CHARACTERISATION AND REPRODUCTIVE BIOLOGY OF FOUR *PHYSALIS* L. SPECIES FROM ILE-IFE, NIGERIA

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ABSTRACT

The nutritional, medicinal and economic values of many *Physalis* species have been established. However, some *Physalis* species in Nigeria have been used interchangeably or misidentified. Consequently, there is the need to re-affirm the taxonomic status of these species. Morphological characters and SDS-PAGE were employed to identify and reveal the genetic relationship that exits among the four *Physalis* species studied. Furthermore, pollen fertility, pollination and germination studies were carried out in order to compile relevant information on reproductive biology of these *Physalis* species. The results revealed that all the four species studied are annuals. *Physalis peruviana* has the longest life span. The mean height of the four species studied ranged from 48.08±6.11 to 97.20±6.51 cm with the highest recorded in *P. peruviana.* The morphological characters subjected to SLCA grouped *P. pubescens* and *P. peruviana* together and branched at higher levels of similarity (0.98). In addition, SDS-PAGE showed that *P. peruviana*, *P. angulata* and *P. pubescens* are distantly related to *P. micrantha*. High pollen stainability (80.6% - 92.6%) was recorded in all the *Physalis* species studied. Therefore, from this study, it can be concluded that all the four species studied are distinct species with a common origin and they are highly fertile.

Keywords: Morphology, Genetic Relationship, SDS-PAGE, Pollen grains, Trichomes, Germination

INTRODUCTION

The genus *Physalis* L. which has its centre of diversity in Mexico belongs to the Family Solanaceae. It comprises annuals and perennial herbs. The genus is distributed in both the tropical and temperate regions of the world. The generic boundary is clearly marked by persistent fruiting calyx with pulpy berry enclosed (Menzel, 1951; Hutchinson and Dalziel, 1963, Olorode, 1984). All the four *Physalis* species present in Nigeria are self-compatible. Three out of these are sometimes found growing sympatrically in Nigeria without the evidence of natural hybrid (Azeez and Faluyi, 2018).

Physalis angulata L. has chromosome number of 2n = 48 while 2n = 24 was reported for *P. micrantha* Link and *P. pubescens* L. Different chromosome numbers: 2n =24, 36 and 48 have been documented for *P. peruviana* (Menzel, 1951; Huisaini and Iwo, 1990; Burkill, 2000; Rodriguez and Bueno,

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2006; Olorode *et al.*, 2013; Azeez and Faluyi, 2019a; Azeez *et al.*, 2019). In Nigeria, *P. pubescens* and *P. micrantha* have been used interchangeably in the past. In addition, 2n = 24 recorded in *P. angulata* was considered to be a case of misidentification (Menzel, 1951; Olatunji, 1985, Olorode *et al*., 2013; Wahua and Sam, 2013).

Tricolporate, prolate or oblate spheroidal monad pollen grains are well known in the genus *Physalis* (Peerven and Qaizer, 2007; Azeez *et al*., 2019). A 3-zonocolporate monad has also been reported in Northeast of Indian species (Bhat *et al*., 2018). Besides, Azeez *et al*. (2019) described tetrad pollens from an accession of *P. micrantha* from Nigeria. Uniseriate, multiseriate, glandular and tectorial trichomes have been described by some researchers in the genus (Wahua and Sam, 2013; Rodriguez *et al*., 2014; Lea Ferreira, 2019). Many *Physalis* species have been acknowledged for their food and medicinal values. Moreover, some species in the genus can also serve as an alternative source of income for the farmers (Burkill, 2000; Oladele *et al*., 2013; Hassan and Ghoneim, 2013; Muniz *et al*., 2014; Bertoncelli *et al*., 2017; Bhat *et al*., 2018; Azeez and Faluyi, 2019b; Zimmer *et al*., 2019).

Morphological characters remain significant and relevant in systematics for delimiting plant species even though there has been a decline in the attention given to it in recent years (Schonenberger and Balthazar, 2012; Iroka *et al*., 2015). Additionally, trichome density, distribution and morphology are of immense taxonomic value and have played a good role in establishing relationship among the plant species (Prabhakar *et al.,* 1985; Khalik, 2005; Khokhar *et al.*, 2012; Talebi *et al.,* 2018). Morphological characters along with other methods such as sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), molecular markers etc. have been used effectively to delimit plant species as well as to reveal phylogenetic relationship that exists among the plant species (Mirali *et al.,* 2007; Schonenberger and Balthazar, 2012). The data from reproductive biology usually reveal the appropriate time and methods of planting and harvesting as well as pollination mechanism which can be employed in breeding programmes in order to improve the agronomic characters of interest in the concerned plant species (Muniz *et al*., 2014; Bertoncelli *et al.,* 2017, Figueiredo *et al*., 2020).

In view of the above and having recognised the nutritional, medicinal and economic values of *Physalis* species, it is, therefore, pertinent to characterise the four *Physalis* species and to investigate their reproductive biology in order to document necessary data that can be employed in the *Physalis* breeding programme in Nigeria. This study employed morphological characters, trichome density and morphology coupled with electrophoresis to identify and elucidate the genetic relationship that exists among the four *Physalis* species in Nigeria. Pollen stainability, pollination and germination studies were carried out so as to gather useful information on the reproductive biology of *Physalis* species under consideration.

MATERIALS AND METHODS

Plant Material Collection

The study was carried out at the experimental site of Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria. The seeds were collected from four *Physalis* species at different places within Ile-Ife, Nigeria (7º 35’ 25.84” N, 4º 44’ 00.81” E).

Morphological Studies

Qualitative and quantitative characters were observed and measured for each *Physalis* species studied. The characters studied include habit, longevity, leaf, stem, flower and fruit. The leaf area was estimated using the graph method.

Reproductive Biology

Pollen fertility was estimated from pollen stainability according to standard method (Olorode and Baquar, 1976). The time of anthesis and anther dehiscence were also monitored. Pollination as well as potential pollinators were monitored.

Germination studies

One hundred fresh and dried seeds of each *Physalis* species were planted separately in plastic bowls. Planted seeds were checked every day for seedling emergence. Percentage seedling emergence over a period of 30 days was calculated. After germination studies, some seedlings were transplanted into 11-litre buckets at the experimental site. Then, days from the emergence of the seedling to the first flowering as well as days to complete the life cycle when the leaves started drying up were documented.

Leaf and Seed Protein Electrophoresis

The proteins of the blended leaves and seeds of four *Physalis* species that were studied were extracted by separation technique. The supernatant obtained was boiled at 100 ºC for 3-4 minutes and electrophoresis was then run according to Weber and Osborn (1975). Sokal and Sneath similarity coefficient (1963) was calculated as follows:

$$Similarity\ coefficient = \frac{a}{a+b+c} \times 100$$

Where,

a is the number of common bands in a pair of species

b is the number of unique bands in one of the pairs of species

c is the number of unique bands in the other pair of species

Epidermal Studies

The epidermal peels from the species studied were obtained and stained according to the method of Ibrahim and Ayodele (2013). The trichome types were documented and trichome index (T.I) was calculated according to the method of Wahua and Sam (2013) as follows:

$$Trichome\ Index\ (T.I) = T\ /\ (T+E) \times 100$$

Where,

T is the number of trichomes per unit area

E is the number of epidermal and subsidiary cells in the same unit area

Statistical Analysis

The means and standard errors were calculated for quantitative that were collected and one-way ANOVA was used where necessary using Statistical Package for Social Sciences (SPSS). Then qualitative data were coded and used along with quantitative data in Single Linkage Cluster Analysis.

RESULTS

Morphological Studies

All the four *Physalis* species studied were observed to be growing in open places, dump sites and ruderal areas. *Physalis angulata* and *P. peruviana* are erect while *P. pubescens* and *P. micrantha* may either be erect or prostrate depending on whether they are growing among other plants or in the open (Figure1). All the species studied are annuals. The mean height of the four *Physalis* species studied ranged between 48.08±6.11 and 97.20±6.51cm. *Physalis peruviana* is the tallest among the four species studied and is the only one with terete stem. The flowers are solitary and hang down with the exception of *P. angulata* where the flowers are solitary but face up. Each flower possesses five epipetalous anthers. The fruit shape is round to ovate. The seeds are reniform in shape (Figure 2a). Slimy substance was observed on the freshly harvested seeds. The highest and lowest number of seeds/fruit was documented in *P. peruviana* (186.00 ±14.11) and *P. micrantha* (30.00±1.61), respectively; seed diameter ranged from 1.4 mm to 1.6 mm. Superficial placentation, a multilocular ovary with whole inner wall of ovary lined with placenta was observed in all the four species studied (Figure 2b, Tables 1-3).

The Single Linkage Analysis of both quantitative and qualitative attributes grouped the species studied into two main clusters. The first cluster branched at 0.83; it was made up of *P. micrantha* and *P. angulata*. The second cluster which comprises *P. pubescens* and *P. peruviana* branched at 0.98. The latter cluster showed a higher level of similarity (Figure 3).

Figure 1: Habits of *Physalis* species studied.
A. *Physalis angulata* B. *Physalis peruviana* C. *Physalis*

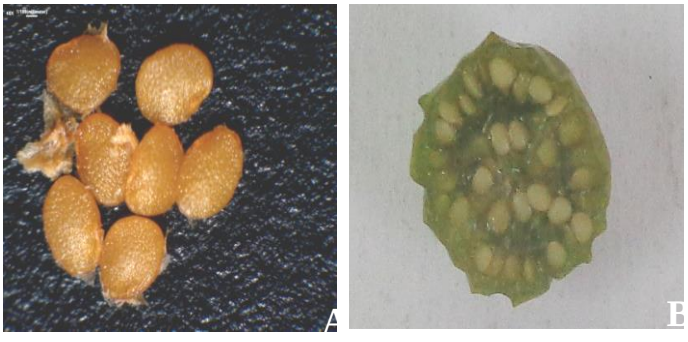

Figure 2: Seed Morphology and Plancentation
A. Seeds of *Physalis* species
B. Superficial Plancentation in the *Physalis* species

Table 1: Floral Characteristics of the four *Physalis* species studied

Species Character	*P. angulata*	*P. pubescens*	*P. micrantha*	*P. peruviana*
Colour of corolla	cream with inner brown patches	yellow with inner brown patches	cream	yellow
Colour of anther	grey	Grey	cream	light yellow
Colour of filament	purplish	Whitish	whitish	whitish
Colour of style	green	Green	green	green
Colour of stigma	green	Green	green	green
Colour of calyx	green with purple stripes	Green	green	Green
Colour of seed	cream	Golden	cream	golden
Colour of pedicel	purple	Green	green	green
Fruit colour at maturity	greenish yellow to yellow	greenish purple to purple	greenish purple to purple	greenish purple to yellow
Fruit Shape	round	Ovate	round to ovate	ovate
Anther Attachment	basifixed	Basifixed	basifixed	basifixed
Corolla Pubescence	slightly pubescent around the inner brown patches only	both inner and outer parts are pubescent	inner is pubescent and outer part is slightly pubescent	both inner nd outer parts are highly pubescent
Calyx Pubescence	slightly pubescent	Pubescent	pubescent	pubescent
Pedicel Pubescence	glabrous	Pubescent	pubescent	pubescent
Stigma Pubescence	glabrous	Glabrous	glabrous	glabrous
Style Pubescence	glabrous	Glabrous	glabrous	glabrous
Ovary Pubescence	glabrous	Glabrous	glabrous	glabrous
Anther Pubescence	glabrous	Glabrous	glabrous	glabrous
Stigma Type	simple	Simple	simple	simple
Style Type	simple	Simple	simple	simple
Placentation	superficial	Superficial	superficial	superficial
Ovary Type	perigynous	Perigynous	perigynous	perigynous
Fruit Calyx	glabrous	Glabrous	pubescent	slightly pubescent
Fruiting calyx pigmentation	pigmented along the veins on the fruiting calyx	no pigmentation	no pigmentation	no pigmentation

Table 2: Vegetative Characteristics of the Four *Physalis* Species Studied

Species Characters	*P. angulata*	*P. pubescens*	*P. micrantha*	*P. peruviana*
Leaf Venation	cladodromous	eucamptodromous	brochidodromous	brochidodromous
Leaf Base	oblique	Oblique	oblique	oblique
Leaf Margin	Entire to serrated	Serrated	entire	entire
Leaf Apex	acuminate	Acuminate	acuminate	acuminate
Leaf Shape	ovate	Ovate	ovate	ovate
Leaf Arrangement	opposite	Opposite	opposite	opposite
Leaf Pubescence	glabrous	slightly pubescent	pubescent	slightly pubescent
Growth Pattern	indeterminate	Indeterminate	indeterminate	indeterminate
Branching Angle	acute	Acute	acute	acute
Life Form	herb	Herb	herb	herb
Longevity	annual	Annual	annual	annual
Plant Habit	erect	erect to prostrate	erect to prostrate	erect
Stem Shape	angled	Angled	angled	terete
Stem	glabrous	slightly pubescent	highly pubescent	pubescent
Stem Pigmentation	purple	purple	purple on the nodes/entire stem in some	purple

Table 3: Quantitative Characters of the Four *Physalis* Species Studied

Species Characters	*P. angulata*	*P. pubescens*	*P. micrantha*	*P. peruviana*
Number of anthers	5	5	5	5
Number of angles in fruiting calyx	10	5	10	5
Seeds/fruit	*122.00±6.95^{b}	102.00±11.44^{c}	30.00±1.61^{d}	186.00±14.11^{a}
Corolla length (mm)	9.25±0.36^{a}	8.40±0.25^{a}	6.25±0.25^{b}	8.90±0.25^{a}
Corolla diameter (mm)	13.35±0.51^{a}	9.00±0.32^{b}	4.63±0.18^{c}	9.20±0.37^{b}
Pedicel length (mm)	6.33±0.62^{a}	4.40±0.25^{b}	2.69±0.30^{c}	4.80±0.37^{b}
Stem diameter (cm)	2.67±0.33^{b}	2.40±0.26^{b}	2.50±0.22^{b}	3.38±0.24^{a}
Seed diameter (mm)	1.5±0.00^{b}	1.6±0.00^{a}	1.5±0.00^{b}	1.4±0.00^{c}
Leaf area (cm^2)	19.30±2.01^{b}	15.64±1.32^{c}	2.07±0.19^{d}	44.87±4.79^{a}
Petiole length (cm)	4.53±0.47^{b}	3.40±0.25^{c}	1.07±0.11^{d}	5.73±0.59^{a}
Number of secondary branches	21.00±2.40^{c}	32.00±4.38^{b}	54.00±5.85^{a}	33.00±2.55^{b}
Number of primary branches	2.00±0.00^{b}	6.00±0.34^{a}	6.00±0.40^{a}	2.00±0.00^{b}
Plant height (cm)	68.30±7.42^{b}	48.08±6.11^{c}	58.25±5.73^{c}	97.20±6.51^{a}
Fruit weight (g)	12.09±0.01^{a}	9.30±0.01^{c}	1.57±0.15^{d}	10.66±0.00^{b}
Fruit diameter (cm)	1.99±0.12^{a}	1.81±0.08^{b}	0.98±0.05^{c}	1.93±0.10^{a}

Means within each row with different superscripts are significantly different ($P< 0.05$)

***** Mean ± Standard Error

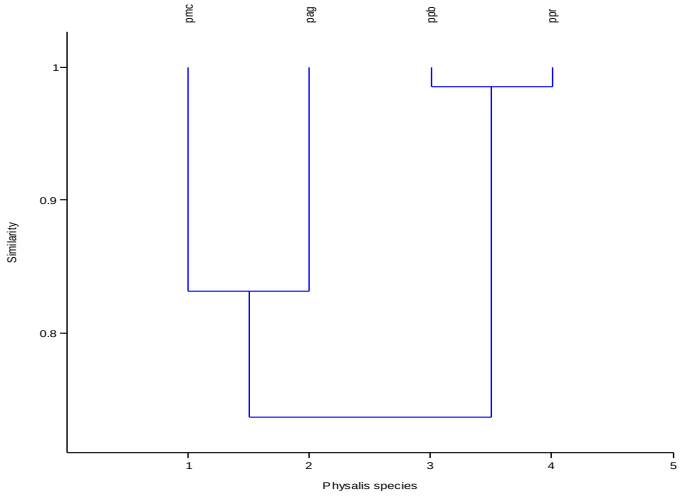

Figure 3: Dendrogram of Morphological Characters in the *Physalis* Species Studied Using Single Linkage Cluster Analysis
Key: Pag-*P. angulata*; Ppr- *P. peruviana*; Ppb- *P. pubescens*; Pmc- *P. micrantha*

Reproductive Biology of *Physalis*

Pollination in *Physalis* species

The flowers of each *Physalis* species studied open between 8 am and 11.30 am. The anther dehiscence starts around 8:30 am. The dehiscence of all the five anthers takes about 2-3 days after anthesis. The anthers slit longitudinally to release the pollen grains. It was observed that before anthesis occurs, the anthers are usually at a lower level to the stigma. The anthers then gradually grow towards the stigma at different rates for pollination to occur in all the species studied (Figure 4). All the four *Physalis* species were observed to be self-pollinating. There was no specific pollinator noticed around the four *Physalis* species.

Pollen Stainability

Generally, the percentage pollen stainability in the species studied was high, ranging from 80.6 to 92.6 %. The highest and lowest percentages were observed in *P. peruviana* and *P. micrantha*, respectively (Table 4).

Germination Studies

The *Physalis* species were observed to be growing in the field between April and October during rainy season. It was noted that fresh seeds failed to germinate; in contrast, those that were well dried germinated in all the species studied. *Physalis angulata* showed the highest germination percentage while the least was

observed in *P. peruviana*. *Physalis micrantha* flowered within 26-28 days after planting. However, flowering was initiated in *P. peruviana* between 60-62 days after planting. The flowering and fruiting occur simultaneously. *P. angulata*, *P. pubescens* and *P. micrantha* spent approximately 4 months to complete their life cycles. *Physalis peruviana* spent a longer period (average of 139 days) (Table 4).

Figure 4: Pollination in *P. angulata* (Typical for all studied *Physalis* species studied)
A. Before anther dehiscence
B. After anther dehiscence and pollination had occurred

Table 4: The Germination Parameters and Pollen viability of the Four *Physalis* Species Studied

Species Parameters	*Physalis angulata*	*Physalis pubescens*	*Physalis micrantha*	*Physalis peruviana*
Percentage seed germination for period of 30 days	89.00	79.00	73.00	25.00
Days to seedling emergence	6	6	5	9
Days to initiation of flowering*	44-46	39	26-28	60-62
Number of days spent to complete life cycle*	117	114	119	139
Percentage Pollen stainability	92.30	80.60	89.90	89.30

* After planting

Epidermal Studies

Simple uniseriate, multicellular trichomes were observed in all the *Physalis* species studied except in adaxial surface of *P. angulata*. In addition, multicellular glandular trichomes were observed in abaxial and adaxial surfaces of *P. micrantha* and *P. pubescens*, respectively, while multicellular glandular trichomes were observed on the abaxial surface of *P. peruviana* and abaxial and adaxial surfaces of *P. pubescens* and *P. angulata* (Figure 5). Both multicellular glandular and unicellular uniseriate trichomes were observed on the adaxial surface of *P. angulata*. Unicellular and bicellular uniseriate trichomes were found on both the leaf adaxial and abaxial surfaces of *P. pubescens*. Peltate scale was observed on the adaxial surface of *P. pubescens* only. The highest trichome index (0.11) was obsrved in *P. peruviana* and a significant difference in the trichome indices was observed across the species (Table 5)

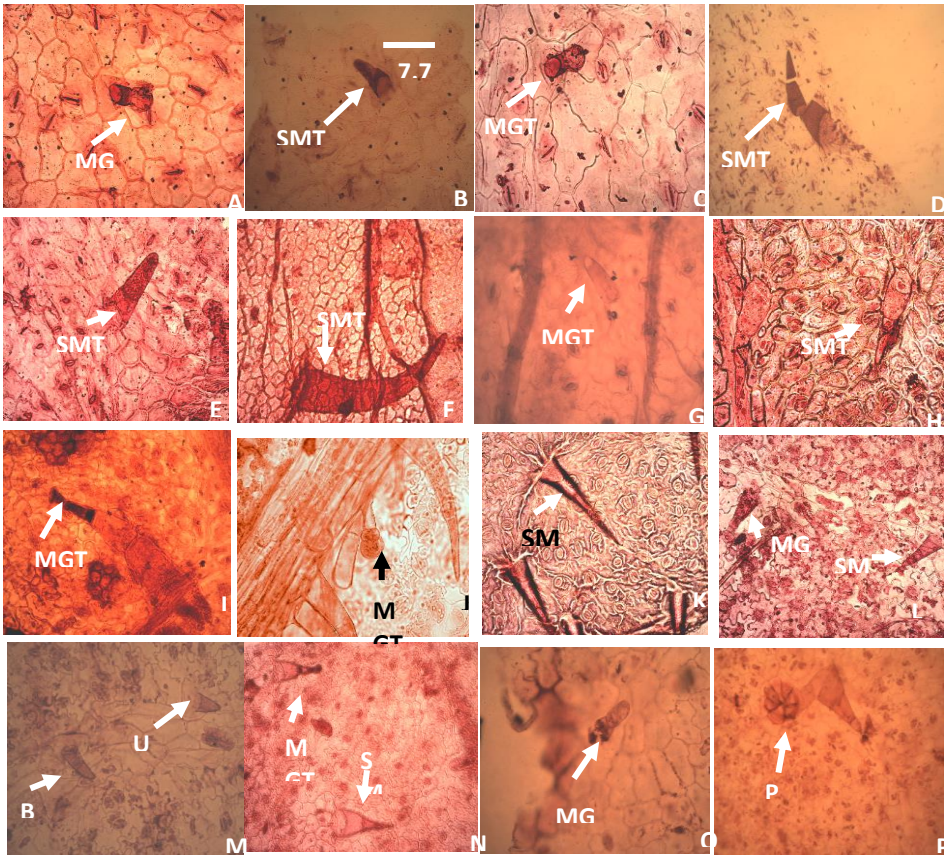

Figure 5: Trichome Morphology in the *Physalis* species studied
A-D: *P. angulata* ; E-G: *P. micrantha*; H-K: *P. peruviana*; L-P: *P. pubescens*
MGT: Multicellular Glandular Trichome
SMT: Simple Multicellular Trichome
UT: Unicellular Trichome
BT: Bicellular Trichome
PS: Peltate Scale

Table 5: Trichome Density and Morphology of Four *Physalis* Species Studied

Species Surface	Trichome Density	Trichome Type
P. micrantha Adaxial	0.06[c]	Simple uniseriate multicellular
P. micrantha Abaxial	0.02[f]	Simple uniseriate multicellular/ multicellular glandular
P. pubescens Adaxial	0.04[d]	Unicellular/ Simple multicellular/multicellular glandular/peltate scale
P. pubescens Abaxial	0.04[d]	Unicellular/ bicellular/Simple uniseriate multicellular/ multicellular glandular trichome
P. peruviana Adaxial	0.08[b]	Simple uniseriate multicellular
P. peruviana Abaxial	0.11[a]	Simple uniseriate multicellular/ multicellular glandular
P. angulata Adaxial	0.03[e]	Unicellular/multicellular glandular
P. angulata Abaxial	0.03[e]	Unicellular/simple uniseriate multicellular/multicellular glandular

Means within each row with different superscripts are significantly different ($p < 0.05$)

Electrophoresis

Leaf Protein Electrophoresis

A total of 61 protein bands were observed in the leaf crude protein profiles of four *Physalis* species. In these species, the bands varied in number and intensities with the slow bands accounting for 24.9% of the total bands, 40.9% of the bands were intermediate bands while 34.4% were fast bands. Four bands (2.1, 4.5, 5.5 and 5.8) were common to all the four *Physalis* species, though the degrees of intensities varied (Figures 6 and 7). These bands are known as generic bands. Interspecific bands as well as unique bands were also observed among the species studied.

A low level of similarity was observed in the leaf protein profiles of the four *Physalis* species studied. The highest level of similarity was observed between *P. peruviana* and *P. angulata* (36%) and lowest between *P. angulata* and *P. micrantha* (28%) (Tables 6 and 8). The Single Linkage Cluster Analysis of the leaf protein profiles showed that the four *Physalis* species were separated into two main clusters. The first main cluster branched at 0.982 which consists of *P. angulata* and *P. peruviana* while the second main cluster branched at 0.983. It includes *P. pubescens* and *P. micrantha* (Figure 8).

Seed Protein Electrophoresis

The seed crude protein profile of the four *Physalis* species showed a total of 49 bands of varied intensities and numbers, out of which only 10.2% accounted for the slow bands. The intermediate bands showed the highest percentage (Figures 6 and 7). The generic bands were observed at 3.6, 3.8 and 5.8. Interspecific bands and unique bands were additionally documented among the species studied. The level of similarity among these species was very low ranging between 20% and 38% (Tables 7 and 9). The Single Linkage Cluster Analysis showed that two main clusters existed. The first main cluster separated into two sub-clusters. The first sub-cluster branched at 0.970 and consists of only *P. pubescens* whereas the second sub-cluster which branched at 0.971 includes *P. angulata* and *P. peruviana*. The second main cluster separated *P. micrantha* from the rest at 0.940 (Figure 9).

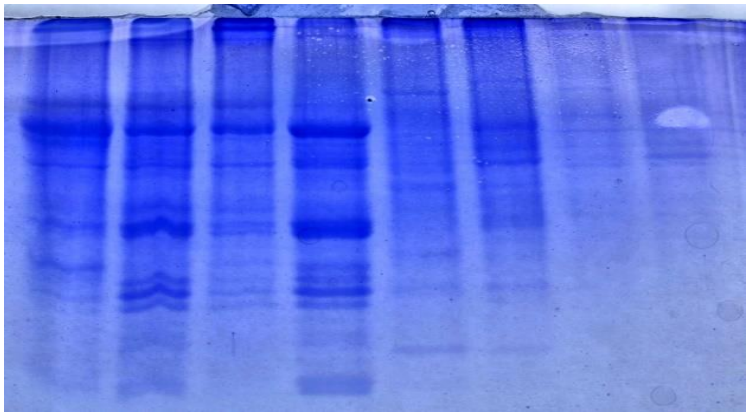

Figure 6: The Leaf and Seed Protein Profiles of the Four *Physalis* Species Studied

A *Physalis micrantha* Leaf

B *Physalis pubescens* Leaf

C *Physalis angulata* Leaf

D *Physalis peruviana* Leaf

E *Physalis micrantha* Seed

F *Physalis pubescens* Seed

G *Physalis angulata* Seed

H *Physalis peruviana* Seed

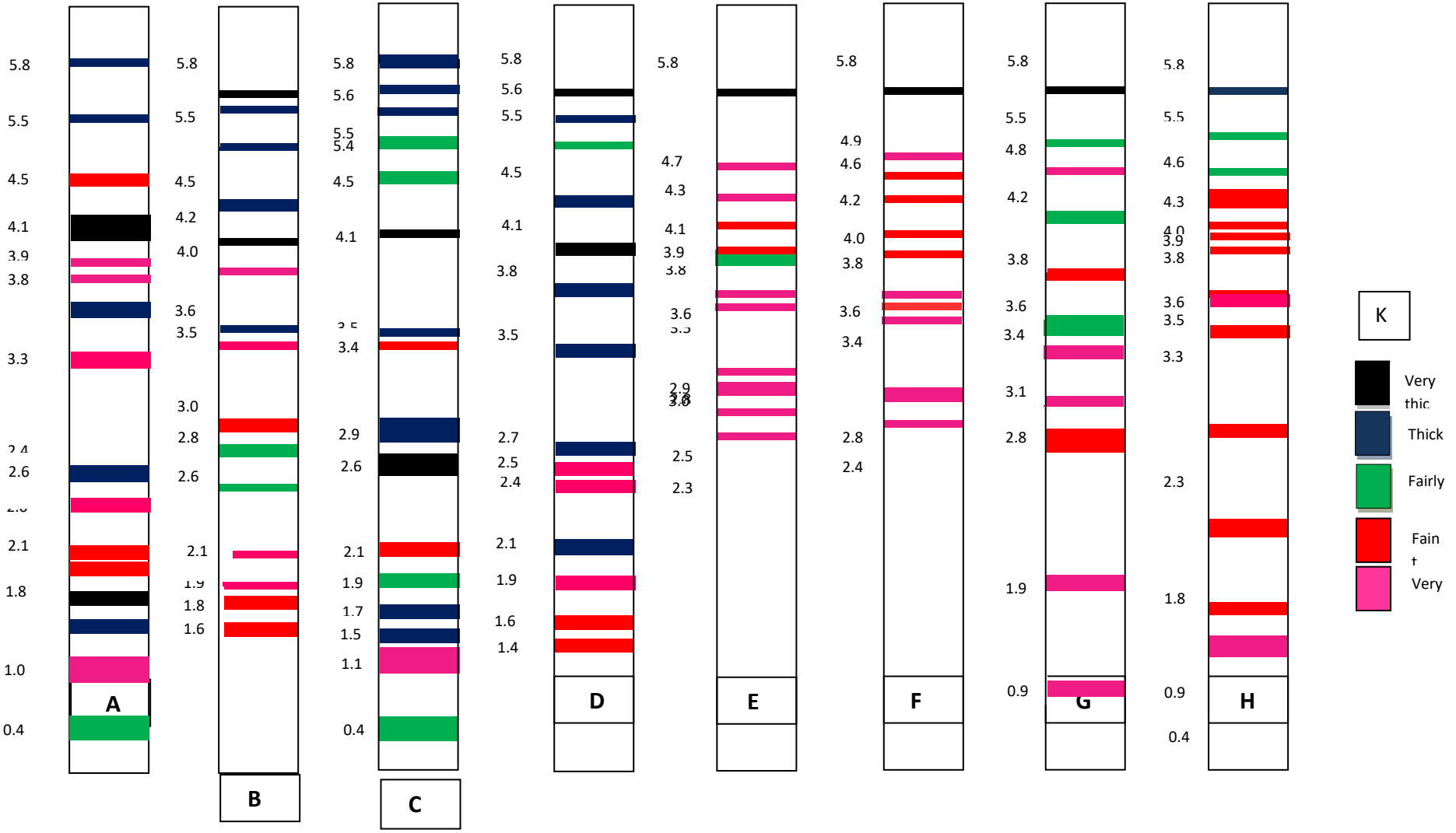

Figure 7: Schematic Diagrams of Leaf and Seed Protein Profiles of the Four *Physalis* Species Studied

Table 6: Leaf Protein Profiles of the *Physalis* Species Studied

Species	Slow bands (0-1.9 cm)	Intermediate bands (2.0-3.9 cm)	Fast bands (4.0-5.8 cm)	TNB*	Unique bands
P. micrantha	4	8	4	16	4
P. pubescens	3	6	6	15	5
P. angulata	5	5	6	16	6
P. peruviana	3	6	5	14	3

* TNB- Total Number of Bands

Table 7: Seed Protein Profiles of the *Physalis* Species Studied

Species	Slow bands (0-1.9 cm)	Intermediate bands (2.0-3.9 cm)	Fast bands (4.0-5.8 cm)	TNB	Unique bands
P. micrantha	-	9	4	13	5
P. pubescens	-	6	5	11	2
P. angulata	2	5	4	11	3
P. peruviana	3	6	5	14	3

* TNB- Total Number of Band

Table 8: Sokal and Sneath Similarity Coefficient among the *Physalis* Species Studied in the Leaf Protein Profiles

Species	*P. micrantha*	*P. pubescens*	*P. angulata*	*P. peruviana*
P. micrantha	-	-	-	-
P. pubescens	0.35	-	-	-
P. angulata	0.28	0.29	-	-
P. peruviana	0.33	0.33	0.36	-

Table 9: Sokal and Sneath Similarity Coefficient among the *Physalis* Species Studied in the Seed Protein Profiles

Species	*P. micrantha*	*P. pubescens*	*P. angulata*	*P. peruviana*
P. micrantha	-	-	-	-
P. pubescens	0.26	-	-	-
P. angulata	0.20	0.38	-	-
P. peruviana	0.36	0.33	0.25	-

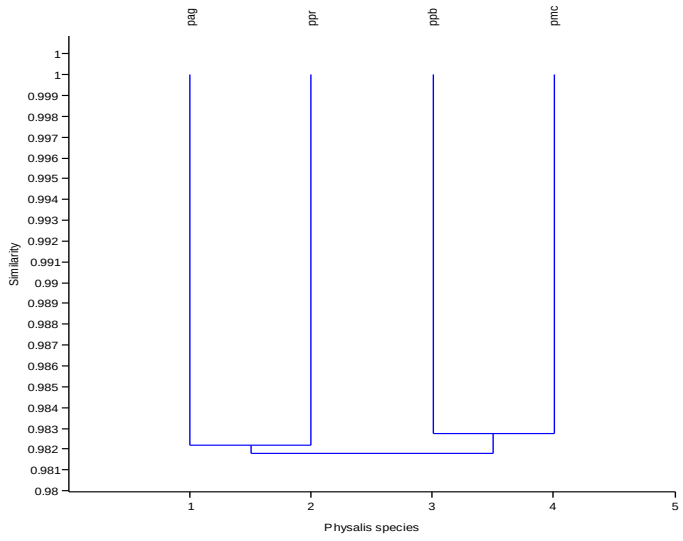

Figure8. Dendrogram of Leaf Protein Profiles of *Physalis* Species Studied Using Single Linkage Cluster Analysis

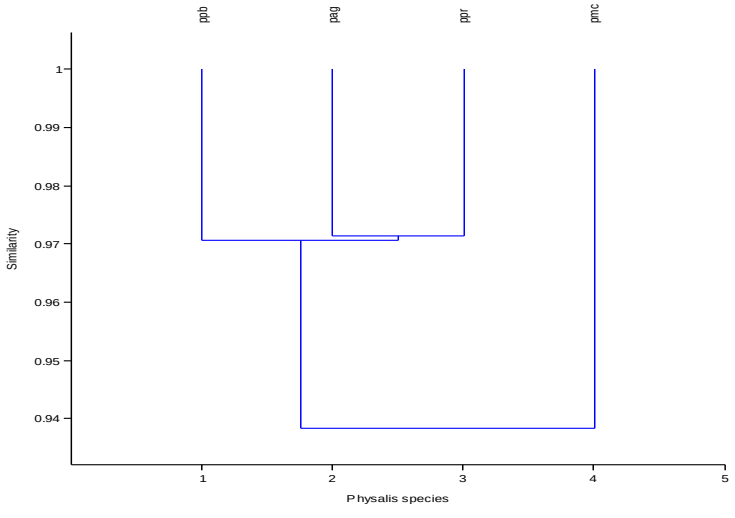

Figure 9: Dendrogram of Seed Protein Profiles of *Physalis* Species Studied Using Single Linkage Cluster Analysis

DISCUSSION

It has been reported by previous workers (Hutchinson and Dalziel, 1963; Olatunji, 1985 and Burkill, 2000) that *P. peruviana* is a perennial herb. However, based on this study, it was observed that in Ile-Ife, Nigeria, *P. peruviana* is an annual herb which completes its life cycle within 5 months. Similarly, Olorode *et al*. (2013) documented *P. peruviana* as annual in Nigeria. In agreement with the previous studies, the other three species studied are annuals (Hutchinson and Dalziel, 1963; Olatunji, 1985 and Burkill, 2000, Olorode *et al*., 2013). There is probability that both annual and perennial varieties of *P. peruviana* exist with different chromosome numbers of 2n=24 and 2n=48, respectively. The report of 2n=36 for *P. peruviana* by some researchers (Rodriguez and Bueno, 2006) might be a natural hybrid between the annual and perennial varieties of the species.

The flower colour of each species studied is diagnostic. The seed diameter for the *Physalis* species studied was between 1.4 mm to 1.6 mm though Martinez (1998) reported 0.6 mm to 3.0 mm for the species she studied. The seed shape as well as the anther attachment observed in this study was in agreement with the observation of Martinez (1998). The flower orientation was the same in all the species studied except in *P. angulata* where the flowers were erect, whereas the flowers hung down in the other three species studied. Olorode *et al*. (2013) observed erect flowers in *P. angulata* and *P. peruviana* while in *P. micrantha* and some accessions of *P. angulata*, reflex flowers were observed.

Some characters such as seed shape and inflated fruiting calyx were common to all the species studied. The inflated fruiting calyx has been described as a syndrome in the genus by Hu and Saedler (2007). This indicated that they are from the same origin as shown by Single Linkage Cluster Analysis. This observation corroborates the findings of Oyelakin and Ayodele (2013) who reported that when some species from the same genus have certain characters in common, it suggests that they have a kind of phylogenetic relationship. In spite of all the characters shared by these species, the leaf shape and size, flower colour and size as well as the number of angles in the fruiting calyx still remain diagnostic features that can be used to separate these species from one another.

The slimy substance that covered the fresh seeds might have hindered germination of the seeds when fresh. Probably, it is a kind of dormancy strategy. *Physalis angulata* which has the highest germination percentage was also the most predominant among the four species studied. Similarly, Olorode *et al*. (2013) reported that *P. angulata* has a wide range of distribution with large populations.

The occurrence of facultative autogamy cannot be ruled out in the species studied even though they are self-compatible (Azeez and Faluyi, 2018), the anther dehiscence took 2-5 days in the *Physalis* species as observed in this study and reported by Menzel (1951). The pollen fertility recorded in this study (80.6-92.3%) is very similar to what was reported (85-98%) by Sullivan (1984) in *P. viscosa* complex. It was noted that no pollinators were seen on and/or around the *Physalis* species studied; probably the pollinators have preference for the *Ocimum* species growing in large numbers very close to the experimental site. However, solitary bees had been identified by Sullivan (1984) as pollinators in the genus which were reported to visit *P. viscosa* for nectar and pollen.

Simple uniseriate multicellular non-glandular and multicellular glandular trichomes were observed in all the species studied. Wahua and Sam (2013) also reported uniseriate trichomes in these *Physalis* species. Non-glandular trichomes have been reported to complement the works of glandular trichomes which are for production and storage and/or secretion of biological active substances in addition to their physical protection

functions in plants (Tozin *et al*., 2016; Lea Ferreira *et al*., 2019). Therefore, the trichomes observed in the four *Physalis* species studied must have been relevant in the storage of various secondary metabolites documented by Azeez and Faluyi (2019b) in these *Physalis* species.

It was observed that the number of bands in the leaf crude protein profiles of all the four *Physalis* species studied was higher than that of seed crude protein profiles. This is in agreement with the findings of Adenegan (2014) in four *Basella* forms that were analysed. On the contrary, Olatunji and Morakinyo (2015) reported higher number of protein bands in the seed protein profiles than that of the leaves in varieties of *Capsicum annum* and *Capsicum frutescens*. Many researchers had successfully used seed crude protein profiling for identification of varieties of plants. Also, seed protein profiling has been used to show the occurrence of genetic diversity among different species (Rogl *et a*l., 1996; Mustafa *et al*., 2006; Khoshroo, 2011; George *et al*., 2013; Omonhinmim and Ogunbodede, 2013; Alege *et al.,* 2014 and Swapan *et al.,* 2015). Available evidence suggests that seeds are highly physiologically stable and species specific because they are direct product of genes and they may serve as markers of these genes. Hence, the difference observed in protein profiles is thought to be proportional to genetic diversities that exist among the species that were compared. Also, the seed protein phenotype was not influenced by the environmental changes (Rogl *et al*., 1996).

Remarkably, this study demonstrated that no two species have the same protein profiles and that each of the species possesses unique protein bands. The presence of unique bands in each of these species is an evidence of speciation which might have resulted from genetic reorganization of the gene pool, eventually leading to development of new species. Furthermore, the presence of a specific band at the same position on the gel across all the species studied suggests that the gene encoding the enzyme at that band is the same. In addition, Oladipo and Illoh (2012) in their study on *Jatropha* observed that no two species showed completely the same protein profiles and subsequently concluded that variation in the combination of protein bands at the anode is taxon-specific. Moreover, the generic bands in the four *Physalis* species studied suggest that they are likely to have evolved from a common ancestor. The shared protein bands by different species in the same cytological sub-group are thought to have been inherited from the same ancestor of the whole group (Gottlieb, 1971).

The low level of similarity that was observed in both the leaf and seed protein band profiles of the four *Physalis* species studied suggests that a high level of genetic diversity exists among them. Azeez and Morakinyo (2004) reported that the degree of variation in the protein bands observed in *Lycopersicon* and *Trichosanthes* species could be interpreted as a measure of genetic divergence. Also, Atoyebi *et al.* (2014) suggested that diversity of protein bands is an indication of genetic diversity. The cluster analysis from both the leaf and seed protein profiles grouped *P. angulata* and *P. peruviana* together, thus indicating that these two species are very close genetically. It is, therefore, not surprising that the *P. pubescens* and *P. micrantha* were clustered by leaf protein profiles because they belong to the same section, though seed protein profiles suggested that they are distantly related to each other. Furthermore, seed protein profiles indicated that *P. angulata*, *P. pubescens* and *P. peruviana* are more closely related. Interestingly, hybridization studies conducted by Azeez and Faluyi (2018) among these species also showed that *P. angulata*, *P. pubescens* and *P. peruviana* are more related. The data from this study showed that all the four *Physalis* species studied are distinct species with a common origin.

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