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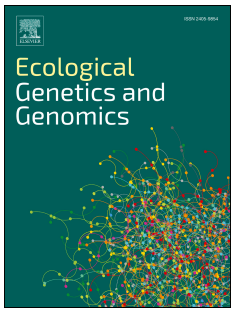
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ASSESSMENT OF EARLY MORPHOLOGICAL TRAITS AND MOLECULAR CHARACTERS OF *Psophocarpus tetragonolobus* (L.) DC. (Winged Bean) USING ISSR and SCOT MARKERS

***Olawuyi, O.J.,[1] Mutiu, H.A.,[1] Akinyoola O. I [2] ., Mudashiru, O.M [3]., [4]Odedeji J. O., Azeez A. A[5,6].**

[1]Department of Botany, Genetics and Molecular Unit, University of Ibadan, Nigeria.

[2]Biotechnology Unit, National Horticultural Research Institute, Ibadan, Nigeria.

[3]Department of Pure and Applied Botany, Federal University of Agriculture, Abeokuta, Nigeria

[4]Food Science and Technology Department, University of Medical Sciences Ondo City, Ondo State. Nigeria.

[5]Rainforest Research Station/Forestry Research Institute of Nigeria, Jericho Ibadan.

[6]Department of Forest Sciences, University of Helsinki, P.O Box 27, 00014, Helsinki Finland.

***Corresponding author: olawuyiodunayo@yahoo.com *Phone number: 07037638364**

Abstract

Psophocarpus tetragonolobus (Winged bean) is an underutilized legume with significant economic potential but is constrained by a prolonged maturation period. This study aimed to identify early maturing accessions using qualitative and quantitative traits, and molecular markers (ISSR and SCoT) in a randomized complete block design. Vegetative and yield characters including leaf shape, corolla colour, pod formation, plant height, leaf length and stem diameter, varied significantly among accessions, with TPt-6 exhibiting the highest plant height (71.96 cm) and TPt-11 showing the largest stem diameter (1.9 cm). Accessions TPt-19, TPt-6 and TPt-125 exhibited early flowering at 41, 42, and 45 days after planting, respectively, while TPt-6 and TPt-11 showed early pod formation at 54 and 58 days, respectively. Among the three ISSR and SCoT markers tested, UBC-811 (ISSR) and SCoT-3 (SCoT) exhibited the highest polymorphic information content (69.75%) and gene diversity (0.75), indicating their effectiveness in detecting genetic variation. The accessions were grouped into two major

clusters A and B, with TPt-19 and TPt-9 forming a subclade within Cluster B which suggests genetic distinctiveness. These findings highlight TPt-6, TPt-11, TPt-19 and TPt-125 as promising candidates for breeding programs focused on improving early maturity in winged bean.

Keywords: *Psophocarpus tetragonolobus*, early maturity, genetic diversity, molecular markers, polymorphism

INTRODUCTION

Psophocarpus tetragonolobus (L.) DC. belonging to the Fabaceae family, is a tropical dicotyledonous leguminous plant commonly known as winged bean, recognized for its nutritional values. It is widely cultivated in Papua New Guinea, Malaysia, Thailand, Bangladesh, Ghana, Indonesia, and to a lesser extent, in Nigeria (Sriwichai *et al.*, 2021). Despite its adaptability to hot and humid climates, the crop remains underutilized in global agriculture (Ojuederie *et al.*, 2020). A distinguishing feature of winged bean is its climbing growth habit and complex plant structure, which allows it to thrive in diverse environmental conditions (Thapa *et al.*, 2024).

Nutritionally, winged bean stands out as a highly valuable crop, as all its parts (seeds, pods, leaves, and tubers are edible and rich in essential nutrients. The protein content of its seeds surpasses that of common legumes such as cowpea, pigeon pea, and lima bean and is comparable to soybeans (Thapa *et al.*, 2024). Additionally, its immature pods are excellent sources of vitamins A, B, and C, along with dietary fiber, iron, and calcium, while its young leaves can be consumed as leafy vegetables, similar to spinach (Bepary *et al.*, 2023). These nutritional attributes make winged bean diversifying diets addressing malnutrition, especially in regions where protein deficiency is prevalent.

Understanding the genetic diversity of winged bean is crucial for improving its agronomic traits, particularly its long maturation period, which limits widespread cultivation. Advances in molecular biology have facilitated the study of genetic variation through molecular markers, which provide insights into genetic relationships, diversity and breeding potential (Zhao *et al.*, 2023). Inter-Simple Sequence Repeats (ISSRs), introduced by Bornet and Branchard (2001), has been widely used DNA-based marker system that amplifies non-coding regions between

microsatellites, making them effective for assessing genetic diversity and evolutionary relationships (Olawuyi *et al.*, 2023; 2024).

In addition to ISSR markers, Start Codon Targeted (SCoT) markers have emerged as valuable tools for genetic analysis. SCoT markers are based on conserved regions surrounding the ATG translation initiation codon, offering high reproducibility and efficiency in evaluating genetic polymorphism and gene expression (Collard and Mackill, 2009). Their applications in molecular breeding studies have been highlighted in recent research, emphasizing their reliability in assessing genetic uniformity and diversity within plant populations (Thakur *et al.*, 2021; Odesola *et al.*, 2024). The integration of multiple molecular markers provides a more comprehensive understanding of genetic variation, as it allows researchers to capture genetic differences at various levels and establish clearer relationships between accessions (Sinha *et al.*, 2023).

Considering the potential of winged bean as a highly valuable crop and the need to enhance its desirable agronomic and yield traits, this study aims to characterize 20 accessions of winged bean using early maturity morphological (qualitative and quantitative) traits and molecular (ISSR and SCoT) markers.

MATERIALS AND METHODS

Planting Materials and Experimental Design

In December 2023, twenty-winged bean accessions were sourced from the Genetic Resources Center (GRC) of the International Institute of Tropical Agriculture (IITA) for the field study. The experiment was conducted at the Nursery Farm of the Department of Botany, University of Ibadan, Oyo State, Nigeria located within 7°02'49" and 7°43'21"N and Longitude 3°31'58" and 4°08'20"E. The experiment followed a randomized complete block design (RCBD) with four replications with polythene bags spaced 1m between accessions and 50 cm within replicates. Each hole was initially sown with two seeds, later thinned to one plant per stand after three weeks. The plants were watered daily, and staking was done after six weeks. Manual weeding and twining were performed, with no additional treatments applied.

Morphological Data Collection

A total of 17 morphological traits (7 quantitative and 10 qualitative) were assessed over five months, starting two weeks after planting. Quantitative traits like leaflet length, petiole length, and plant height were measured using rulers and vernier calipers, while qualitative traits such as leaf shape and stem color were observed phenotypically. Vegetative growth data were

collected for six weeks prior to staking, while phenological traits (days to flowering and pod formation) were recorded continuously after staking, until the end of the experiment. Heritability and genetic variance were subsequently calculated.

DNA Extraction and Quality Assessment

Three fresh leaf samples (totalling 60 samples) were collected from each winged bean accession early in the morning and subsequently lyophilized at -80°C. Genomic DNA was extracted from the young leaf tissues following the modified Cetyltrimethylammonium Bromide (CTAB) protocol for DNA isolation as described by Doyle and Doyle (1990), with modifications from Borges *et al.* (2009). The DNA purity was evaluated using gel electrophoresis and Nanodrop spectrophotometry. A 2µl aliquot of genomic DNA was extracted to achieve a 1.8 ratio at the 260/280 absorbance level, and this concentration was used to prepare working dilutions for Polymerase Chain Reaction (PCR). To assess DNA quality and RNA removal, 4µl of the DNA mixture (2µl DNA + 2µl 1X loading dye) was loaded onto a 1% agarose gel with lambda-DNA markers and visualized under UV light using a Transilluminator (Model-2, Upland, CA, USA). DNA concentration was further quantified using a Nanodrop spectrophotometer (Beckman Coulter DU530) at 260nm.

Morphological Data analysis

The morphological data were subjected to Analysis of Variance (ANOVA) using the Statistical Analysis System (SAS) software version 9.4 (SAS, 2012). Following confirmation of ANOVA assumptions, mean differences were determined using Duncan's Multiple Range Test (DMRT) at a 95% confidence level ($p < 0.05$).

Molecular Assay and Analysis

The twenty accessions of *Psophocarpus tetragonolobus* were analyzed using six primers (SCoT-2, SCoT-3, SCoT-13, UBC-811, UBC-824, and UBC-825), selected from 10 of the 11 linkage groups on the winged bean genome map for their high polymorphism and reproducibility (Andargie *et al.*, 2017). PCR amplifications were performed using an Applied Biosystems GeneAmp PCR System 9700 (USA), and the amplified products were stored at -20°C until gel electrophoresis. Polymorphic primers were scored visually for each genotype based on band presence (1) or absence (0). Molecular data were analyzed using NTSYSpc software to determine allelic frequency, allele number, gene diversity, and Polymorphic

Information Content (PIC). Hierarchical clustering was performed using a UPGMA dendrogram generated in Power marker software version 3.25.

RESULTS

Morphological Variation in *Psophocarpus tetragonolobus* Accessions

The result in Table 1 presents the qualitative traits observed in *Psophocarpus tetragonolobus* accessions, highlighting variations in leaf shape, corolla color, and pod characteristics. Leaf size was predominantly medium, except in TPt-51 and TPt-2, which exhibited small leaves. Three distinct leaf shapes were identified: deltoid (12 accessions), ovate (4 accessions), and ovate-lanceolate (3 accessions). Stem and calyx colors were consistently green across all accessions.

Corolla color exhibited variability, with purple (9 accessions), blue (3 accessions), and nil pigmentation (8 accessions). Pod characteristics also displayed diversity. Most accessions had green pods with green pod wings and a flat shape with smooth surfaces. However, six accessions (TPt-43, TPt-9, TPt-51, TPt-2, TPt-16 and TPt-5) lacked pod wing coloration and speckling. Pod speck presence was recorded in 10 accessions, primarily among those with purple corolla coloration. The remaining accessions exhibited no pod specks or additional structural modifications.

Additionally, the result in Plate 2 shows the early flowering of TPt-19 accession, while the result in Plate 3 presents the early pod formation of TP-6 accession, further illustrating the morphological variation observed among the accessions.

Genotypic Performance of Growth Characters in *Psophocarpus tetragonolobus* Accessions

The result in Table 2 presents the genotypic variation in growth parameters among *Psophocarpus tetragonolobus* accessions, evaluated using Duncan's Multiple Range Test (DMRT) at $p < 0.05$. The accessions exhibited significant differences across leaflet length (LL), leaf width (LW), number length (NL), plant height (PH), number of branches (NB), petiole length (PL), and stem diameter (SD).

Leaflet length ranged from 2.83 cm (TPt-2) to 5.31 cm (TPt-3), while leaf width varied between 2.61 cm (TPt-2) and 5.63 cm (TPt-3). Notably, TPt-19 exhibited the tallest plant height (71.96

cm), whereas TPt-2 had the shortest (25.78 cm). The number of branches was highest in TPt -19 (19.67), followed by TPt-11 (19.17), and lowest in TPt-2 (8.67).

Petiole length showed substantial variation, with TPt-43 (7.54 cm) displaying the longest petiole, whereas other accessions ranged between 1.53 cm and 3.56 cm. Stem diameter was relatively uniform across accessions, though TPt-11 (0.33 cm) had the largest stem diameter, while TPt-2 (0.16 cm) had the smallest.

DNA Concentration and Molecular Characterization of *Psophocarpus tetragonolobus* Accessions

The data shown in Table 3 indicates that accession TPt 12 exhibited the highest DNA concentration, measuring 1.93 µl with a total genomic DNA yield of 1329.7 ng/µl. In contrast, accession TPt-9 had the lowest DNA concentration, at 1.81 µl and a genomic DNA total of 1121.6 ng/µl. Accession TPt-43 produced the highest total genomic DNA yield at 1406.9 ng/µl, while TPt-2 yielded the least genomic DNA at 1113.3 ng/µl, corresponding to a DNA concentration of 1.82 µl. The gel electrophoresis of the twenty *Psophocarpus tetragonolobus* accessions are illustrated in Plate 1.

The results of the molecular analysis presented in Table 4, where three SCoT primers and three ISSR primers exhibited polymorphism across the accessions, identifying a total of 23 alleles. The number of alleles ranged from 3 (SCoT 2) to 4 (SCoT 3, SCoT 13, UBC-811, UBC-824, and UBC-825), with a mean of 3.83 alleles across all primers. The major allele frequency ranged from 0.3 (SCoT 3, SCoT 13, and UBC-811) to 0.45 (UBC-825), with an average of 0.38. Gene diversity values ranged from 0.66 (SCoT 2) to 0.75 (SCoT 3 and UBC-811), yielding an average of 0.70. Polymorphic Information Content (PIC) ranged from 58.05% (SCoT 2) to 69.75% (SCoT 3 and UBC-811), with an average of 64.81%. The highest allele number (4) was observed for SCoT 3, SCoT 13, UBC-811, UBC-824, and UBC-825, while SCoT 2 revealed the lowest allele number (3). The highest PIC (69.75%) and gene diversity (0.75) were recorded for SCoT 3 and UBC-811 primers, respectively.

The dendrogram generated using the SCoT and ISSR markers revealed two main clusters, A and B, as shown in Figure 1. Cluster A comprises 12 accessions (TPt-33, TPt-6, TPt-5, TPt-11, TPt-14, TPt-125, TPt-154, TPt-2, TPt-16, TPt-3 and TPt-31), Cluster B comprises 8 accessions

(TPt-19, TPt-9, TPt-15, TPt-12, TPt-126, TPt-18, TPt-43 and TPt-53) and accessions TPt-19 and TPt-9 grouped together in a subclade within Cluster B.

DISCUSSION

Morpho-Genetic Variation in *Psophocarpus tetragonolobus* accessions and its Insights

This study revealed substantial morphological and genetic variability among *Psophocarpus tetragonolobus* accessions, particularly in corolla pigmentation and pod traits. This observation is in accordance with the reports made by Yulianah *et al.* (2020) and Afridatul *et al.* (2021) on the Indonesian varieties of *P. tetragonolobus*, suggesting the potential use of these traits as genetic markers for taxonomic classification and breeding programs aimed at improving agronomic traits of the plants at the variety level.

Comparable findings of environment-driven trait variation have also been reported in tomato, where Anakaa *et al.* (2025) demonstrated significant variability in growth, flowering, and yield traits across different ecological zones in Nigeria, underscoring the importance of ecological context in trait expression and adaptation. Likewise, Oyedoyin *et al.* (2025) reported successful hybridisation of wild yam species (*Dioscorea abyssinica* and *Dioscorea praehensilis*) and observed significant variation in progeny growth and yield traits, reinforcing the role of genetic diversity and hybridisation in enhancing crop improvement strategies.

Significant genetic variation in growth traits was observed among accessions, with notable differences in leaf shape, and plant height. These variations highlight the genetic diversity within the species, which can be harnessed to improve desirable traits. The morphological assessment aligned with previous report by Assefa *et al.* (2013) which identified pod traits as key selection criteria in improvement of leguminous crops.

Genomic DNA Yield and Purity

The genomic DNA analysis of twenty *P. tetragonolobus* accessions exhibited substantial variation in DNA yield and purity, influenced by factors such as plant material quality, developmental stage, and genetic background. This finding is similar to the report made by Ajayi *et al.* (2019) and Olawuyi and Azeez (2019) on DNA quality of *Mangifera indica* varieties and *Garcinia kola* accessions respectively. In this study, some accessions, such as TPt-9, exhibited lower purity levels, necessitating further optimization of DNA extraction protocols. The observed variation in DNA yield is consistent with findings from Odesola *et al.* (2024) on *Macrotyloma geocarpum* accessions. Enhancing purification steps or adopting alternative extraction methods may improve DNA quality, ensuring suitability for downstream molecular applications (Azeez *et al.*, 2024).

Molecular Marker Analysis and Genetic Diversity

Molecular analysis confirmed significant genetic diversity among the accessions, as evidenced by the high polymorphism levels detected using ISSR and SCoT markers. Each primer generated at least three alleles, with a minimum gene diversity value of 0.66. The polymorphic information content (PIC) averaged 64.81%, with SCoT-2 being the least informative primer. These findings align with similar reports on *Camellia oleifera* and *Celastrus orbiculatus* genetic diversity assessments using ISSR and SCoT markers (Xiao *et al.*, 2020; Yang *et al.*, 2022). The major allele frequency ranged from 0.30 to 0.50, indicating a broad allele distribution and reaffirming the suitability of these markers for genetic mapping and breeding efforts (Al-Yasi *et al.*, 2024).

The clustering analysis grouped the accessions into two distinct clusters, corroborating previous studies on legume germplasm diversity (Susmitha *et al.*, 2023). Such clustering patterns often reflect evolutionary relationships and selection pressures that influence genetic

structure (Azeez *et al.*, 2020). The high gene diversity and PIC values further validate ISSR and SCoT markers as effective tools for assessing genetic variability in *P. tetragonolobus* accessions.

It is important to note that this study utilized only three ISSR and three SCoT primers. Although these markers were sufficiently informative to detect meaningful patterns of genetic diversity, a more comprehensive assessment would typically involve a larger panel of polymorphic markers to enhance genome coverage and strengthen the robustness of the results. Therefore, the findings presented here should be considered a valuable first step that provides preliminary insights, while highlighting the need for future studies incorporating additional markers to validate and expand upon these results.

Relationship Between Morphological and Molecular Variation in *Psophocarpus tetragonolobus* Accessions

The observed morphological variation among *Psophocarpus tetragonolobus* accessions, particularly in leaf traits, plant height, branch number, and pod characteristics, reflects the underlying genetic variability within the species. Variations in developmental timing, such as early flowering and pod formation, suggest inherent differences in growth regulation among accessions, which could influence adaptability and agronomic performance.

Molecular analysis using ISSR and SCoT markers corroborated the presence of substantial genetic diversity, revealing allelic variation and clustering patterns that broadly align with morphological trends. This concordance suggests that certain phenotypic traits may be strongly influenced by genetic factors captured by these markers. However, discrepancies between morphological and molecular patterns indicate that environmental effects, gene interactions, and epigenetic factors also contribute to trait expression.

The integration of morphological and molecular insights underscores the importance of a holistic approach to germplasm characterization. While morphological traits reflect immediate phenotypic expression and agronomic relevance, molecular markers uncover latent genetic variation that can be harnessed in breeding. Accessions combining desirable phenotypic traits with high genetic diversity represent valuable resources for selection, highlighting the potential to improve yield, growth dynamics, and developmental traits through informed breeding strategies.

Ultimately, these findings emphasize that the relationship between phenotype and genotype in winged bean is complex, shaped by both heritable genetic variation and environmental modulation. Understanding this interplay is crucial for optimizing selection and guiding future genetic improvement efforts.

Implications for Breeding and Future Research

The substantial genetic variability observed in Winged bean accessions is similar to the observation by Olawuyi and Fawole (2005) on *Cajanus cajan*, Azeez *et al.* (2020) on *Garcinia kola* and Ajayi *et al.* (2022) on *Mangifera indica*. Similarly, Anakaa *et al.* (2025) emphasized the role of ecological variability in shaping growth, flowering, and yield performance in tomato, which aligns with the present findings and further highlights the importance of identifying accessions with superior adaptability and performance under diverse environmental conditions. In line with this, Oyedoyin *et al.* (2025) highlighted how hybridizing wild yam species broadened the genetic base and produced progenies with valuable growth and yield traits, demonstrating how wild or underutilised germplasm can be exploited to strengthen breeding programs.

The study presents valuable opportunities for breeding programs focused on traits such as early maturity, disease resistance, and enhanced nutritional content. Accessions TPt-11, TPt-6, TPt-

19, and TPt-12 exhibited superior genetic traits, making them promising candidates for future breeding initiatives. Further research incorporating next-generation sequencing (NGS) technologies, such as genotyping-by-sequencing (GBS) and whole-genome sequencing, may provide deeper insights into key genomic regions associated with desirable agronomic traits (He *et al.*, 2014). Additionally, integrating genome-wide association studies (GWAS) with traditional marker-based approaches could facilitate the identification of candidate genes for targeted breeding, accelerating genetic enhancement efforts in *P. tetragonolobus*.

CONCLUSION AND RECOMMENDATIONS

This study successfully assessed the genetic diversity of *P. tetragonolobus* accessions using ISSR and SCoT markers. The results revealed significant genetic variation, with SCoT-3 and UBC-811 primers demonstrating the highest PIC and gene diversity, confirming their effectiveness in distinguishing genetic differences. Accessions TPt-11, TPt-6, TPt-19 and TPt-125 were identified as promising genotypes with superior traits, making them suitable candidates for future breeding programs focused on yield improvement and other agronomic traits, particularly those targeting early maturation and enhanced adaptability.

It should be noted, however, that the study employed only three ISSR and three SCoT primers. Although these markers provided useful insights, the relatively small number limits the comprehensiveness of the diversity analysis. Expanding the number of markers in future studies will provide more robust and representative results.

Based on these findings, TPt-11, TPt-6, TPt-19 and TPt-125 should be prioritized for further breeding efforts to enhance traits such as yield potential, disease resistance, and drought tolerance. Given the high genetic diversity observed, continued use of ISSR and SCoT markers in future molecular studies is recommended for detailed genetic analysis and marker-assisted selection. Future studies should also expand the number of accessions analyzed to capture a broader range of genetic variation, ensuring the identification of additional promising genotypes for improved crop performance.

Table 1: Qualitative traits in *Psophocarpus tetragonolobus* accessions

S/N	Accessions	LS	LSh	SC	CC	CrC	PC	PWC	PS	PSS	PSp
1	TPt-48	Medium	Deltoid	Green	Green	Purple	Green	Green	Flat on sides	Smooth	Presence

2	TPt-12	Medium	Deltoid	Green	Green	Purple	Green	Green	Flat on sides	Smooth	Presence
3	TPt-43	Medium	Ovate-lanceolate	Green	Nil	Nil	Nil	Nil	Nil	Nil	Nil
4	TPt-9	Medium	Ovate	Green	Green	Purple	Nil	Nil	Nil	Nil	Nil
5	TPt-126	Medium	Deltoid	Green	Green	Purple	Green	Green	Flat on sides	Smooth	Presence
6	TPt-51	Small	Deltoid	Green	Green	Blue	Nil	Nil	Nil	Nil	Nil
7	TPt-11	Medium	Deltoid	Green	Green	Purple	Green	Green	Flat on sides	Smooth	Presence
8	TPt-33	Medium	Ovate	Green	Green	Blue	Green	Green	Flat on sides	Smooth	Presence
9	TPt-53	Medium	Deltoid	Green	Green	Blue	Green	Green	Flat on sides	Smooth	Presence
10	TPt-3	Medium	Deltoid	Green	Green	Purple	Green	Green	Flat on sides	Smooth	Presence
11	TPt-6	Medium	Deltoid	Green	Green	Purple	Green	Green	Flat on sides	Smooth	Presence
12	TPt-2	Small	Ovate	Green	Nil	Nil	Nil	Nil	Nil	Nil	Nil
13	TPt-15	Medium	Ovate-lanceolate	Green	Green	Purple	Green	Green	Flat on sides	Smooth	Presence
14	TPt-16	Medium	Ovate-lanceolate	Green	Nil	Nil	Nil	Nil	Nil	Nil	Nil
15	TPt-5	Medium	Deltoid	Green	Nil	Nil	Nil	Nil	Nil	Nil	Nil
16	TPt-154	Medium	Deltoid	Green	Nil	Nil	Nil	Nil	Nil	Nil	Nil
17	TPt-125	Medium	Deltoid	Green	Green	Purple	Nil	Nil	Nil	Nil	Nil
18	TPt-31	Medium	Ovate	Green	Green	Purple	Green	Green	Flat on sides	Smooth	Presence
19	TPt-14	Medium	Deltoid	Green	Nil	Nil	Nil	Nil	Nil	Nil	Nil
20	TPt-19	Medium	deltoid	Green	Green	Purple	Green	Green	Flat on sides	Smooth	Presence

Leaf size, LSh: Leaf shape, SC: Stem colour; CC: Calyx colour, CrC: Corolla colour, PC: Pod colour, PWC: Pod wing colour, PS: Pod shape, PSp: Pod speck, PSS: Pod surface structure.

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Table 2: Genotypic Performance of Growth Characters in *Psophocarpus tetragonolobus* Accessions

Acc	LL (cm)	LW (cm)	NL (cm)	PH (cm)	NB (cm)	PL (cm)	SD (cm)	DF (days)	DP (days)
TPt-19	5.01^{ab}	4.79^{ab}	54.17^{a}	71.96^{a}	19.67^{a}	3.56^{b}	0.28^{ab}	41^{h}	63^{fgh}
TPt-48	3.78^{d}	3.00^{de}	33.83^{def}	37.44^{efg}	12.11^{cd}	1.98^{b}	0.23^{bc}	55^{cde}	66^{cde}
TPT-3	5.31^{a}	5.63^{a}	41.94^{abcde}	57.52^{b}	15.50^{abc}	2.28^{b}	0.28^{ab}	52^{f}	64^{efg}
TPt-14	4.86^{abc}	3.87^{bcde}	38.50^{cde}	43.99^{bcdef}	14.33^{bc}	2.46^{b}	0.28^{ab}	53^{f}	65^{def}
TPt-33	4.74^{abc}	4.38^{bc}	45.86^{abcde}	47.24^{bcdef}	17.83^{ab}	3.11^{b}	0.25^{ab}	54^{def}	77^{a}
TPt-5	4.91^{abc}	4.01^{bcd}	51.83^{ab}	42.03^{cdef}	17.17^{ab}	2.32^{b}	0.27^{ab}	56b^{cd}	68^{bc}
TPt-6	4.64^{abcd}	4.08^{bcd}	41.94^{abcde}	55.50^{bc}	15.72^{abc}	2.39^{b}	0.28^{ab}	42^{h}	54^{k}
TPt-12	4.78^{abc}	3.07^{bcd}	46.50^{abcd}	53.21^{bcd}	17.72^{ab}	3.54^{b}	0.30^{ab}	53^{ef}	65d^{ef}
TPt-11	4.52^{abcd}	3.48^{bcde}	41.94^{abcde}	40.39^{def}	19.17^{a}	1.98^{b}	0.33^{a}	54d^{ef}	58^{j}
TPt-9	4.5^{abcd}	3.87^{bcde}	34.17^{def}	46.37^{bcdef}	14.17^{bc}	2.46^{b}	0.24^{ab}	56^{bc}	59^{ij}
TPt-53	4.79^{abc}	3.80^{bcde}	41.17^{bcde}	57.52^{b}	15.39^{abc}	2.59^{b}	0.28^{ab}	57^{bc}	59^{ij}
TPt-125	4.44^{abcd}	3.87^{bcde}	44.00^{abcde}	39.77^{def}	15.94^{abc}	2.02^{b}	0.24^{ab}	45^{g}	61^{hi}
TPt-31	4.48^{abcd}	4.04^{bcd}	44.78^{abcde}	45.89^{bcdef}	17.28^{ab}	3.02^{b}	0.28^{ab}	58^{ab}	61^{hi}
TPt-16	4.30^{bcd}	3.43^{cde}	42.11^{abcde}	39.74^{def}	16.22^{abc}	2.17^{b}	0.26^{ab}	56^{bcd}	68^{bc}
TPt-51	4.07^{cd}	3.39^{cde}	42.28^{abcde}	44.82^{bcdef}	15.28^{abc}	2.19^{b}	0.24^{b}	57^{bc}	60^{ij}
TPt-2	2.83^{e}	2.61^{e}	23.5^{f}	25.78^{g}	8.67^{d}	1.53^{b}	0.16^{c}	60^{a}	62^{ghi}
TPt-126	4.23^{bcd}	4.00^{bcd}	49.94^{abc}	32.92^{fg}	17.00^{ab}	2.32^{b}	0.27^{ab}	55^{cde}	66^{cde}
TPt-154	4.41^{abcd}	4.17^{bcd}	33.06^{ef}	39.08^{def}	13.17^{bc}	2.81^{b}	0.25^{ab}	56^{bcd}	67^{cd}
TPt-43	4.28^{bcd}	3.87^{bcde}	41.8^{abcde}	51.04^{bcde}	17.11^{ab}	7.54^{a}	0.26^{ab}	54d^{ef}	66^{cde}
TPt-15	4.78^{abc}	3.66^{bcde}	45.27^{abcde}	48.88^{bcde}	16.22^{abc}	2.19^{b}	0.27^{ab}	55^{cde}	70^{b}

LL: leaflet length, LW: leaf width, PH: plant height NL: Number length NB: Number of branches PL: petiole length SD: Stem diameter, DF: days to flowering, DP: days to pod formation. Means with the same letter in the same column are not significantly different at $p<0.05$ using Duncan's Multiple Range Test (DMRT)

Table 3: Nano drop and DNA concentration of *Psophocarpus tetragonolobus* accessions

S/N	Accessions	Total Genomic DNA Extracted (ng/µl)	DNA Concentration (260/280 gl)
1	TPt-2	1113.30	1.82
2	TPt-3	1129.20	1.82
3	TPt-5	1122.10	1.89
4	TPt-6	1145.30	1.83

5	TPt-9	1121.60	1.81
6	TPt-11	1157.90	1.89
7	TPt-12	1329.70	1.93
8	TPt-14	1316.30	1.92
9	TPt-15	1268.90	1.90
10	TPt-16	1156.70	1.90
11	TPt-48	1152.20	1.86
12	TPt-19	1214.10	1.92
13	TPt-31	1352.00	1.92
14	TPt-33	1171.60	1.87
15	TPt-43	1406.90	1.92
16	TPt-51	1304.40	1.85
17	TPt-53	1131.80	1.89
18	TPt-125	1126.00	1.88
19	TPt-126	1310.20	1.92
20	TPt-154	1276.70	1.89

Table 4: Allele Frequency, Gene Diversity and Polymorphic Information Content (PIC) of *Psophocarpus tetragonolobus* Accessions

Marker	Major Allele Frequency	Allele Number	Gene Diversity	PIC (%)
SCoT 2	0.40	3.00	0.66	58.05
SCoT 3	0.30	4.00	0.75	69.75
SCoT 13	0.30	4.00	0.74	68.54
UBC-811	0.30	4.00	0.75	69.75
UBC-824	0.50	4.00	0.67	61.79
UBC-825	0.45	4.00	0.67	60.99
Mean	0.38	3.83	0.70	64.81

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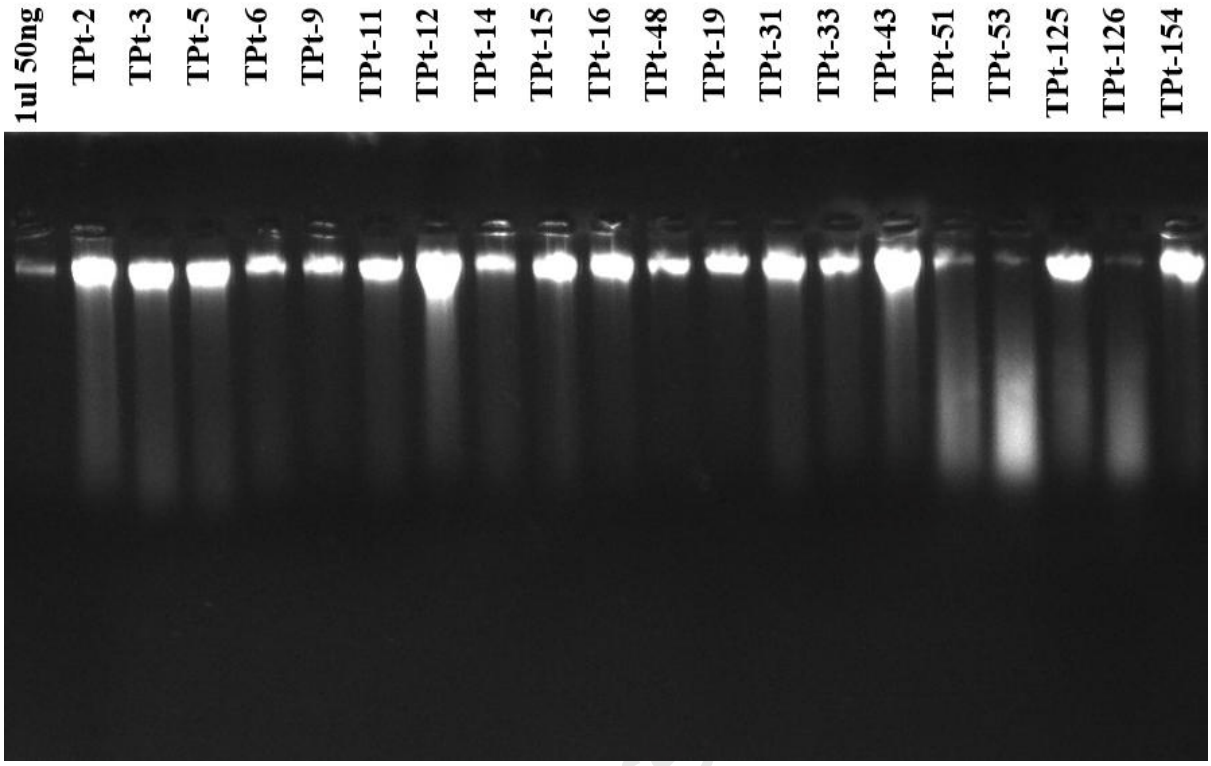


Plate 1: Gel electrophoresis showing the 20 accessions of *Psophocarpus tetragonolobus* Accessions

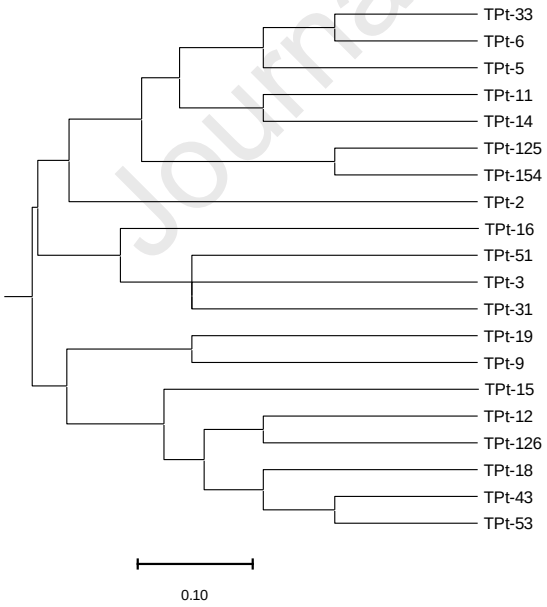


Fig. 1: Dendogram showing the genetic relationship of *Psophocarpus tetragonolobus* accessions



Plate 2: Photograph showing the early flowering of TPt 19 accession



Plate 3: Photograph showing the early pod formation of TPt-6 accession

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CRediT Author Statement:

- **Olawuyi, O.J.:** Conceptualization, Methodology, Supervision, Project administration, Formal analysis, Funding acquisition, Supervision, Writing – review & editing.
- **Mutiu, H.A.:** Data curation, Formal analysis, Visualization, writing, Resources, Validation, Data curation.
- **Akinyoola, O.I.:** Writing – review & editing.
- **Mudashiru, O.M.:** Investigation, review & editing.
- **Odedeji, J.O.:** Writing – review & editing.
- **Azeez, A.A.:** Formal analysis, Investigation, Visualization, Writing – review & editing.

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