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Morphological characterization and cytological studies of the green-stemmed and the red-stemmed *Basella alba*

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Abstract

Basella alba is an underutilized vegetable with ethnobotanical importance used for culinary as well as medicinal purposes in many parts of the world. Morphological characterization and chromosome studies of the mitotic and meiotic cells of the green stemmed and the red-stemmed *B. alba* was carried out with a view to filling the knowledge gaps that exist in their morphological characterization and also to provide insightful information on their chromosome numbers and meiotic behaviour. The *B. alba* accessions studied were characterized with respect to their habit, leaves, inflorescence, fruits and seeds. Mitotic and meiotic studies were carried out on the *Basella* accessions using standard techniques. The morphological studies revealed significant differences between the green-stemmed and red-stemmed *Basella alba* with respect to the green/red colour of their stems, colour of the flower bud apex, mean plant height at flower bud initiation, mean leaf length, mean leaf width, mean petiole length, mean flower per spike, mean fruit length and mean fruit diameter. The cytological study revealed a chromosome number of $2n=4x=44$ for both the green-stemmed and the red-stemmed *B. alba* studied. It also revealed the occurrence of chromosomal aberrations such as stickiness and precocious migration of chromosomes to the poles during meiosis, which could lead to irregular chromosome segregation that could result in chromosome instability and aberrant meiotic products within the species.

Keywords: bivalents chromosomes; descending dysploidy; Malabar spinach; meiotic segregation; mitotic studies; underutilized vegetables

Introduction

Basella alba L. is the most common species in the family Basellaceae (Ozela *et al*., 2007) and it comprises the green-stemmed (*Basella alba* var. *alba*) and purple-stemmed (*Basella alba* var. *rubra*) *Basella alba* forms. It is native to tropical Southern Asia and probably originated from India or Indonesia (Saroj *et al.,* 2012). *B. alba* is particularly abundant in Malaysia, Philippines, Thailand, Nepal, India, tropical Africa, the Caribbean and tropical South America (Palada and Crossman, 1999).

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Basella alba is an underutilized vegetable often cultivated in home gardens (Bolaji *et al*., 2022a) and used for culinary and medicinal purposes. It is a very popular vegetable in many communities of South-western Nigeria and is also one of the chief sources of the major ingredients in the Northern Nigeria and North-eastern Nigerian foods (Izonfuo *et al.,* 2006). It is useful as medicinal plant in the treatment of various ailments including anaemia (Rahmatullah *et al.,* 2010), pelvic inflammation disease, threatened abortion (Focho *et al*., 2009), hypertension (Olowokudejo *et al*., 2008), earache and sore throat (Chatchawal *et al.,* 2010; Paul *et al.,* 2011), gonorrhoea (Singh *et al*., 2010), diabetes (Shantha *et al.*, 2016) and ulcer (Dixit and Goyal, 2011).

Chromosomes are key building blocks of eukaryotic genomes (Tiang *et al.*, 2012) and the cytogenetic characterisation of species require in-depth studies of the chromosome numbers and chromosomal behaviours of the species and their morphogenic varieties (Silva *et al*., 2017). Meiosis, a process by which sexually reproducing organisms reduce their genome from diploid to haploid (John, 1990) is a highly coherent and genetically controlled process (Pessim *et al.*, 2015) which involves the process of commitment and initiation, homologous chromosome pairing, synapsis, inter-homologous reciprocal recombination, disjunctive segregation and haploid gamete formation (Murphy and Bass, 2012). There were knowledge gaps concerning the morphological characteristics of the green-stemmed and the red-stemmed *Basella alba.* Previous reports had focused on their stem colour, leaf shapes, floral arrangement and fruit shape (Palada and Chang, 2003; Adeyemi, 2007; Kumar *et al*., 2013). Previous cytological studies on the genus *Basella* indicated varying reports on the chromosome numbers of the green-stemmed and the red-stemmed *B. alba*., with the reports varying from chromosome numbers 2n = 44 to 2n = 45 and 2n = 48 (Hanson *et al.,* 2005; Silva e*t al.*, 2017). There is no known report of chromosome numbers of *B. alba* morphotypes from Nigeria. There is also paucity of information about the meiotic behaviour of *B. alba.* The specific objectives of these study were therefore to characterize the green-stemmed and red-stemmed *B. alba* and establish their chromosome numbers as well as investigate their meiotic behaviours.

Materials and Methods

Plant source and identification

The seeds of the *Basella alba* accessions studied were collected from various locations in Nigeria (Table 1) and authenticated at the IFE Herbarium, Department of Botany, Obafemi Awolowo University, Nigeria. The seeds were sown in polythene bags filled with sterilized top soil laid out in a completely randomized design and raised to maturity in the screen house of the Department of Botany, Obafemi Awolowo University Ile-Ife, Nigeria. Normal agronomic practices such as watering, weeding and staking were performed.

Morphological characterization of plants studied

The qualitative and quantitative attributes of the green-stemmed and the red-stemmed *Basella alba* accessions studied were characterized with respect to their habit, leaves, inflorescence, fruits and seeds. The quantitative data were collected in five replicates for thirty-eight (38) accessions for both the green-stemmed and the red-stemmed. The quantitative morphological attributes of the green-stemmed and red-stemmed *B. alba* were compared by subjecting the data obtained to the Generalized Linear Model (GLM) analysis of variance (ANOVA) and the differences between the means that were significantly different at $p<0.05$ were evaluated by Duncan Multiple Range Test (DMRT) using System Analysis Software (SAS) version 9.0. The means were presented as mean and standard error (mean ± SE).

Table 1. Sources of the green-stemmed and red-stemmed *Basella alba* studied

Accession	*Basella alba* form	Source	Location	Comment
BAIWO (10)*	Green-stemmed	Iwo	7.629444 °N 4.191111 °E	Cultivated in home gardens
BAIFE (8)	Green-stemmed	Ile-Ife	7.523056 °N 4.515833 °E	Cultivated in School gardens
BAONDO (5)	Green-stemmed	Ondo	7.236111 °N 5.239722 °E	Cultivated in home gardens
BAEKITI (5)	Green-stemmed	Ekiti	7.616389 °N 5.218333 °E	Cultivated in home gardens
BAOYO (5)	Green-stemmed	Oyo	7.419167 °N 3.964722 °E	Cultivated in Church gardens
BALAG (5)	Green-stemmed	Ijede	6.942778 °N 4.191111 °E	Cultivated beside home
BRIWO (5)	Red-stemmed	Iwo	7.629444 °N 4.191111 °E	Cultivated in home gardens
BRIFE (5)	Red-stemmed	Ile-Ife	7.523056 °N 4.515833 °E	Cultivated in School gardens
BRONDO (10)	Red-stemmed	Ondo	7.236111 °N 5.239722 °E	Cultivated in home gardens
BREKITI (7)	Red-stemmed	Ekiti	7.616389 °N 5.218333 °E	Cultivated in home gardens
BROYO (5)	Red-stemmed	Ogbomoso	8.146111 °N 4.259167 °E	Cultivated in School gardens
BRLAG (6)	Red-stemmed	Ijede	6.942728 °N 3.098056 °E	Cultivated in home gardens

*Note: Numbers in brackets represent number of plants within each of the accessions studied

Pollen viability and seed set study

The pollen viability study was carried out on 100 pollen grains following the method of Bolaji *et al.* (2022b). Pollen grains from freshly dehisced anthers were harvested on to microscope slides and were stained with cotton-blue-in-lacto-phenol for 30 minutes. The well-formed and deeply stained pollens were considered as viable; while those with collapsed outline, partially stained or not stained were considered to be non-viable. The percentage of viable pollens were documented.

The seed set study was carried out on 10 randomly selected plant. The percentage seed set was determined by dividing the number of seeds obtained by the number of seeds expected (i.e., number of flowers obtained) multiplied by 100, following the method of Idowu and Oziegbe (2017).

Mitotic chromosome studies

Young whole plants of the green-stemmed and the red-stemmed *B. alba* were placed in plastic bottles containing water and allowed to root. The root tips were excised and fixed in 1: 3 acetic-ethanol after four days between 9:00 a.m. and 11:00 a.m. The fixed root tips were left at room temperature for 48 hours before being used for preparation of mitotic cells. The root tips were hydrolysed in 18% HCl, squashed and stained with modified Orcein for 15 minutes following the methods of Bolaji *et al.* (2019). Slides prepared were observed under the light microscope and chromosome counts were made for each of the *Basella* forms. Good mitotic spreads were photomicrographed using Amscope MT Microscope camera version 3.0.0.1 attached to light microscope.

Meiotic chromosome studies

Young flower buds were harvested into vias containing 1: 3 acetic-ethanol between 9:00 a.m and 11:00 am. The anthers were excised, squashed and stained in FLP orcein (2g of Orcein in 100 cm^3 of solution containing equal parts of formic acid, lactic acid, propionic acid and distilled water). Pollen mother cells were examined to investigate meiotic events and photomicrographs of good meiotic spreads were taken using Amscope MT microscope camera version 3.0.0.1 attached to a light microscope.

Results

Description of the green-stemmed and red-stemmed Basella alba studied

The green-stemmed (Figure 1A) and red-stemmed (Figure 1B) *Basella alba* studied were perennial, herbaceous twinning, with glabrous stem (Table 2). The mean plant height was 87.65 ± 5.32 cm for the green-stemmed, while it was 119.49 ± 6.99 cm for the red-stemmed. The red-stemmed had significantly greater plant height (Table 3) than the green-stemmed at P = 0.0005. The mean stem diameter for both forms of *B. alba* were not significantly different. The green-stemmed had a mean stem diameter of 3.38 ± 0.09 cm, while the red-stemmed had 3.64 ± 0.15 cm. The mean number of primary branches was 5.26 ± 0.45 for the green-stemmed and 4.34 ± 0.29 for the red-stemmed with no significant difference between them. The mean internode distance was 4.58 ± 0.23 cm for the green-stemmed and 5.05 ± 0.31 cm for the red-stemmed., with no significant difference between them.

Figure 1. The green-stemmed and red-stemmed *Basella alba* studied and their inflorescence, fruits and seeds; A: Green-stemmed *B. alba* studied; B: Red-stemmed *B. alba* studied; C: Inflorescence of the green-stemmed *B. alba* studied; D: Inflorescence of the red-stemmed *B. alba* studied; E: Mature fruits of the green-stemmed *Basella alba* studied;; F: Mature fruits of the red-stemmed *B. alba* studied; G: Seeds of the green-stemmed *B. alba* studied; H: Seeds of the red-stemmed *B. alba* studied

The leaves were green, simple, glabrous, succulent, cordate, margin entire, apex acute and round, venation pinnate, leaf arrangement opposite at seedling stage, alternate at mature stage, leaf attachment petiolate; leaves exstipulate for both forms of *B. alba* (Table 2). The mean leaf length was 6.62 ± 0.37 cm for the green-stemmed and 7.75 ± 0.31 cm for the red stemmed, with the red-stemmed having significantly longer leaves than the green-stemmed at P = 0.0218 (Table 3). The mean leaf width was 5.23 ± 0.19 cm for the green-stemmed and 6.67 ± 0.26 for the red-stemmed, with the red-stemmed having significantly wider leaves at P = 0.0001. The mean petiole length was 1.71 ± 0.06 cm for the green-stemmed and 1.95 ± 0.08 cm for the red-stemmed with the red-stemmed having significantly longer petiole than the green-stemmed at P = 0.0172.

Table 2. Comparison of morphological attributes of the green-stemmed and the red-stemmed *Basella alba* studied

Characters	Green-stemmed *Basella alba*	Red-stemmed *Basella alba*
Habit	Perennial, herbaceous, twinning, stem green, glabrous, mean plant height at flower bud initiation 87.65 ± 5.32 cm, mean stem diameter 3.38 ± 0.09 cm, mean number of primary branches 5.26 ± 0.45, mean internode distance 4.58 ± 0.23 cm	Perennial, herbaceous, twinning, stem red, glabrous, mean plant height at flower bud initiation 119.49 ± 6.99 cm, mean stem diameter 3.64 ± 0.15 cm, mean number of primary branches 4.34 ± 0.29, mean internode distance 5.05 ± 0.31 cm
Leaves	Green, glabrous, succulent, cordate, margin entire, venation pinnate, leaf arrangement alternate, leaf attachment petiolate, mean length 6.62 ± 0.37 cm, mean width 5.23 ± 0.19 cm, petiole length 1.71 ± 0.06 cm	Red, glabrous, cordate, margin entire, venation pinnate, leaf arrangement alternate, leaf attachment petiolate, mean length 7.75 ± 0.31 cm, mean width 6.67 ± 0.26 cm, petiole length 1.95 ± 0.08 cm
Inflorescence	Racemose, flowers bisexual, pedicel sessile and glabrous, symmetry radial; one perianth with 5 segments joined at the base, perianth cream with pink apex, bracts attached to base of perianth, anther 5, pollen light yellow, powdery; filament white with adnate attachment; stigma simple, glabrous; style of unequal length, white, simple, glabrous; ovary superior, placentation basal, mean flower per spike 22.16 ± 0.93	Racemose, flowers bisexual, pedicel sessile and glabrous, symmetry radial; one perianth with 5 segments joined at the base, perianth cream with deep purple apex, bracts attached to base of perianth, anther 5, pollen deep yellow, powdery; filament white with adnate attachment; stigma simple, glabrous; style of unequal length, white, simple, glabrous; ovary superior, placentation basal, mean flower per spike 16.45 ± 0.82
Fruits	Drupe, glabrous, spherical, apedicelate on green spike; four lobed, one-seeded, unripe colour green, ripen colour deep purple, mean length 1.07 ± 0.03 cm, mean diameter 2.12 ± 0.02 cm, mean number of fruits per spike 12.21 ± 0.69	Drupe, glabrous, spherical, apedicelate on green spike; four lobed, one-seeded, unripe colour green, ripen colour deep purple, mean length 1.15 ± 0.03 cm, mean diameter 2.25 ± 0.03 cm, mean number of fruits per spike 10.24 ± 0.74
Seeds	Brown, spherical, mean length 0.66 ± 0.10 cm, mean diameter 1.25 ± 0.01 cm, mean number of seed per spike 12.21 ± 0.69	Brown, spherical, mean length 0.64 ± 0.02 cm, mean diameter 1.26 ± 0.01 cm, mean number of seed per spike 10.24 ± 0.74

The inflorescence was racemose with each of the flowers consisting of one perianth with 5 segments joined at the base for both forms of *B. alba* (Table 2). The perianth was cream with pink apex (Figure 1C) in the green stemmed, while it was cream with deep purple apex (Figure 1D) for the red-stemmed. Bracts were attached to the base of the perianth in both forms. The flowers were bisexual, pedicel sessile and glabrous, symmetry radial; anther 5, pollen yellow, powdery; filament white with adnate attachment; stigma simple, glabrous; style of unequal length, white, simple, glabrous; ovary superior, placentation basal for both forms of *B. alba* studied. The mean flower per spike was 22.16 ± 0.93 for the green-stemmed while it was 16.45 ± 0.82 for the red stemmed, with the green stemmed having significantly higher number of flowers per spike than the red-stemmed at $P = 0.0001$ (Table 3).

Table 3. Comparison of morphometric characteristics between the green-stemmed and the red stemmed *Basella alba* studied

Characters	Green stemmed *Basella alba*	Red stemmed *Basella alba*
Mean plant height at flower bud initiation (cm)	87.65 ± 5.32 b	119.49 ± 6.99 a
Mean stem diameter (cm)	3.38 ± 0.09 a	3.64 ± 0.15 a
No. of primary branches	5.26 ± 0.45 a	4.34 ± 0.29 a
Mean internode distance (cm)	4.58 ± 0.23 a	5.05 ± 0.31 a
Mean leaf length (cm)	6.62 ± 0.37 a	7.75 ± 0.31 a
Mean leaf width (cm)	5.23 ± 0.19 b	6.67 ± 0.26 a
Mean petiole length (cm)	1.71 ± 0.06 b	1.95 ± 0.08 a
Mean flower per spike	22.16 ± 0.93 a	16.45 ± 0.82 b
Mean fruit length (cm)	1.07 ± 0.03 b	1.15 ± 0.03 a
Mean fruit diameter (cm)	2.12 ± 0.02 b	2.25 ± 0.03 a
Mean number of fruits per spike	12.21 ± 0.69 a	10.24 ± 0.74 a
Mean seed length (cm)	0.66 ± 0.10 a	0.64 ± 0.02 a
Mean seed diameter (cm)	1.25 ± 0.01 a	1.26 ± 0.01 a
Mean number of seed per spike	12.21 ± 0.69 a	10.24 ± 0.74 a

*Notes: Different letters between cultivars denote significant differences (Duncan test, $p < 0.05$)

The fruits were berry, glabrous, spherical, apedicelate on green spike; four lobed, one-seeded, unripe colour green, ripen colour deep purple (Figure 1E and Figure 1F) for both forms of *B. alba* studied. The mean fruit length was 1.07 ± 0.03 cm for the green stemmed while it was 1.15 ± 0.cm for the red stemmed, with the red-stemmed having significantly longer fruits than the green-stemmed at P = 0.0398 (Table 3). The mean fruit diameter was 2.12 ± 0.02 cm for the green-stemmed while it was 2.25 ± 0.03 cm for the red-stemmed with the red-stemmed having significantly wider fruits than the green-stemmed at P = 0.0002. The mean number of fruits per spike was 12.21 ± 0.69 for the green-stemmed, while it was 10.24 ± 0.74 for the red-stemmed with no significant difference between them.

The seeds were brown (Figure 1G and Figure 1H), spherical in shape for both forms of *B. alba* studied. The mean seed length was 0.66 ± 0.10 cm for the green-stemmed, while it was 0.64 ± 0.02 cm for the red-stemmed with no significant difference between them (Table 3). The mean seed diameter was 1.25 ± 0.01 cm for the green-stemmed, while it was 1.26 ± 0.01 cm for the red-stemmed with no significant difference between them. The mean number of seed per spike was 12.21 ± 0.69 for the green-stemmed while it was 10.24 ± 0.74 for the red-stemmed with no significant difference between them.

Chromosome studies

The study revealed a diploid chromosome number of $2n = 44$ and haploid chromosome number of $n = 22$ for the green-stemmed (Figure 2A, Figure 2B, Figure 2C) and the red-stemmed *Basella alba* (Figure 2D) studied. The meiotic chromosome study also revealed the occurrence of many quadrivalent chromosomes amidst bivalent chromosomes at diakinesis (Figure 2C) and metaphase I (Figure 2D). It also revealed the occurrence of chromosome stickiness (Figure 2E) and precocious migration of chromosomes to the poles (Figure 2E). The frequency of the chromosome stickiness was 2.5% in the green-stemmed *B. alba*; while it was 3.5% in the red-stemmed (Table 4). The frequency of the precocious migration of chromosomes to the poles was 5.7% in the green-stemmed; while it was 6.3% in the red-stemmed.

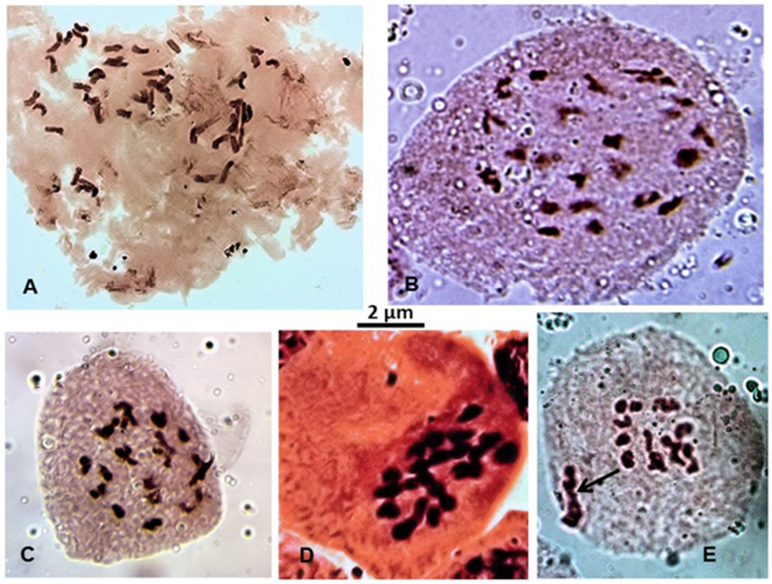

Figure 2. Mitotic and meiotic chromosomes of the green-stemmed and red-stemmed *Basella alba* studied; A: Mitotic metaphase of green-stemmed *B. alba* showing 2n = 44 chromosomes; B: Diakinesis of green-stemmed *B. alba* showing n = 22 bivalent chromosomes; C: Diakinesis of green-stemmed *B. alba* showing n = 22(7IV+8II); D: Metaphase I of red-stemmed *B. alba* showing n = 22(9IV+4II) chromosomes; E: Diakinesis of green-stemmed *B. alba* showing chromosome stickiness and precocious migration to the pole (arrow)

Table 4. Number of pollen mother cells analysed and percentage of abnormal cells affected by chromosome stickiness, laggards and precocious migration to the poles in the *Basella alba* accessions studied

Accessions	Green-stemmed *B. alba*	Red-stemmed *B. alba*
No. of pollen mother cells analysed	955	1255
cells with chromosome stickiness (%)	2.5	3.5
cells with precocious migration of chromosomes to the poles (%)	5.7	6.3
Pollen viability (%)	93.70	94.5
Seed set (%)	65.8	58.43

Discussion

The green-stemmed and red-stemmed *Basella alba* studied were similar with respect to many of their vegetative and reproductive attributes (Table 3) and this is in support of their taxonomic classification as members belonging to same species as reported by Roy *et al.* (2010), Bolaji *et al.* (2022a), and Bolaji *et al.* (2022b). However, there were significant differences between them with respect to the green/red colour of their stems, colour of the flower bud apex (i.e., colour of the perianth apex), mean plant height at flower bud initiation, mean leaf length, mean leaf width, mean petiole length, mean flower per spike, mean fruit length and mean fruit diameter. These significant differences further support their delimitation as varieties belonging to same species. This is corroborated by the reports of Henry *et al.* (1987), Roy *et al.* (2010) and Bolaji *et al.* (2022a).

Chromosome counting from the floral and vegetative tissues of plants remains the most reliable method to establish their chromosome numbers (Mayrose *et al.*, 2020). In this study, the diploid chromosome count of

$2n = 44$ and haploid chromosome count of $n = 22$ indicates a basic chromosome number of $x = 11$ thereby indicating a chromosome number of $2n = 4x = 44$ for the green-stemmed and the red-stemmed *Basella alba* studied. This is in line with the reports of Silva *et al*. (2017) for *Basella alba* species obtained from Brazil. The chromosome numbers obtained for the green-stemmed and red-stemmed *Basella alba* in this study contradicts those of Hanson *et al.* (2005) who reported chromosome numbers $2n = 48$, 41 and 44 for those obtained from England. It also contradicts the reports of Sperling and Bittrich (1993) who reported the basic chromosome number of $x = 12$ for *B. alba.* According to Silva *et al.* (2017) the chromosome number $2n = 44$ observed in *B. alba* may have been derived from descending disploidy from the number $2n = 4x = 48$.

The occurrence of many quadrivalent chromosomes amidst bivalent chromosomes at diakinesis and metaphase I further indicates that the green-stemmed and red-stemmed *Basella alba* studied were tetraploids. The precocious migration of some of the chromosomes to the pole and stickiness of the chromosomes during meiosis could lead to irregular chromosome segregation and chromosome instability within the species. Mendes-Bonato *et al*. (2001) noted that though the cause and biochemical basis for chromosome stickiness is not known, it is characterised by intense clustering and in severe cases makes it impossible for the chromosomes to separate leading to formation of single or varying numbers of pycnotic nuclei that culminate into full chromatin degeneration. Potapova and Gorbsky (2017) also noted that the full consequences of chromosome segregation errors are vast in scope and could produce aneuploid or polyploid cells as well as progeny with altered chromosome content. It is however noteworthy that in this study the frequencies of the chromosome stickiness and precocious migration of chromosomes to the pole during meiosis was very low (Table 4), hence could have been the reason why the pollen viability was still high and the percentage seed set was as well moderately high.

Conclusions

This study characterized the accessions of the green-stemmed and red-stemmed *Basella alba* and established their chromosome as $2n = 4x = 44$. The study also revealed significant differences between the green-stemmed and red-stemmed *B. alba* with respect to the green/red colour of their stems, colour of the flower bud apex, mean plant height at flower bud initiation, mean leaf length, mean leaf width, mean petiole length, mean flower per spike, mean fruit length and mean fruit diameter. The occurrence of many quadrivalent chromosomes amidst bivalent chromosomes suggest that they are tetraploids. The stickiness of the chromosomes and precocious migration to the poles during meiosis in the *B. alba* accessions studied could result in irregular segregation, which could possibly lead to chromosomal instability within the species. However, the frequencies of this aberrant chromosomal events were quite low in the *B. alba* accessions studied, hence, did not result in low pollen viability and low seed set.

Authors' Contributions

Conceptualization: AOB; Data curation: AOB and OIA; Formal analysis: AOB, ASO and OIA; Investigation: AOB, ASO and OIA; Methodology: AOB, ASO and OIA; Project administration: AOB, ASO and OIA; Resources: AOB, ASO and OIA; Software: AOB; Supervision: AOB; Validation: AOB; Visualization: AOB, ASO and OIA; Writing - original draft: AOB and OIA; Writing - review and editing: AOB and ASO.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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