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Cytological, foliar epidermal and pollen grain studies in relation to ploidy levels in four species of *Physalis* L. (Solanaceae) from Nigeria

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ABSTRACT

The relationship in the ploidy levels, pollen grain and epidermal parameters among four *Physalis* species (*P. angulata* Linn., *P. peruviana* Linn., *P. micrantha* Link. and *P. pubescens* Linn) present in Nigeria was investigated from meiotic chromosome, foliar epidermal and pollen grain studies using standard techniques. The study revealed that *P. angulata* has chromosome number of $2n=4x=48$ as against $2n=2x=24$ recorded in each of the other species. The chromosomes of all the four *Physalis* species studied were well paired at pachynema as ring II or rod II with two simultaneous cytokineses observed in all. Tetrad pollens were observed in an accession of *P. micrantha*. Significantly higher pollen diameter, guard cell area and stomata Index were recorded in *P. angulata* when compared with the diploid species investigated. The results indicated that the tetraploid nature of *P. angulata* correlates with its higher guard cell area and pollen grain diameter respectively.

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Keywords: Chromosome association, tetraploid, diploid, pollen grains, tricolporate, stomata.

INTRODUCTION

Four *Physalis* (*P. angulata*, *P. peruviana*, *P. micrantha* and *P. pubescens*) species have been reported in West Africa and they are all represented in Nigeria (Azeez and Faluyi, 2018). Husaini and Iwo (1990) and Wahua and Sam (2013) reported two different chromosome numbers of $2n=4x=48$ and $2n=2x=24$ respectively for *P. angulata* in Nigeria. Burkill (2000) documented $2n=4x=48$ for West African *P. peruviana* whereas $2n=2x=24$ was observed in the Nigerian population for this species (Olorode

et al., 2013). However, there is no known record of chromosome count for *P. pubescens* in Nigeria.

Many researchers have used stomatal size and pollen grain diameter which were described to be simple and less stressful methods of determining the ploidy levels in many plants. It has been reported that as ploidy level increases, the stomatal size as well as pollen diameter increases (Jaochimiak and Grabowska-Jaochimiak, 2000; Kadota and Niimi, 2002; Chen et al., 2009; Ye et al., 2010; Ghasemi et al., 2014).

Some *Physalis* species have been reported to have food value or one medicinal use or the other such as antidiabetic and antioxidative activities (Burkill, 2000; Oladele et al., 2013). In Nigeria, *P. angulata* is a potential vegetable; it served as food for the people of Northener Nigeria in 1972/1973 during the lean year (Burkill, 2000). Considering the economic importance of *Physalis* species, it is very crucial to ascertain the chromosome number/ploidy level(s) of these species in Nigeria as a result of the lack of consensus on chromosome number of *P. angulata* and *P. peruviana* in Nigeria so as to prevent misidentification. Hence, this study investigated the relationship in chromosome count/ ploidy level, pollen grain diameter and guard cell area among four *Physalis* species in Nigeria.

MATERIALS AND METHODS

Herbarium studies were carried out at the IFE Herbarium and the Flora of West Tropical Africa by Hutchinson and Dalziel (1963) was consulted to provide information on the species identification and distribution. *Physalis* species were collected from different locations in South Western Nigeria (Table 1) and were identified, planted and nursed to maturity. Seeds were recovered from matured fruits from each species and planted separately. The seedlings from each species were transplanted into five 11-litre plastic buckets filled with top soil for each species.

Meiotic chromosome study

The young flower buds were harvested from each of species for meiotic chromosome study between 9.00 am and 12.00 pm when the cell activities were believed to be at the peak and stored in 1:3 Acetic acid: alcohol. The anthers were later removed, squashed and stained in FLP Orcein by the squash technique method according to Azeez and Faluyi (2018). The Pollen Mother Cells were examined and

scored for chromosome associations. Good meiotic chromosome spreads were photographed at x1000 under BK series system microscope.

Pollen grain study

Fresh pollen grains from each species were collected, stored in 70% ethanol and subjected to acetolysis according to Azeez and Folorunsho (2014). Photomicrographs of the pollen grains were documented at x400 phase contrast and oil immersion illumination under BK series system microscope (BKSS microscope) attached with Digital Camera for Microscope (DCM510, CMOS chips). The diameters of the acetolysed pollens were measured at x400 and their morphologies were described. The pollen fertility was estimated using the method of Bolaji and Nwokeocha (2013).

Foliar epidermal study

The epidermal peels for each of the *Physalis* species were obtained according to techniques of Ibrahim and Ayodele (2013) and Nwokeocha (2015). The epidermal peels were stained with Safranin 'O' for 3-5 minutes, rinsed in distilled water and then mounted in 25% glycerine for examination. Photomicrographs of the epidermis of each of the species were taken for both adaxial and abaxial surfaces at x1000 under BK series system microscope attached with a digital camera (DCM 510, CMOS chips). The stomata type, the stomata density per mm², the Stomata Index (I), trichome type and Trichome Index (T) were calculated according to the method of Wahua and Sam (2013) and the guard cell area was estimated for the two leaf surfaces using standard method.

Statistical analysis

The means and standard errors were calculated for the quantitative data collected. One-way ANOVA was employed and Duncan multiple range test was carried out to separate the means.

Table 1: Germplasm Sources Used for the Studies.

Species Code	Location	Longitudes and Latitudes
PMR 1201	Dump site at Odoje Ojubo on Ogbomoso-Ajawa Road, Ogbomosho, Oyo State.	8° 08'00.00"N 4° 15'00.00"E
PMR 1301	Along the road at Omuo-Ekiti, Ekiti State.	7° 45'46.85"N 5° 43'28.98"E
PMR 1401	Fallow land at New Market, OAU, Ile-Ife	7° 29'12.07"N 4° 29'35.42"E
PMR 1402	Along Road 7, Ile-Ife	7° 29'12.07"N 4° 29'35.42"E
PAG 1302	Dump site around Pottery Museum, Moore, Ile-Ife	7° 35'25.84"N 4° 44'00.81"E
PAG 1202	Fallow land around Music Department, OAU, Ile-Ife	7° 29'12.07"N 4° 29'35.42"E
PAG 1203	Along the road, Oranfe area, Ile-Ife	7° 35'25.84"N 4° 44'00.81"E
PPR 1204	Dump site around Town Hall, Iloko-Ijesa	7° 39'08.48"N 4°49'24.78"E
PPR 1204	Fallow land around Pottery Museum, Moore, Ile-Ife	7° 35'25.84"N 4° 44'00.81"E
PPR1303	Dump site at Akinyele LGA, Ibadan	7° 31'49.87"N 3°54'39.20"E
PPB 1206	Around Music Department, OAU, Ile-Ife	7° 29'12.07"N 4° 29'35.42"E
PPB 1207	Dump site around Pottery Museum, Moore, Ile-Ife	7° 35'25.84"N 4° 44'00.81"E
PPB 1304	Dump site at Akinyele LGA, Ibadan	7° 31'49.87"N 3°54'39.20"E

*PAG- *Physalis angulata*; PPR- *Physalis peruviana*; PPB- *Physalis pubescens*; PMR- *Physalis micrantha*

RESULTS

Meiotic chromosome study

The meiotic chromosome studies revealed that *P. angulata* has a chromosome number of $2n=4x=48$ (Figure1a-b) while *P. micrantha*, *P. peruviana* and *P. pubescens* have the same chromosome number of $2n=2x=24$ (Figure1c-h). The chromosomes of all the four *Physalis* species were well paired at pachynema and presented as ring II or rod II at diakinesis. Two simultaneous cytokineses were observed in all the four *Physalis* species. There was no abnormality observed in all the stages of meiotic cell division. The events of cell division were asynchronous in all the four *Physalis* species, though these events were synchronous in each anther. This is evident from the fact that the pollen mother cells found together were at the same stage of cell division.

Pollen grain study

All the pollen grains observed in the four *Physalis* species studied were

tricolporate, small to medium in size with polar axis ranging between 23.94 μm in *P. peruviana* to 30.73 μm in *P. angulata* (Figure 2a-d). The shapes of the pollen grains were largely prolate, spheroidal or oblate spheroidal. The pollens were monads except in one accession (PMC 1301) of *P. micrantha* in which the pollens occurred mostly as tetrads (Figure 2d and Table 2). In addition, triads and dyads were present while monads were very rare in this accession. *Physalis angulata* has significantly larger pollen grains with equatorial diameter of 30.73 μm . The equatorial diameter of the other three diploid species was between 24.06 μm in *P. peruviana* and 25.04 μm in *P. pubescens*. Both the polar axis and equatorial diameter of *P. angulata* pollen grains were significantly higher than those of the other three species studied (Table 2).

Foliar epidermal study

The most predominant stomata types observed in the *Physalis* species studied were

anomocytic and anisocytic though tetracytic stomata were observed occasionally in the four species, with stomata generally closed (Figure 3a-h). Multicellular trichomes were observed in all the four species. The highest trichome index of 0.1% was recorded in *P. peruviana*. *Physalis angulata* has the highest stomata index and largest guard cell area (Table 3). There was no significant difference

in the stomata indices among the diploid species. In this study, higher guard cell area was recorded in the adaxial surfaces than abaxial surfaces in *P. angulata* and *P. peruviana*. In contrast, it was higher on the abaxial surface of *P. micrantha* though no significance difference was observed on the two surfaces of *P. pubescens*.

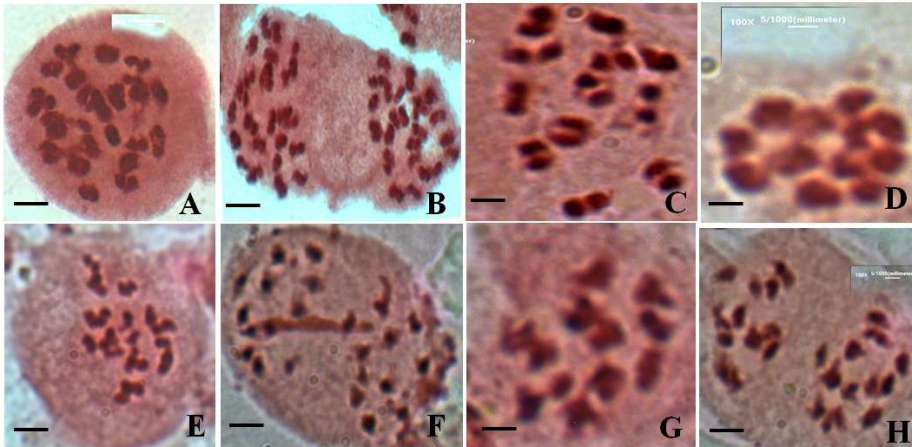


Figure 1a-h: Stages of meiotic cell divisions in the Four *Physalis* species studied

- A. Late diakinesis in *P. angulata* showing 24 II
- B. Metaphase II in *P. angulata* showing n=24 at each pole
- C. Diakinesis in *P. pubescens* showing 12II
- D. Metaphase I *P. pubescens* showing 12 II
- E. Late diakinesis in *P. micrantha* showing 12II
- F. Metaphase II in *P. micrantha* showing n=12 at each pole
- G. Metaphase I in *P. peruviana* showing 12II
- H. Metaphase II in *P. peruviana* showing n= 12 at each pole . Scale bar = 3µm

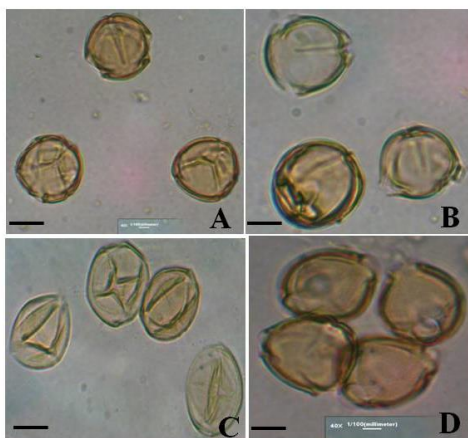


Figure 2a-d: Pollen grains of the four *Physalis* species studied.

- a-c Monad pollen in *P. angulata*, *P. pubescens* and *P. peruviana*
- d. Tetrad pollen in *P. micrantha* Scale bar = 4µm

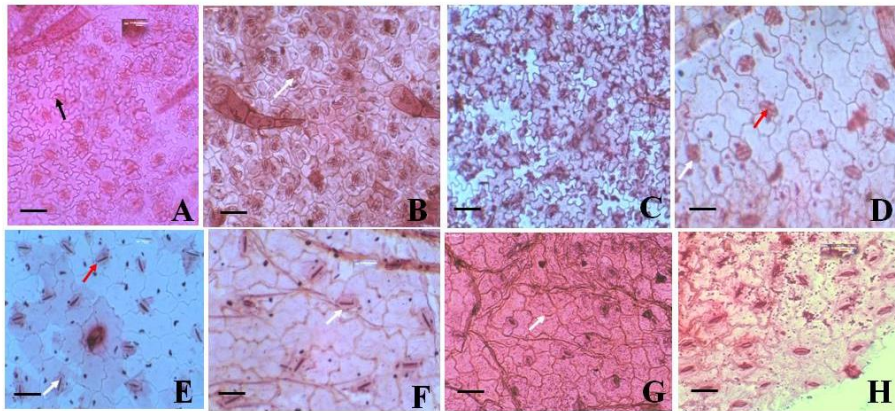


Figure 3a-h: Foliar epidermal features of the four *Physalis* species studied.

- A. Abaxial of *P. peruviana*
- B. Adaxial of *P. peruviana*
- C. Abaxial of *P. pubescens*
- D. Adaxial of *P. pubescens*
- E. Abaxial of *P. angulata*
- F. Adaxial of *P. angulata*
- G. Abaxial of *P. micrantha*
- H. Adaxial of *P. micrantha*

*Black arrows indicate anomocytic stomata, white arrows indicate anisocytic, red arrows indicate tetracytic stomata. Scale bar = 4µm

Table 2: Pollen grain parameters in the *Physalis* species studied.

Pollen Characters	<i>Physalis peruviana</i>	<i>Physalis micrantha</i>	<i>Physalis pubescens</i>	<i>Physalis angulata</i>
Polar axis (µm)	23.94 ^a	24.43 ^{ab}	25.40 ^b	30.73 ^c
Equatorial Diameter (µm)	24.06 ^a	24.56 ^{ab}	25.40 ^b	30.73 ^c
Colpi (µm)	22.57 ^a	22.57 ^a	22.81 ^b	22.81 ^b
Ora (µm)	5.82 ^c	6.88 ^d	4.54 ^b	0.00 ^a
Exine (µm)	2.28 ^d	1.90 ^b	2.33 ^c	1.37 ^a
Exine Pattern	Finely reticulate	Reticulate	Reticulate	Finely reticulate
Shape	Prolate spheroidal	Prolate spheroidal	Oblate spheroidal	Oblate-or Prolate spheroidal
Pollen Stainability (%)	92.30	80.60	89.60	89.30

*Means within each row with different superscripts are significantly different at P < 0.05

Table 3: Epidermal features of the *Physalis* species studied.

Species	Trichome Index (%)	Stomata Index (%)	Guard Cell Area (µm ²)
<i>P. micrantha</i>	0.02 ^c	0.24 ^b	2.17 ^d
<i>P. pubescens</i>	0.04 ^b	0.25 ^b	2.75 ^c
<i>P. peruviana</i>	0.10 ^a	0.23 ^b	3.03 ^b
<i>P. angulata</i>	0.03 ^b	0.27 ^a	6.23 ^a

*Means within each row with different superscripts are significantly different at P < 0.05.

DISCUSSION

The pairing pattern for each *Physalis* species studied was regular. Ganapathi et al. (1991) reported regular pairing pattern for *P. angulata*, *P. peruviana*, *P. minima* and *P. pubescens* in India. The asynchronous events of cell division that were observed in this study might be as a result of different rates of development observed among the five anthers in each of the flower bud of *Physalis* species studied because the anthers do not dehisce at the same time. About 2-3 days is usually required for all the anthers to dehisce. This observation corroborates the report of Menzel (1951). The chromosome number of $2n=48$ for *P. angulata* observed in this study is in agreement with previous studies (Menzel, 1951; Husaini and Iwo, 1990; Olorode et al., 2013). However, Wahua and Sam (2013) reported $2n=24$ for *P. angulata* in Nigeria. Menzel (1951) stated that the report of $2n=24$ for *P. angulata* can be considered as a case of misidentification. Burkill (2000) documented $2n=48$ for *P. peruviana* whereas Rodriguez and Bueno (2006) recorded chromosome counts of $2n=24$, $2n=32$ and $2n=48$ for *P. peruviana*. The chromosome number of $2n=24$ was reported for *P. peruviana* in this study corroborates the report of Olorode et al. (2013). Menzel (1951) suggested that *P. peruviana* may possibly have more than one chromosome number. It has been reported that this species is stable and delimited in its place of origin which seemed to be tetraploid in that same population. The report of $2n=24$ for *P. micrantha* in this present study supports the findings of Wahua and Sam (2013).

The pollen morphology observed in this study is in agreement with previous report for the genus *Physalis* (Perveen and Qaizer, 2007). Remarkably, tetrad pollen grains were observed only in an accession of *P. micrantha* out of all the accessions of the four species investigated. *Physalis angulata*, a tetraploid, has the largest pollen grains. An increase in pollen diameter as ploidy level increases has been documented by many researchers in angiosperms (Venessa et al., 2004; Jones and

Reed, 2007; Zlesak, 2009). The increase in ploidy level in *Iris* was associated with a significant increase in the length of stomata guard cells and the pollen grain diameter when compared with *Iris* species with lower ploidy levels of chromosome (Ghasemi et al., 2014). The higher pollen size as well as wider guard cell area observed in *P. angulata* is as a result of gigas effects which are characteristics of polyploids. In addition, chromosome number and bivalent pairing in *P. angulata* showed that the species is a tetraploid with a diploid like behaviour ($2n=4x=48$).

Amphistomatic leaves were observed in all the four *Physalis* species in this study which is in agreement with the previous studies (Savulescu and Hoza, 2011 and Wahua and Sam, 2013). The stomata indices observed in this study were significantly higher than earlier reports. Anomocytic stomata observed in this study corroborates previous findings in *P. micrantha*, *P. peruviana* and *P. angulata* (Savulescu and Hoza, 2011; Thepsithar and Thongpukdee, 2013; Wahua and Sam, 2013). Simple uniseriate multicellular trichomes were observed in all the species studied, with the exception of the adaxial surface of *P. angulata* where unicellular uniseriate and simple unicellular glandular trichomes were observed. Wahua and Sam (2013) also reported uniseriate trichomes in these *Physalis* species. Differences in the width of stomata guard cells recorded on adaxial and abaxial surfaces of *Physalis* species in this present study have also been documented in some species of *Corchorus* studied by Maity and Datta (2011).

Physalis angulata has the highest guard cell area. Jones and Reed (2007) observed that stomatal guard lengths in *Hydragea macrophylla* ssp. *macrophylla* diploid cultivars were lesser than those of triploid cultivars. This study showed that *P. angulata* which is characterized by the largest guard cell area has the highest Stomata Index. According to khazaei et al. (2010), higher

stomatal frequency as well as lower stomata length and width were observed in diploid wheat. However, as the ploidy level increases, the stomatal frequency decreases while the stomatal size increases. In addition, the same trend was noted by Venkatesh et al. (2014) in two mulberry varieties. Also, Wang and Lei (2012), reported significantly larger stomata size and lower stomata density in tetraploids when compared with the diploids in *Clivia miniata* Regel. According to the findings of Hansen et al. (2007), polyploidy occasionally results in an increase in plant size which is probably due to reduction in the number of cell divisions during development. Beaulieu et al. (2008) reported a close relationship between genome size and guard cell length as well as epidermal cell area; while inverse relationship was reported between genome size and stomata density. This shows that the traits are undergoing correlated evolution with the genome size and stomata density in the angiosperm (Beaulieu et al., 2008). Joachimiak and Grabowska-Joachimiak (2000) stated that the measurement of the stomata length is useful for proper taxonomic identification as well as determining the ploidy level in morphologically non distinct species belonging to closely related pairs of taxa in *Phleum*.

Conclusion

The relationship in chromosome counts, ploidy level, pollen grain diameter and stomatal guard cell area among four *Physalis* species in Nigeria has been investigated. It was confirmed in this present study that *P. angulata* is a tetraploid with $2n=4x=48$ while *P. micrantha*, *P. pubescens* and *P. peruviana* are diploids with $2n=2x=24$. Tetrad pollen grains were observed in an accession of *P. micrantha*. The pollen grain diameter and guard cell were significantly higher in the tetraploid when compared with the diploid species. It can therefore be concluded that the tetraploid nature of *P. angulata* is responsible for the highest pollen diameter as well as largest guard cell area observed in this study.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

The first author carried out pollen grain and foliar epidermal studies. The idea of the work came from second author, supervised by him and he took all the photomicrographs in the manuscript. The chromosome study aspect of the work was jointly carried out by all the authors and they jointly prepared the manuscript.

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