

# Hybridization in Four Nigerian *Physalis* (Linn.) Species

Sekinat Okikiola AZEEZ\*, Julius Olaoye FALUYI

Obafemi Awolowo University, Faculty of Science, Department of Botany,  
Nigeria; [sekinatokiki@gmail.com](mailto:sekinatokiki@gmail.com) (\*corresponding author); [jfaluyi@gmail.com](mailto:jfaluyi@gmail.com)

## Abstract

Interspecific reciprocal crosses among four Nigerian *Physalis* species (*Physalis angulata*, *P. micrantha*, *P. pubescens* and *P. peruviana*) were carried out by transferring desired pollen grains to ovalate parents, bagged and labelled to prevent contamination by external pollen grains and for easy identification. Pollen cells of the F<sub>1</sub> hybrid combination from the only viable cross (*P. angulata* x *P. pubescens*) were studied using standard cytogenetic techniques. The F<sub>1</sub> hybrid obtained in the current investigation produced few flower buds and no matured fruit was harvested. Its average pollen grain diameter was found within the range of that of the two genitors and the pollen stainability was less than 50%. The hybrid was also characterized by meiotic irregularities. Based on the results obtained, the study concluded that *P. pubescens* is closely related to *P. angulata* and *P. peruviana*, while *P. micrantha* is more distant from them since the formal individuals were able to cross reciprocally among each other, though no matured fruit was produced except in one cross (*P. angulata* x *P. pubescens*).

**Keywords:** interspecific hybridization; meiotic irregularities; pollen grains

## Introduction

The genus *Physalis* Linn. species belongs to the night shade family called Solanaceae (Olorode, 1984; Jagatheeswari, 2014). This is a family of herbs, shrubs, rarely small trees, climbers, vines, epiphytes (Olorode, 1984). The genus includes about 100 species (Sultana *et al.*, 2008; El-Sheikha *et al.*, 2009) which were divided into three sections: *Physalis* (Euphysalis), *Microphysalis* and *Megista* (Sullivan, 1984). In West Africa, four species (*P. angulata* L., *P. micrantha* Link., *P. pubescens* L. and *P. peruviana* L.) were reported and all are present in Nigeria (Hutchinson and Dalziel, 1963; Olatunji, 1985). The genus constitutes a natural group of annual and perennial herbs characterised by solitary flowers borne on the axils of leaves and branches (Menzel, 1951; Olatunji, 1985; Shu, 1994).

Olorode *et al.* (2013) opined that *Physalis angulata* L. ( $2n = 48$ ) might be of hybrid origin from a cross between *P. peruviana* ( $2n = 24$ ) and *P. pubescens* ( $2n = 24$ ) based on their results from pollen grain, morphological and preliminary cytological studies. In addition, they observed that *P. angulata* has a wider latitudinal range of distribution with usually large populations, which is a characteristic of the distribution of successful hybrid genomes (Olorode *et al.*, 2013).

There is no report on artificial hybridization among Nigerian *Physalis* so far, hence this study. The objective of the study was therefore to carry out hybridization among

the four Nigerian *Physalis* species to elucidate the phylogenetic relationship that exists among them.

## Materials and Methods

### Germplasm collection and cultivation of *Physalis* species

Whole plants of all the *Physalis* species studied except *P. micrantha* were collected from different locations in South-Western Nigeria (Table 1 and Fig. 1). Accession numbers were given to the specimens, identified at the IFE Herbarium and voucher specimens were deposited. The whole plants collected were planted and nursed to maturity. Fruits of *P. micrantha* were also collected from different locations in South-Western Nigeria. The seeds were recovered from the fruits and planted separately. The seedlings were transplanted into 11-litre plastic buckets filled with top soil, in an experimental garden field beside the screen-house of the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria.

### Hybridization

Each of the four *Physalis* species was selfed before the hybridization was carried out, in order to determine their self-compatibility. Self-fertility test was carried out by randomly bagging twenty flower heads on plants of each species prior to the opening of the flowers.

Interspecific reciprocal crosses were carried out by physical emasculation of matured flower buds of each

ovulate genitor before the occurrence of self-pollination. The pollen grains were transferred to the ovulate parent, bagged and labelled to prevent contamination by external pollen grains and for easy identification.

The crosses were monitored for fruit production and seed set. The F<sub>1</sub> hybrid from the successful cross was raised along with its genitors and characterized for all morphological differences and similarities.

#### *Meiotic chromosome study*

Young flower buds from the F<sub>1</sub> hybrid were collected for meiotic chromosome study between 9.00 am and 12.00 pm,

when the cell activities were considered to be at the peak (Jackson, 1962) and stored in 1:3 Acetic acid:alcohol. The anthers were later removed, squashed and stained in FLP Orcein by the squash technique according to Lasebikan and Olorode (1972). The pollen cells were examined and good meiotic chromosome spreads were photographed at x1000 under BK series system microscope. The pollen stainability was carried out according to the method of Olorode and Baquar (1976) using Cotton Blue in Lactophenol.

Table 1. Germplasm sources used for the study

Accession No.	Location	Longitudes and Latitudes
PMR 1201	Open place isolated in fallowed farmland at the University of Abuja Permanent site, FCT, Abuja.	8° 57'24.21"N 7° 04'30.56"E
PMR 1202	Dump site at Odoje Ojubo on Ogbomoso-Ajawa Road, Ogbomoshosho, 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 the 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 1203	Fallow land around Music Department, OAU, Ile-Ife	7° 29'12.07"N 4° 29'35.42"E
PAG 1204	Along the road at Oranfe area, Ile-Ife	7° 35'25.84"N 4° 44'00.81"E
PPR 1205	Dump site around, Town Hall, Iloko-Ijesa	7° 39'08.48"N 4° 49'24.78"E
PPR 1206	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 1207	Around Music Department, OAU, Ile-Ife	7° 29'12.07"N 4° 29'35.42"E
PPB 1208	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*; PMC- *Physalis micrantha*



Fig. 1. Habits of *Physalis* species studied A. *P. angulata* (inset right is flower and fruit); Scale = 10 cm; B. *P. peruviana* (inset right is flower and fruit); Scale = 3 cm; C. *P. pubescens* (inset right is flower and fruit); Scale = 10 cm; D. *P. micrantha* (inset right is flower and fruit); Scale = 5 cm

## Results

All the four species studied were self-compatible. No natural hybrid was observed where *P. angulata*, *P. pubescens* and *P. peruviana* were growing sympatrically, whereas *P. micrantha* was observed to be growing allopatrically in ruderal and waste places. Of all the crosses that were carried out, including the reciprocal crosses (Table 2 and 3), no successful hybrid combination was obtained, except in one cross between *P. angulata* and *P. pubescens* (Fig. 2). Most of the fertilized ovaries were aborted few weeks after fertilization. Some fruiting calyces were enlarged with immature fruits which occasionally contained aborted seeds. A few full-sized seeds which failed to germinate were recovered in some fruits.

Some matured fruits with full-sized seeds were harvested. These seeds germinated; however, the F<sub>1</sub> hybrid plants showed no influence of pollen donor. From *P.*

*angulata* x *P. pubescens* (tetraploid x diploid), 15 seeds were recovered and all germinated. However, only one plant showed hybrid characteristics. Few flower buds were developed on this F<sub>1</sub> hybrid plant and no matured fruit was harvested.

The pollen diameter of the hybrid plant (25.40-30.72 µm) fell within the range of that of the two genitors. The percentage pollen stainability of the F<sub>1</sub> hybrid plant was 43.25% which was about 50% less than that of the parents. The flower colour, as well as the flower orientation, took after the staminate parent (Table 4).

The pollen cells of the F<sub>1</sub> hybrid plant were characterized by meiotic irregularities (Fig. 3). The F<sub>1</sub> hybrid plant was a triploid, having chromosome number of  $2n = 36$ . The chromosomes were unequally distributed to the two poles at anaphase I; precocious chromosome movement and multiple laggards were observed at anaphase I and II. Unpaired chromosomes were also observed in some pollen mother cells.

Table 2. The crosses involving the four *Physalis* species studied, including the reciprocal crosses

♀ \ ♂	<i>P. peruviana</i>	<i>P. pubescens</i>	<i>P. angulata</i>	<i>P. micrantha</i>
<i>P. peruviana</i>	-	x	x	x
<i>P. pubescens</i>	x	-	x	x
<i>P. angulata</i>	x	x	-	x
<i>P. micrantha</i>	x	x	x	-

Table 3. The summary of the crosses in relation to the percentage of fertilized ovules and fruit set

Crosses	% Fertilized ovules	% Fruit set
<i>P. peruviana</i> x <i>P. pubescens</i>	28.9	21.1
<i>P. peruviana</i> x <i>P. angulata</i>	15.2	9.1
<i>P. peruviana</i> x <i>P. micrantha</i>	-	-
<i>P. pubescens</i> x <i>P. peruviana</i>	37.8	36.5
<i>P. pubescens</i> x <i>P. angulata</i>	18.0	18.0
<i>P. pubescens</i> x <i>P. micrantha</i>	9.1	-
<i>P. angulata</i> x <i>P. peruviana</i>	14.1	-
<i>P. angulata</i> x <i>P. pubescens</i>	17.8	6.7
<i>P. angulata</i> x <i>P. micrantha</i>	-	-
<i>P. micrantha</i> x <i>P. peruviana</i>	35.7	21.4
<i>P. micrantha</i> x <i>P. pubescens</i>	24.0	-
<i>P. micrantha</i> x <i>P. angulata</i>	22.7	-

Table 4. Characteristics of the F<sub>1</sub> hybrid plants and its genitors

Species \ Characters	<i>P. angulata</i>	<i>P. pubescens</i>	F <sub>1</sub> hybrid
Colour of corolla	cream with inner brown patches	yellow with inner brown patches	light-yellow with inner brown patches
Fruiting calyx pigmentation	pigmented along the veins on the fruiting calyx	no pigmentation	pigmented along the veins on the fruiting calyx
Leaf Venation	cladodromous	eucamptodromous	cladodromous
Leaf Texture	glabrous	coriaceous	coriaceous
Calyx Angles	10	5	10
Corolla Diameter (mm)	13.35	9.00	10.00
Leaf Area (cm <sup>2</sup> ) (Mean ± SE)	19.30 ± 2.01	15.64 ± 1.32	38.09 ± 9.51
Petiole Length (cm) (Mean ± SE)	4.53 ± 0.47	3.40 ± 0.25	6.30 ± 0.63
No. of secondary branches	21.00	32.00	28.00
No. of primary branches	2.00	6.00	2.00
Plant Height (cm)	68.30	48.08	45.00
Days to flower initiation after planting	44-46	39	40
Days spent to complete life cycle after planting*	117	114	102
Pollen stainability	89.3	89.6	43.3

\*The end of life cycle was taken when the leaves started drying up

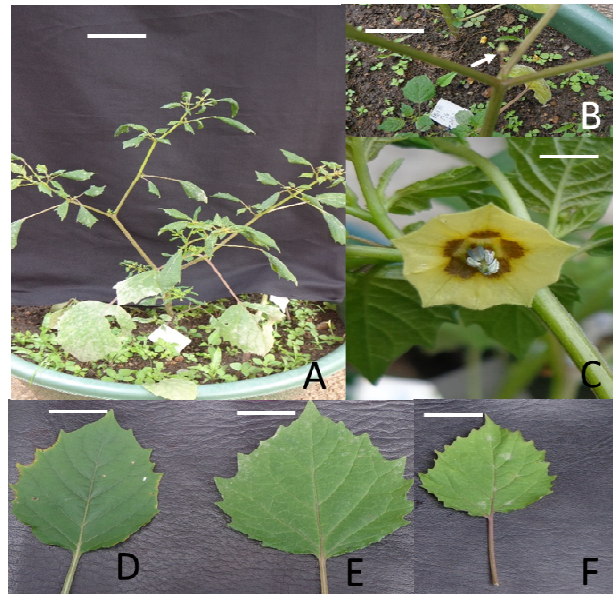


Fig. 2. F<sub>1</sub> Hybrid between *P. angulata* x *P. pubescens* A. The habit of the F<sub>1</sub> hybrid plant (Scale = 8 cm); B. Fertilized ovary which slightly enlarged and later dropped after few days (arrowed) (Scale = 3 cm); C. Flower of the hybrid plant (Scale = 0.25 cm); D. Leaf of *P. angulata*; E. Leaf of F<sub>1</sub> hybrid plant; F. Leaf of *P. pubescens* (all Scale = 2 cm)

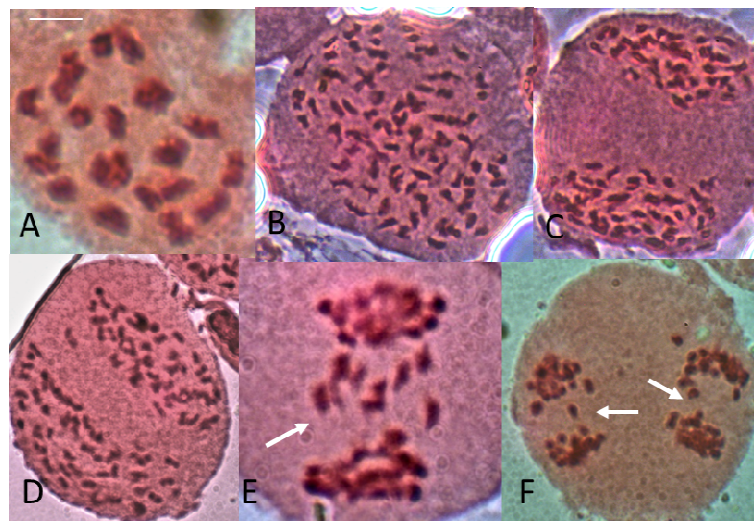


Fig. 3. Stages of cell division in *P. angulata* x *P. pubescens* F<sub>1</sub> hybrid (Scale = 1.8 μm); A. Metaphase I (showing 18 II); B. Complete univalent (36 I) in metaphase II; C. Unpaired chromosomes in anaphase I (no laggard); D. Unequal distribution of chromosome in anaphase I (the pole at the bottom has more chromosomes than at the one at the top); E. Multiple laggards in anaphase I (arrowed); F. Laggard chromosomes in anaphase II

## Discussion

It was observed from the present study that the species that were found growing sympatrically produced no natural hybrids. The lack of hybrids among closely-related sympatrically-distributed species showed that strong pre- and post-zygotic isolated mechanism has developed among these species and therefore prevented hybridization (Pringle and Murry, 1991; Pascarella, 2007). Fourteen out of fifteen seeds recovered from *P. angulata* and *P. pubescens* that germinated did not show the influence of pollen donor. Menzel (1951) obtained no hybrid with *P. angulata* as a seed parent and also reported that no species combination

resulted in both matroclinal and hybrid populations they investigated, which was in contrast to what was observed in the F<sub>1</sub> hybrid plants of *P. angulata* x *P. pubescens* in the present study.

In most crosses, heavy flower drop, early ovary abortion where fruiting calyces were slightly enlarged with or without ovary enlargement, as well as numerous aborted and few full-sized seeds, which failed to germinate, were observed. The unsuccessful hybridization recorded in the present study is similar to the report of Menzel (1951). Ganapathi *et al.* (1991) also recorded unsuccessful hybridization between *P. pubescens* and *P. peruviana*, and between *P. pubescens* and *P. angulata* including their reciprocal crosses.

Such a result can be attributed to the fact that these species belong to different sections; even intrasectional crosses were sometimes difficult, as it was observed between *P. pubescens* and *P. micrantha* in the present study. The difficulties encountered over these crosses might also be due to the well-established barriers to hybridization in annuals (Menzel, 1951).

The embryo abortion observed in the study might be as a result of early nutrition barrier reported by Geerts *et al.* (2002) in the crosses between *Phaseolus vulgaris* and *Phaseolus polyanthus*. Moreover, the lack of coordination in endosperm may result in imbalanced production of growth-relating substances which in turn cause embryo abortion (Abbo and Ladizinsky, 1994). On the other hand, there might be incompatibilities between the parental genomes at cellular level which might have distorted the normal nuclear and cellular activities in the hybrid cell. Also, the parental genome may not function properly within the maternal cytoplasm (Pringle and Murry, 1991).

From the current study, one of the pollen mother cells of the hybrid obtained from the cross between *P. angulata* and *P. pubescens* showed 18 bivalents, which was a rare event. This association can be explained by the fact that *P. pubescens* designated "BB" was probably one of the genitors of *P. angulata* designated "AB" (Olorode *et al.*, 2013). The hybrid might have undergone chromosome doubling resulting in AABB, restoring its fertility. Perhaps the restoration of fertility in *P. angulata* by polyploidization gave rise to regular segregation that was seen in *P. angulata*. By this same reasoning, the hybrid between *P. angulata* and *P. pubescens* was ABB. The two genitors shared similar genome, B known as pivotal genome (Kimber and Yen, 1988). Therefore, the homologous chromosomes in the B genome would have been able to pair, giving rise to 12 bivalents. The disruption in the meiotic mechanism governing bivalent pairing enabled the chromosomes of A genome to pair autosyndetically, producing 6 bivalents on a basic number of  $x = 6$ . This is supported by the idea of Stebbins (1971) saying that the basic chromosome number of 12 and above in some genera and families might have evolved through chromosome doubling from groups with lower numbers.

The irregularities observed in the course of meiosis in the pollen cells of the  $F_1$  hybrid must have been responsible for malformation observed in some of the pollen grains, as well as the low pollen stainability recorded in the  $F_1$  hybrid plant. The  $F_1$  hybrid plant obtained from the present study failed to set fruit. This was similar to the observation of Oziegbe and Faluyi (2011) in a hybrid obtained from tetraploid and diploid crosses in *Ludwigia*. This observation was attributed to genetically imbalanced gametes that originated from the parents of the  $F_1$  hybrid which eventually led to hybrid sterility observed (Oziegbe and Faluyi, 2011).

## Conclusions

It can be concluded from the present study that *P. pubescens* is closely related to *P. angulata* and *P. peruviana*, while *P. micrantha* is more distant from them. This is

because *P. pubescens* was able to cross reciprocally with both *P. angulata* and *P. peruviana* even though fruits were not set, except in the cross between *P. angulata* and *P. pubescens*.

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