2n = 40
14	TDr 23019-59	30	30	30	30	30	30	30	30	30	2n = 30
15	TDr 23020-30	40	40	40	40	40	40	40	40	40	2n = 40
16	TDr 23007-16	25	25	25	25	25	25	25	25	25	2n = 25
17	TDr 23021-1	40	40	40	40	40	40	40	40	40	2n = 40
18	TDr 23000-68	25	25	25	25	25	25	25	25	25	2n = 25
19	TDr 23032-9	40	40	40	40	40	40	40	40	40	2n = 40
20	TDr 23001-68	25	25	25	25	25	25	25	25	25	2n = 25
21	TDX 23011-6	40	40	40	40	40	40	40	40	40	2n = 40
22	**TDX 23009003	40	40	40	40	40	40	40	40	40	2n = 40
23	*TDabp2101006	20	20	20	20	20	20	20	20	20	2n = 20
24	*TDabp2101046	20	20	20	20	20	20	20	20	20	2n = 20
25	*TDabp2101016	20	20	20	20	20	20	20	20	20	2n = 20
26	*TDabp2101060	32	32	32	32	32	32	32	32	32	2n = 32

Single asterisk (*): parental accessions of F₁ hybrids.

Double asterisks (**): surviving F₂ hybrid from F₁ self-crosses.

The others were F₂ progenies from F₁ hybrids x *D. rotundata*.

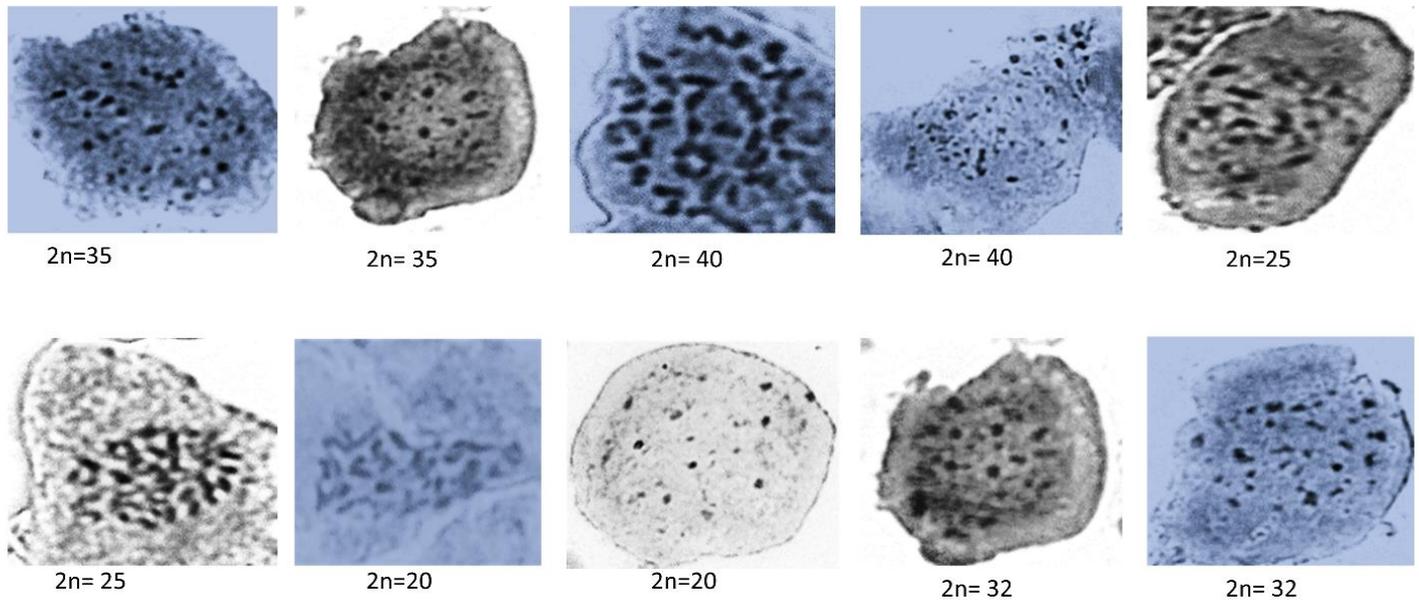


Figure 2: Photographs of metaphase chromosome variability in wild yam F₁ and F₂ progenies

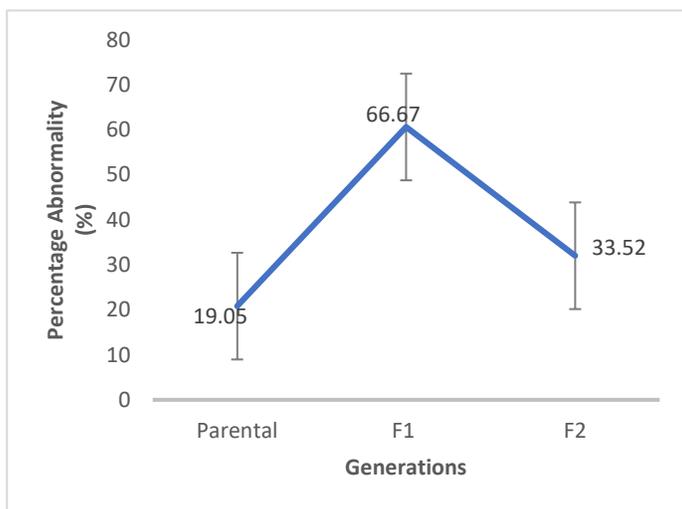


Figure 3: Comparative line chart of chromosomal abnormalities across generations. The comparative line chart illustrates the percentage of chromosomal abnormalities observed across different generations (Parent, F₁, and F₂). The trend in the chart revealed that abnormalities increased from Parent to F₁ progenies but drastically decreased in the F₂ progenies (Figure 3).

DISCUSSION

Chromosome counting and ploidy analysis are crucial techniques in plant breeding and genetic research (Hrabovský *et al.*, 2025). The chromosome counting results presented in Tables 1 and 2, TDabwild (Maternal parent), showed that TDPr 1018 (paternal parents), and TDr9518544 (*D. rotundata*) had a consistent chromosome count of 40, indicating diploidy (2n = 2x = 40). These findings align with prior observations in breeding programs involving hardy geraniums, where Akbarzadeh *et al.* (2021) emphasised that knowledge of chromosome numbers plays a crucial role in predicting hybrid

stability and guiding selection. Similarly, in yams (*Dioscorea* spp), these stable parental lines serve as a cytogenetic reference for evaluating hybrid progeny performance and guiding the selection process (Darkwa *et al.*, 2020). Nevertheless, most of the F₁ progenies showed chromosome count of 20 (2n = 20), suggesting haploidy in chromosome number. Interspecific hybridisation often leads to genomic instability, including chromosome aberrations, which can result in haploid or near-haploid chromosome numbers in the progenies, even from diploid parents. This phenomenon is driven by several mechanisms, primarily rooted in genome incompatibility between the parental species, including abnormal spindle attachment, lagging chromosomes, and unequal segregation during meiosis. Such chromosomal aberrations are well-documented in a variety of plant interspecific hybrids, impacting fertility and genetic stability (Pershina & Trubacheeva, 2017; Majka *et al.*, 2023). However, further studies using complementary cytogenetic approaches are required to confirm the exact mechanisms involved. One progeny, TDabp2101017, exhibited a chromosome count of 2n = 31, indicating a different ploidy level potential. Such unusual chromosome counts can result from hybridisation and chromosomal rearrangements. This is in agreement with different studies on *Humulus lupulus*, *Brassica rapa*, *Misgurnus anguillicaudatus*, and *Populus* spp. Hybrids, which showed that interploidy crosses often result in aneuploid progeny with chromosome numbers deviating from the expected euploid levels (Trojak-Goluch & Skomra, 2020; Gu *et al.*, 2016; Wang *et al.*, 2017). Other progenies (TDabp2101018, TDabp2101019, TDabp2101020, etc.) consistently exhibit a chromosome count of 25 (2n = 25). Studies have revealed that intermediate chromosome numbers compared to their parents may indicate the presence of

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unbalanced chromosomal segments, possibly resulting from irregular meiosis in hybrids (Van Oost *et al.*, 2021; Fukuhara *et al.*, 2016). These abnormalities, such as deviations from diploid chromosome numbers, suggest potential genomic instability, chromosomal overlap during preparation, or meiotic irregularities. Studies on red clover hybrids by Dluhořová *et al.* (2016) and synthetic allotetraploid wheat by Lv *et al.* (2022) demonstrated significant chromosomal variations and rearrangements in the hybrid progeny. The presence of different chromosome counts in various F₁ genotypes indicated that even in the first generation, the hybrids had a wide range of ploidy levels, suggesting a significant level of genetic variability, which could impact future breeding selection. Chromosomal instability revealed as numerical and structural variations, often resulting from meiotic irregularities, as reported by Zhao *et al.* (2021) and Lv *et al.* (2022).

The F₂ progenies from self-crossing F₁ plants showed a more stable and consistent diploid with most having $2n = 2x = 40$, indicating that the chromosome number was stabilised in the F₂ generation, particularly in those derived from self-crosses. However, this contrasts with the findings of Zhao *et al.* (2021), who stated that instability in chromosomal expression can persist for multiple generations, as seen in synthetic-natural wheat hybrids. This suggests that selfing has helped restore diploid levels or maintain a consistent chromosome number, in accordance with the parental species. This consistency of $2n = 2x = 40$ counts was observed in most F₂ progenies. This contrasts with the greater variability in chromosome counts observed in the F₁ hybrids. Furthermore, the percentage of abnormality was reduced from 66.67% in F₁ progenies to 33.52% in F₂ progenies (Figure 3), suggesting a more stable chromosome count in F₂ progenies. Selfing of F₁ hybrids appeared to reduce the variability in chromosome numbers. This is particularly important for breeding, as more stable chromosome counts could lead to more predictable genetic traits in subsequent generations of the species. Recent studies have explored the potential of selfing in plant breeding, particularly for stabilising hybrid traits. For example, in potatoes, diploid F₁ hybrid breeding using self-compatible lines offers promise for producing genetically uniform cultivars, despite the challenges of inbreeding depression (Sood *et al.*, 2024; Bradshaw, 2022; Bachem *et al.*, 2019). This is also in agreement with Ramzan *et al.* (2020), who demonstrated that self-fertilization in *Lilium* hybrids could lead to genome stabilisation in successive generations. This approach could lead to the fixation of beneficial alleles and improved breeding efficiency. The consistency in chromosome numbers among the F₂ progenies suggests that these lines could be prime candidates for further selection, especially if consistent ploidy correlates with desirable traits. A limitation of this study is the absence of flow cytometry-based genome size estimation, which would provide independent validation of ploidy levels inferred from chromosome counts. Consequently, interpretations of haploidy and aneuploidy are considered putative and would benefit from confirmation using complementary cytogenetic approaches. Nevertheless, the study clearly documents chromosomal

variation and instability across F₁ progenies and the subsequent stabilisation observed in the F₂ generation.

CONCLUSION

This study demonstrates that interspecific hybridisation in *Dioscorea* spp. induces pronounced chromosomal instability in the F₁ generation, characterised by wide variation in chromosome numbers, while subsequent selfing promotes partial restoration and stabilisation of the diploid chromosome complement in the F₂ progenies. The transition from high chromosomal variability in F₁ to increased cytogenetic stability in F₂ highlights selfing as an effective mechanism for genome stabilisation in wild-relative yam breeding. Importantly, this work provides one of the few cytogenetic evaluations tracking chromosome behaviour from parental lines through F₁ and F₂ generations in *Dioscorea*, offering novel insight into how chromosomal instability arising from interspecific crosses can be progressively stabilised through selection.

These findings have direct relevance for yam improvement programmes, as they underscore the necessity of cytogenetic screening when advancing hybrids to later breeding generations. While ploidy inferences are based on mitotic chromosome counts, future studies integrating flow cytometry and molecular cytogenetics will further refine understanding of genome stabilisation in yam hybrids.

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Author Contributions

F. P. Oyedoyin: Conceptualisation, Methodology, Experimentation, Data Collection and Analysis, and Preparation of the first manuscript draft. O. J. Olawuyi: Supervision, Critical Review, and Validation of experimental design. R. T. Feyisola: Methodology Development, Formal Analysis and Manuscript Review. N. Salaudeen: Experimentation and Laboratory Support. M. A. Ajayi: Experimentation, Data Collection and Statistical Support. A. Edemodu: Project Administration and Final Manuscript Review.

Conflicts of Interest

The authors declare no conflicts of interest.

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