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Exploring Spatial Distribution Patterns of Morphological and Genetic Variation in *Talinum triangulare* (Jacq.) Willd. from Mid-Western Nigeria

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ABSTRACT

This study employed a comprehensive approach, combining surveys, morphological characterization, and genetic analysis, to assess the diversity of *Talinum triangulare* in Mid-Western Nigeria. A structured questionnaire was first administered to 50 participants to collect socio-economic information related to the plant's usage in the study area. Next, 20 accessions were randomly collected from ten sampling stations across Edo and Delta States, with mapping conducted using ArcGIS Pro. Morphological traits, including plant height, leaf length, leaf area, number of leaves, stem girth, and plant dry weight, were measured in triplicate. Genetic diversity was evaluated using Simple Sequence Repeat (SSR) markers. Data analysis involved descriptive statistics, ANOVA, and the Least Significant Difference (LSD) test at a 5% significance level, providing insights into both morphological and genetic variation in *T. triangulare*. Survey results highlighted the plant's socio-economic relevance, with 90% of respondents being women and 36% identifying as traders. Morphological analysis revealed significant variation in plant height, leaf length, and leaf area. SSR-based genetic analysis showed moderate genetic diversity (GD = 0.43) and significant molecular variance. Phylogenetic analysis further revealed distinct genetic clusters, indicating both shared ancestry and genetic divergence. These findings highlighted the need to conserve the genetic diversity of *T. triangulare* for sustainable agricultural use and its potential value in breeding programs. This study demonstrated how integrating socio-economic, morphological, and genetic perspectives can effectively assist in understanding and preserving plant diversity for *T. triangulare*.

Abbreviation: Analyses of Variance (ANOVA), Genetic Diversity (GD), International Institute for Tropical Agriculture (IITA), Least Significant Difference (LSD), Polymerase chain reaction (PCR), Sampling stations (SaST), Simple Sequence Repeats (SSR), Unweighted Pair Group Method with Arithmetic Mean (UPGMA), Water Leaf Plant (WLP)

Introduction

The paradox of persistent food insecurity in developing countries, despite Africa's substantial agricultural capacity, highlights the urgent need for innovative strategies to address food security challenges (Asogwa, 2017). One promising approach lies in the utilization of locally available, underutilized vegetables, which offer great potential for enhancing food security and stimulating economic growth. Among these, indigenous leafy greens such as *Talinum triangulare* (Family: Talinaceae) stand out for their numerous contributions to sustainable food systems. *Talinum triangulare* possesses remarkable attributes that make it a valuable asset in the fight against food insecurity. Rich in proteins, vitamins, minerals, and antioxidants, this highly adaptable crop thrives in tropical climates with minimal water and maintenance. Its wide distribution, drought tolerance, and rapid growth cycle make it a readily accessible and sustainable food source. The plant's edible leaves, stems, and roots offer culinary versatility, while its low cultivation cost and cultural significance further enhance its appeal. The genetic diversity of *T. triangulare* plays a critical role in its broader adoption and successful cultivation. Understanding and appreciating its genetic, morphological, temporal, and cultural variability allow the species to adapt to diverse environmental conditions. This inherent genetic variation supports its resilience to climate fluctuations, resistance to diseases, and suitability across various dietary practices. As such, conserving and sustainably utilizing this resource is essential for achieving long-term food security and positions *T. triangulare* as a key component in addressing nutritional challenges. Advancing its development through modern biotechnological tools presents a promising research frontier. Such efforts could lead to the creation of sustainable, high-yielding cultivars that benefit local communities and bolster food security.

Continued research will be instrumental in unlocking the plant's full potential and supporting sustainable agricultural practices. Previous studies have demonstrated significant internal structural differences among *T. triangulare* varieties, such as mesophyll differentiation in leaves, indicating environmental adaptation (Adenegan-Alakinde and Ojo, 2020). Additionally, the plant's production of secondary metabolites, unique chemical compounds that are key to resilience and nutritional value, varies by geographic origin (Budiarti and Fatchiyah, 2022). Incorporating nutrient-rich plants like *Talinum triangulare* can significantly improve diets in mineral-deficient regions (Owoicho et al., 2014). Its traditional medicinal uses for gastrointestinal and hypertensive issues further highlight its importance in regional healthcare (Chibueze and Akubugwo, 2011). Understanding its morphological and genetic diversity is therefore crucial for ensuring its sustained availability and realizing its full potential to address food and health needs, particularly in Mid-Western Nigeria. A multidisciplinary approach, integrating genetic analysis, conservation efforts, and the documentation of traditional knowledge, is essential for fully utilizing *Talinum triangulare*. This is vital given the significant genetic diversity within the species (Nya et al., 2023), which is key for breeding programs aimed at enhancing yield and nutritional value. Research has demonstrated that this genetic diversity allows the plant to adapt to diverse environments and resist diseases. Furthermore, its rich content of bioactive compounds, such as flavonoids and alkaloids, underscores its importance for human health (Ikewuchi et al., 2016; Ilodibia and Igboabuchi, 2016). Consequently, the conservation of this genetic diversity is paramount to maximizing the benefits of *Talinum triangulare*. In conclusion, the morphological and genetic assessments of *Talinum triangulare* highlight a crucial interplay between environmental adaptation and genetic diversity, which underpins the species' sustainability and its value within local cultures. Ongoing research in this area is essential for developing improved cultivars and promoting the conservation of this significant plant resource.

Materials and Methods

Study area

This study was conducted in the botanic garden of the Department of Plant Biology and Biotechnology, University of Benin (Ugbowo campus), Benin City, Edo State. Samples were collected from selected areas within Edo and Delta States (Fig. 1).

Documentary and survey approach for accession collection

In the first step, the study involved the systematic identification and mapping of areas with a notable presence of *Talinum triangulare* in Edo and Delta States, utilizing ArcGIS Pro (Nya et al., 2010). Subsequently, ten sampling stations (SaST) were designated within each state, with individual plant collections from each station referred to as accessions. *T. triangulare* samples were procured from these stations to facilitate efficient plant collection and storage for further investigation. In addition, data on the local utilization and economic importance of the plant was collected, following the methodologies described by Nya and Eka (2007), providing valuable context regarding its cultural and economic significance. To achieve a deeper understanding of the socio-economic and environmental determinants affecting *Talinum triangulare* cultivation and utilization, a CATWOE analysis framework was implemented. This framework facilitated the identification of key stakeholders (customers), value chain participants (Actors), underlying worldviews, responsible entities for sustainable management (owners), and relevant constraints. This comprehensive approach provided significant insights into the socio-economic and environmental factors influencing the cultivation and utilization of *Talinum triangulare* in the study region (Fig. 2).

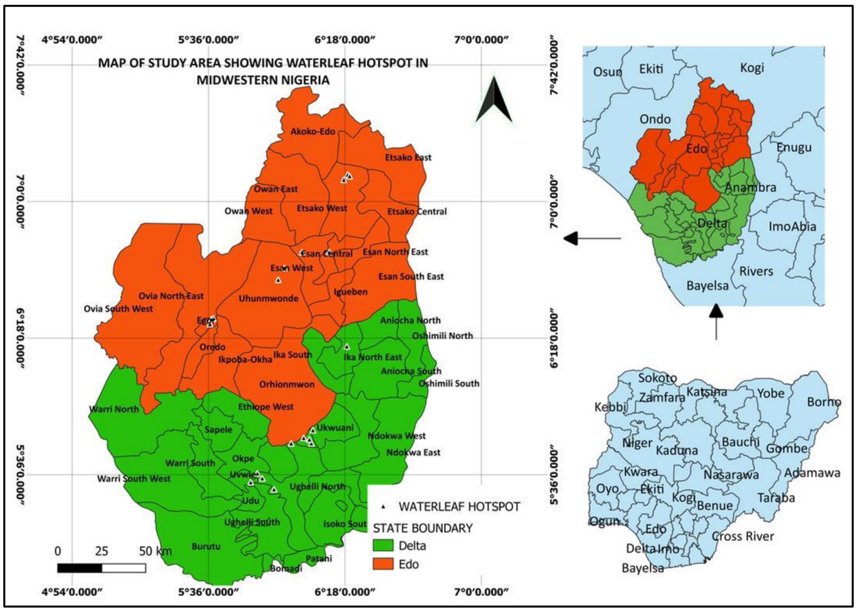

Fig. 1. Map of study area showing water leaf hotspot in midwestern Nigeria.

Fig. 2. Waterleaf plant accessions for the parent plants collected from Delta and Edo States (the numbers attached represent the accession numbers).

Plant data collection per location

Morphological traits of *Talinum triangulare* were assessed by measuring plant height, leaf length, leaf area, leaf count per plant, stem girth, and plant dry weight. Data were collected from three replicates per designated location. Descriptive statistics (mean and standard error) were calculated for each trait. Analysis of variance (ANOVA) was performed to compare means among locations, and means were separated using Least Significant Difference (LSD) at 5% significance level.

Understanding WLP genetic diversity studies using microsatellites

In this study, the genetic diversity of the Waterleaf plant (*Talinum triangulare*) was evaluated using Simple Sequence Repeats (SSRs), also known as microsatellite markers. Primer design was carried out using Primer3 software, and genomic DNA was extracted from leaf tissue using the DNeasy Plant Mini Kit. Additional DNA extraction was performed from 0.01–0.1 g of plant material following the cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1987). DNA concentration and purity were determined using NanoDrop spectrophotometry. Polymerase Chain Reaction (PCR) amplifications were conducted under optimized conditions, using 10–20 ng of template DNA and 0.2–0.5 μM of each primer. Thermocycling conditions consisted of 35–40 cycles of denaturation at 94 °C for 30 s, annealing at 55–60 °C for 30 s, and extension at 72 °C for 1–2 mins, followed by a final extension at 72 °C for 5–10 mins. PCR amplification and sequencing were performed at the International Institute for Tropical Agriculture (IITA) Genetic Resources Laboratory in Ibadan, following standard protocols (Curto et al., 2019; Tibihika et al., 2019). Fragment analysis was conducted using agarose gel electrophoresis and GeneMapper software to assess allele sizes and peak profiles. The SSR primers employed for genotyping are listed in Table 1.

Table 1. SSR primers used in the genetic diversity investigation.

S/N	Primer Name	Forward Sequence (5' - 3' end)	Reverse Sequence (5' - 3' end)
1	EST-SSR9	CAATCATCATGCAGTGCTCT	GTTGTAGGTCACTCCATTCC
2	EST-SSR13	GTTTGGCGGTGGATTGAGA	ATGGTGAGGAGGATGTGGG
3	EST-SSR15	GTCAACAACCACAACCAAAT	GTTGATGGCATTGTTGATCT
4	EST-SSR19	ATCATCATGCAGTGCTCTCT	AACTGGTTGTTCACGTTGTT
5	EST-SSR20	TAAAGTAGGCCAACTGCAAA	ACTTTGATTGGAGGAAGAGG
6	EST-SSR22	TCAACCTGCCAAAGTACAAT	TTTTTCTTGGCTGTAGCTTG
7	EST-SSR23	ACTGACACAAATGGAAATGC	GCTCGTCAATAGTGAGATCG
8	EST-SSR26	GAGAGACGACTGGCATACTG	CCACCTGTTGACATCCTATG
9	EST-SSR41	TAACCCAGCTATGCCTTACA	TCTCCTTCCTCTTTGACCTC
10	EST-SSR46	AGTATCTGGGATTCCTTTCG	ACTGATGTTGGATGAGCAAC

Data analysis

The results were presented as mean values of three replicates. A completely randomized experimental design was employed, based on the assumption that the experimental plot was homogeneous, as soils had been pooled prior to use. Data were analyzed using single-factor analysis of variance (ANOVA). Before performing ANOVA and LSD tests, the assumptions of normality and homogeneity of variances were verified. Statistical analyses were conducted using SPSS® version 23 and, where applicable, PAST® version 2.17c.

Results

Documentary and survey approach for accession collection

Table 2 presents the locations from which plant samples were collected. Prior to sample collection, information on each accession was obtained from local respondents to justify plant selection. Demographic data of the 50 survey participants are shown in Table 3. Ten percent of respondents were under 21 years of age, while over 50% were between 21 and 40 years old. The majority (90%) were female. All participants (100%) reported consuming the vegetable, 36% identified as traders, and 14% as farmers. Survey responses were evaluated using a 5-point Likert scale, with a theoretical cut-off mean of 3.00. The statement that waterleaf is used as a soup vegetable received unanimous agreement, yielding a mean score of 5.0. Most respondents also affirmed its use in herbal preparations for medicinal purposes. Among the 36% who were traders of the vegetable, there was unanimous agreement that selling waterleaf was significantly more profitable than selling other leafy vegetables. However, none of the respondents across the surveyed locations reported any use of waterleaf for horticultural purposes (Table 4).

Table 2. Location of water leaf plant collections in Edo and Delta States.

Accessions	Location of sample collection	GPS Coordinates
WWLP1	Eruemukohwarien, Ughelli North, Delta State	Latitude: N5^{0} 31'31.7946; Longitude: E5^{0} 56'10.4446, Altitude: 11m
WWLP2	Okurowhe, Okpe L.G.A., Delta State	Latitude: N5^{0} 36' 26; Longitude: E 5^{0}51' 1 Altitude: 21m.
WWLP3	Otorho, Abraka Ethiope, Delta State	Latitude: N5^{o} 45' 40.87188; Longitude: E 6^{o} 7' 37.89336 Altitude 8m a.s.l
WWLP4	Obiaruku, Ukwani LGA, Delta State	Latitude: N5^{o} 49' 42.798; Longitude: E6^{o} 8' 7.91412 Altitude: 30m a.s.l
WWLP5	Ofagbe, Isoko North LGA, Delta State	Latitude: N 5^{o} 33' 4.266; Longitude: E 6^{o} 20'47.17788 Altitude: 19m a.s.l
WWLP6	Umunede, Ika North, Delta State	Latitude: N6^{o} 15' 29.69136; Longitude: E6^{o} 18' 33.01488 Altitude: 228m
WWLP7	Ajalomi, Ethiope East LGA, Delta State	Latitude: N5^{o} 47' 19.4294; Longitude: E6^{o} 5' 17.4181 Altitude: 18m a.s.l
WWLP8	Ugbomor Road, Warri, Delta State	Latitude: N5^{o} 33' 42.82236; Longitude: E5^{o} 48^{o} 58.21596 Altitude: 2629m a,s.l
WWLP9	Unity School, Agbarho, Ughelli North, Delta State	Latitude: N5^{o} 34'55.07976; Longitude: E5^{o} 52'32.8422 Altitude: 3m a.s.l
WWLP10	Sanubi, Orogun, Ughelli North, Delta State	Latitude: N5^{o} 45'39.08592; Longitude: E6^{o} 1'26.16924 Altitude: 20m a.s.l
WWLP11	Uselu- Benin City, Edo State	Latitude: N 6^{o} 22'20; Longitude: E 5^{o} 36' 27 Altitude: 3m a.s.l
WWLP12	Faculty Of Agriculture, UNIBEN, Edo State	Latitude: N 6^{o} 24'5; Longitude: E 5^{o} 37' 29 Altitude: 98m a.s.l
WWLP13	Benin-Auchi Road, Edo State	Latitude: N 7^{o} 6' 44; Longitude: E 6^{o} 17'38 Altitude: 265m
WWLP14	Edo State University Iyamo, Edo State	Latitude: N7^{o} 8'19; Longitude: E6^{o} 18'48 Altitude: 213m
WWLP15	Iyamo Location, Auchi, Edo State	Latitude: N7^{o} 7'51; Longitude: E 6^{o} 19'25 Altitude: 152m
WWLP16	Irrua Edo State, Edo State	Latitude: N6^{o} 44' 32.8794; Longitude: E6^{o} 13' 9.17688 Altitude: 404m a.s.l
WWLP17	Ambrose Alli Univ campus, Ekpoma Edo State	Latitude: N6^{o} 44' 27.0528; Longitude: E6^{o} 4'44.9598 Altitude: 358m a.s.l
WWLP18	Uhunmwode, Edo State	Latitude: N6^{o} 39' 36; Longitude: E5^{o} 59'21 Altitude: 261m
WWLP19	Igieduma, Edo State	Latitude: N6^{o} 35'58.3188; Longitude: E5^{o} 57'26.7656 Altitude: 237m a.s.l
WWLP20	Botanical Garden and Environment, University of Benin, Benin City, Edo State	Latitude: N6^{o} 23' 49.146; Longitude: E5^{o} 36'54.46368 Altitude: 112m

Table 3. Demographic information of respondents in the study.

Group (N=50)	(n)	(%)
Age category		
< 21 years	5	10
21 - 30 years	17	34
31 - 40 years	14	28
41 - 50 years	6	12
51 - 60 years	4	8
61 - 70 years	3	6
>70 years	1	2
Gender		
Female	45	90
Male	6	12
Status of usage		
#Grower of the vegetable	7	14
Traders of the vegetable	18	36
Consumers of the vegetable	50	100

#All growers are also traders of the crop. All growers and traders are also consumers of the vegetable.

Table 4. Responses of study participants on the utilization of waterleaf.

S/N	Assertion (*Mean Cut-off of Scales=3.00*) (N=50)	Theoretical Cut-off Mean		
		Consumers (n=48)	Traders (n=18)	Farmers (n=7)
1	Water leaf is used as a soup vegetable	5.000	5.000	5.000
2	Water leaf is used in herbal preparations for medicinal uses	3.163	2.943	3.006
3	Water leaf plants are used in the surroundings for horticultural purposes in environmental aesthetics	0	0	0
4	It is very profitable to sell waterleaf among other leafy vegetables (only traders)	NA	3.746	NA
5	It is highly patronized by the locals (only traders)	NA	3.882	NA

Responses related to waterleaf cultivation by farmers among the study participants are presented in Table 5. To gain a comprehensive understanding of stakeholder perspectives in the waterleaf value chain, the CATWOE analysis technique was employed. CATWOE provides a structured framework for identifying and analyzing stakeholder viewpoints in a business context. Based on this analysis, respondents were categorized into two groups: farmers cultivating waterleaf strictly for commercial purposes and those cultivating it solely for personal consumption. The majority of farmers (85% of the farming population) sourced their planting materials from existing fields, selecting waterleaf plants randomly and propagating them primarily through stem cuttings, which were used by over 90% of the total farming population. In terms of land use, fewer than 15% of the farmers cultivated waterleaf on more than one plot of land. Notably, all farmers, whether engaged in commercial or subsistence cultivation, identified theft as the primary cause of crop loss. This widespread issue likely stems from the vegetable's high acceptability and broad utility, which make it a frequent target for theft. This finding underscored the importance of addressing security concerns to reduce crop losses and further highlights the popularity and economic value of waterleaf among local communities.

Table 5. Responses on cultivation of waterleaf by farmers among the study participants.

Responses from farmers only (n(farmers)=7)		Commercial farmer (n=4)		Subsistence farmer (n=3)	
		(n)	(%)	(n)	(%)
How do you source planting materials?	Market	1	14.29	0	0
	Field	3	42.86	3	42.86
	Nursery	0	0.00	0	0.00
	Others	0	0.00	0	0.00
How do you grow the plants in your farm? (More than one choice is selected)	Stem cutting	4	57.14	3	42.86
	Seed	2	28.57	0	0.00
	Both	3	42.86	0	0.00
What is the extent of land coverage for cultivation of the water leaf farm?	<1 plot	3	42.86	3	42.86
	1 – 3 plots	1	14.29	0	0.00
	> 3 plots	0	0.00	0	0.00
How often do you suffer losses due to theft of waterleaf in your farm?	Frequently	4	57.14	3	42.86
	Often	0	0.00	0	0.00
	Rarely	0	0.00	0	0.00
	None	0	0.00	0	0.00

Following plant collection from the designated locations (Fig. 1 and Table 2), the morphological characteristics of the *Talinum triangulare* (waterleaf) accessions were evaluated and are presented in Table 6. Plant height showed considerable variation, ranging from a minimum of 36.24 cm (WWLP7, Ajalomi, Ethiope East LGA, Delta State) to a maximum of 82.64 cm (WWLP12, Faculty of Agriculture, University of Benin, Edo State). Leaf length ranged from 4.6 to 9.1 cm. The largest leaf area (23.73 cm^2) was recorded in accessions collected along the Benin-Auchi express road, while the smallest (10.04 cm^2) was observed in Ajalomi, Ethiope East LGA. Leaf count per plant ranged from 13 to 21. Statistical analysis revealed significant morphological differences among accessions, particularly in plant height (P = 0.027) and leaf area (P < 0.001). The overall mean whole plant weight was 1.89 g (Fig. 3). Dry weight also varied among accessions, with WWLP1 recording 1.63 g, WWLP showing the highest at 3.15 g, and WWLP20 at 2.05 g. Notably, the dry weight of WWLP differed significantly from that of the WWLP1–11 accessions (P<0.05).

Table 6. Morphological characteristics of water leaf plant accessions collected from the selected locations in Edo and Delta States (mean±SE).

Accessions	Plant height (cm)	Leaf length (cm)	Leaf area (cm^2)	No. of leaves per plant	Stem girth (1cm from root base)
WWLP1	39.67±4.01	5.9±1.3	10.67±2.12	17±3	2.01±0.53
WWLP2	37.33±3.01	5.8±0.9	10.07±1.56	14±3	2.13±0.82
WWLP3	42.67±6.23	4.7±0.8	10.75±1.66	16±3	1.92±0.25
WWLP4	41.33±7.12	4.6±1.4	11.22±2.01	15±4	2.58±0.59
WWLP5	40.33±3.91	5.3±1.6	10.08±2.11	17±3	2.31±0.63
WWLP6	47.31±4.01	5.7±1.7	10.62±2.73	15±4	1.46±0.49
WWLP7	36.24±7.24	4.6±1.2	10.04±1.82	16±3	1.85±0.69
WWLP8	48.00±9.13	6.3±1.9	12.67±3.12	13±4	2.15±0.29
WWLP9	48.00±4.94	6.4±2.1	11.92±2.03	15±4	2.04±0.40
WWLP10	52.67±3.12	6.3±2.8	13.14±2.83	16±5	1.87±0.58
WWLP11	42.67±5.01	5.9±1.6	11.43±3.11	17±2	1.94±0.72
WWLP12	82.67±4.82	9.8±2.1	31.48±6.21	18±3	1.70±0.63
WWLP13	61.33±4.23	9.1±2.3	23.73±4.11	18±3	1.85±0.42
WWLP14	56.33±5.01	8.9±2.9	17.90±3.96	21±4	2.14±0.51
WWLP15	56.00±3.53	8.8±2.1	17.70±4.02	19±2	2.21±0.77
WWLP16	46.67±2.90	6.7±2.5	13.48±2.39	21±4	2.01±0.38
WWLP17	48.00±4.92	6.3±2.3	11.73±1.93	16±1	2.36±0.44
WWLP18	53.01±8.55	6.6±1.9	13.28±2.37	19±3	2.65±0.36
WWLP19	47.33±7.01	6.1±2.6	11.82±2.46	18±4	1.98±0.38
WWLP20	50.33±10.22	6.8±2.3	14.18±2.19	20±3	2.01±0.42
p-value	15.23	3.4	5.03	5	1.26
LSD (0.05)	***0.027***	0.182	***<0.001***	0.303	0.412

Values reduced to the nearest integer.

Understanding WLP genetic diversity

This study investigated the genetic diversity of 20 *Talinum triangulare* accessions collected from various locations within the Midwestern region of Nigeria. DNA samples were extracted from each accession, and their quality was assessed using Nanodrop readings (Table 7). Subsequently, genetic diversity was analyzed using 10 SSR primers, of which five (EST SSR 13, 19, 26, 41, and 46) yielded consistent results (Table 8). The non-responsiveness of primers EST-SSR 9, 15, 20, 22, and 23 may be attributed to several factors, including primer specificity, primer mismatch, SSR region variability, suboptimal PCR conditions, and insufficient DNA quality. These factors may have prevented successful amplification, and further optimization of primer design, PCR conditions, and DNA quality may be necessary to improve amplification success. Table 8 summarizes the genetic statistics, including major allelic frequencies, mean allelic frequency, mean number of alleles per locus, genetic diversity index, and polymorphism information content (PIC) values. The results revealed moderate genetic diversity among the accessions, with an average allelic frequency of 0.65 and a mean number of alleles (2.6) per locus.

The Polymorphism Information Content (PIC) values assessed the level of genetic diversity within *Talinum triangulare* using different EST-SSR primers. EST SSR 13 showed no polymorphism (PIC=0), indicating limited variability at this locus. In contrast, EST SSR 19 exhibited a high PIC value (0.6113), suggesting significant genetic diversity at this locus. EST SSR 26 also demonstrated high polymorphism with a PIC value of 0.5632. Moderate levels of polymorphism were observed in EST SSR

41 (PIC=0.3047) and EST SSR 46 (PIC=0.3725). These findings highlight the varying levels of genetic diversity among the different EST-SSR loci within the *T. triangulare* population. Primers with higher PIC values, such as EST SSR 19 and EST SSR 26, are particularly valuable for differentiating between individuals within the population and provide valuable insights into the genetic structure of *T. triangulare*.

Phylogenetic analyses, including Neighbor-Joining (Fig. 4) and UPGMA (Fig. 5), revealed complex genetic relationships among the accessions. While UPGMA suggested a common ancestry for all accessions, the NJ analysis identified distinct clusters, indicating genetic divergence within the species. These findings provide valuable insights into the genetic diversity and structure of *T. triangulare* in the Niger Delta, which is crucial for developing effective conservation and breeding strategies for this important vegetable crop.

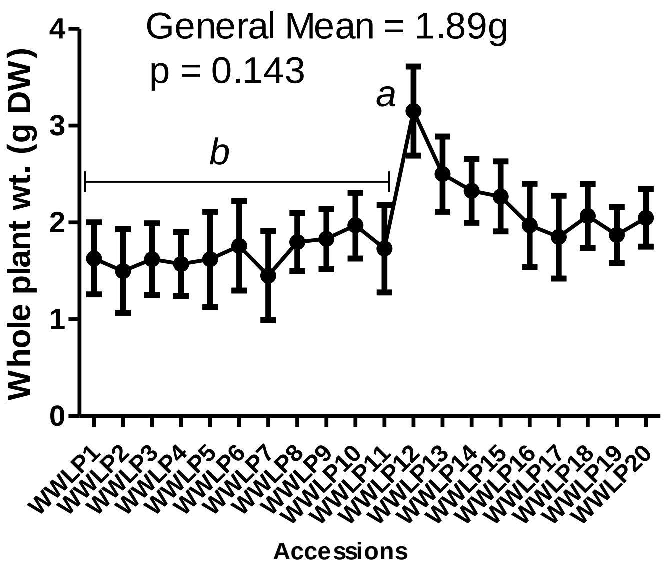

Fig. 3. Whole plant dry weight of water leaf plant collected from the selected locations. Note: Age of plants as at time of collection was unknown.

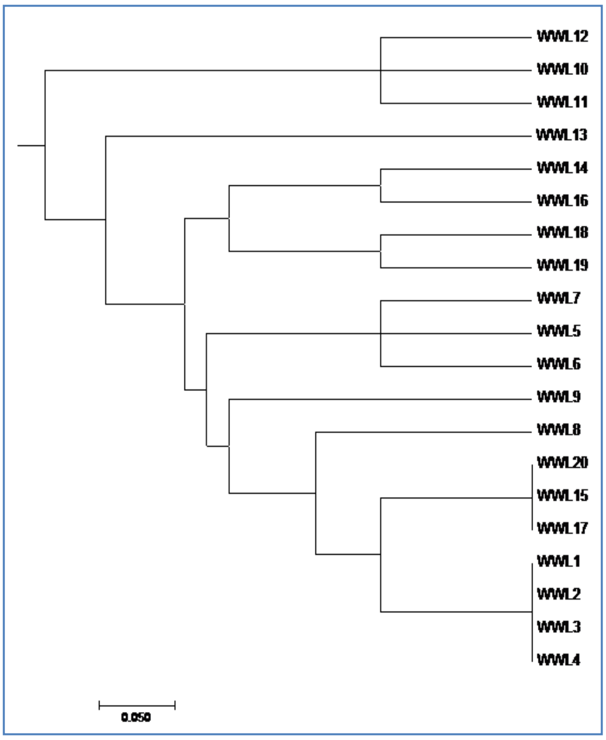

Fig. 4. UPGMA tree (WWL1 to WWL20 represent the 20 accessions of *T. triangulare* from 20 widely dispersed by random locations in the Niger Delta region of Nigeria).

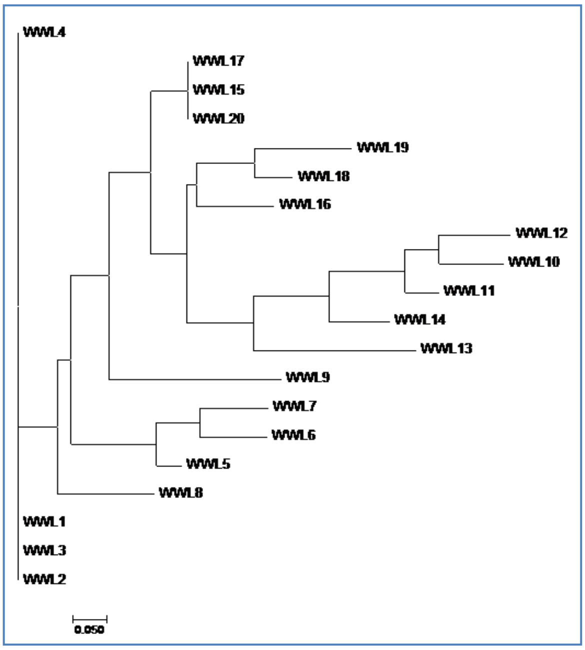

Fig. 5. NJ tree (WWL1 to WWL20 represent the 20 accessions of *T. triangulare* from 20 widely dispersed by random locations in the Niger Delta region of Nigeria).

Table 7. Nanodrop readings for DNA samples of 20 accessions of *T. triangulare* from 20 widely dispersed by random locations in the Niger Delta region of Nigeria.

Sample Code	Nucleic Acid (ng µl^{-1})	260/280	260/230
WWWL1	1951.7	1.89	1.81
WWWL2	962.1	1.9	1.76
WWWL3	529.4	1.89	1.58
WWWL4	960.7	1.89	1.26
WWWL5	1016.7	1.93	1.65
WWWL6	1612	1.9	1.9
WWWL7	1034.7	1.9	1.71
WWWL8	813.9	1.89	1.65
WWWL9	608.4	1.82	1.42
WWWL10	1883.7	1.9	1.89
WWWL11	1535.3	1.9	1.91
WWWL12	1806.6	1.9	1.66
WWWL13	1192.2	1.94	1.64
WWWL14	1216.9	1.91	1.91
WWWL15	1661.5	1.94	1.87
WWWL16	1874.5	1.91	1.84
WWWL17	933.9	1.9	1.61
WWWL18	1674.8	1.97	1.88
WWWL19	1302.9	1.92	1.86
WWWL20	1004	1.97	1.79

Table 8. Summary of genetic statistics.

Marker	Major Allele Frequency	Sample Size	No. of obs.	Allele No	Availability	Gene Diversity	Polymorphism Information Content (PIC)
EST SSR 13	1	20	20	1	1	0	0
EST SSR 19	0.55	20	20	5	1	0.645	0.6113
EST SSR 26	0.4	20	20	3	1	0.64	0.5632
EST SSR 41	0.75	20	20	2	1	0.375	0.3047
EST SSR 46	0.55	20	20	2	1	0.495	0.3725
Mean	0.65	20	20	2.6	1	0.431	0.3703

PCR amplification was successful for the 20 *Talinum* accessions using EST-SSR primers. Specifically, primer 19 yielded positive results with a 50 bp ladder on agarose gel (Fig. 6), displaying at least two bands. Similar amplification patterns were observed with primers EST-SSR 13 (Fig. 7), EST-SSR 26 (Fig. 8), and EST-SSR 41 (Fig. 9). However, no amplification occurred with EST-SSR 46 for accessions WWL2 and WWL8 (Fig. 10). Figure 11 illustrates the molecular variance among waterleaf populations and individual species within each accessional population. The analysis revealed significant genetic diversity, with 35% variance within individuals, 29% among individuals, and 36% variance among waterleaf populations. These findings indicate substantial genetic variation within and among the *Talinum* accessions.

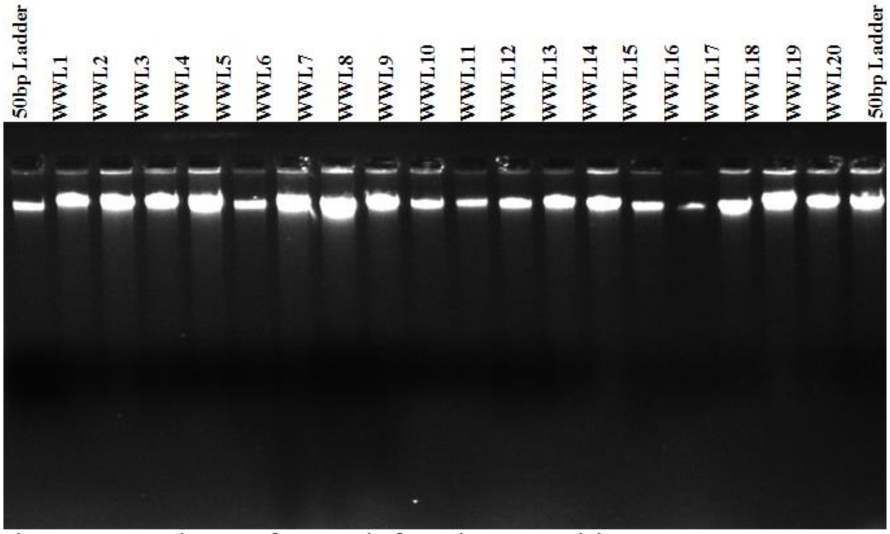

Fig. 6. DNA gel image of 20 waterleaf samples using Gel documentation system.

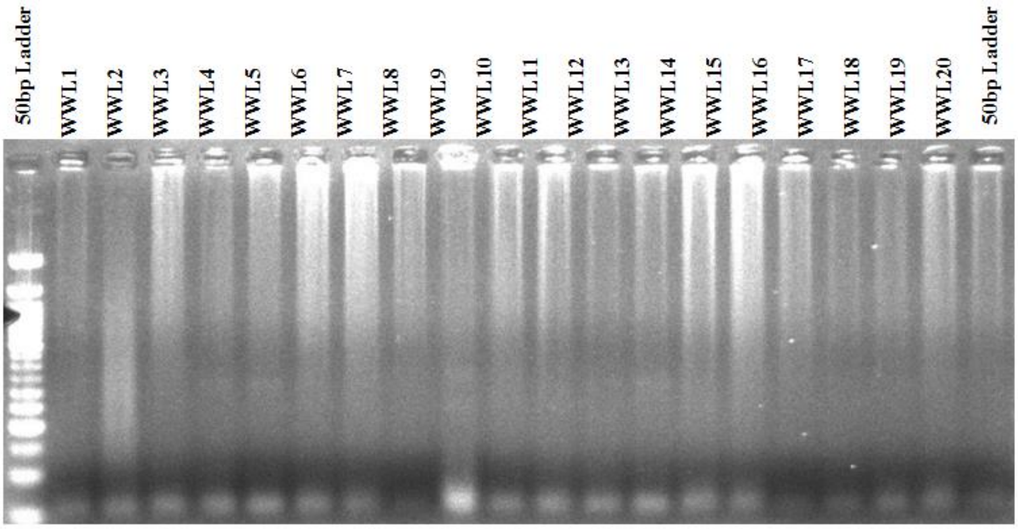

Fig. 7. PCR Amplification of Twenty Talinum accession using EST-SSR primer 13 with a (50bp ladder) on Agarose gel.

Phylogenetic analysis, employing the Neighbor-Joining (NJ) algorithm, revealed genetic divergence among the *T. triangulare* accessions based on genetic distance (Fig. 4). Notably, WWLP15, 17, and 20 clustered closely, while WWLP16, 18, and 19 exhibited distinct genetic profiles. In contrast, the UPGMA tree (Fig. 5) suggested a common ancestry for all accessions, indicating shared evolutionary history. However, this analysis also revealed subsequent genetic divergence, with WWLP10, 11, and 12 forming a separate cluster distinct from WWLP1, 2, 3, and 4. These findings underscore the complex genetic relationships within *T. triangulare*, highlighting both shared ancestry and significant genetic divergence.

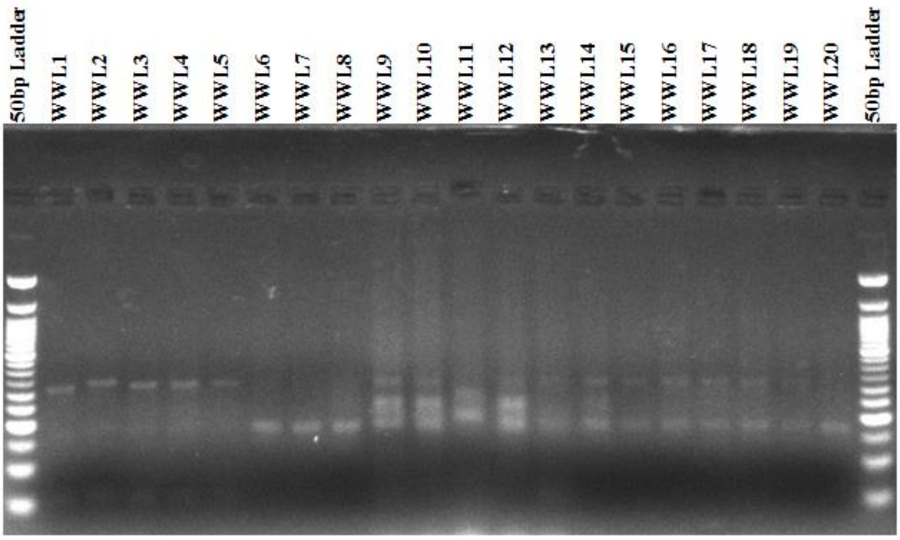

Fig. 8. PCR Amplification of Twenty Talinum accession using EST-SSR primer 19 with a (50bp ladder) on Agarose gel.

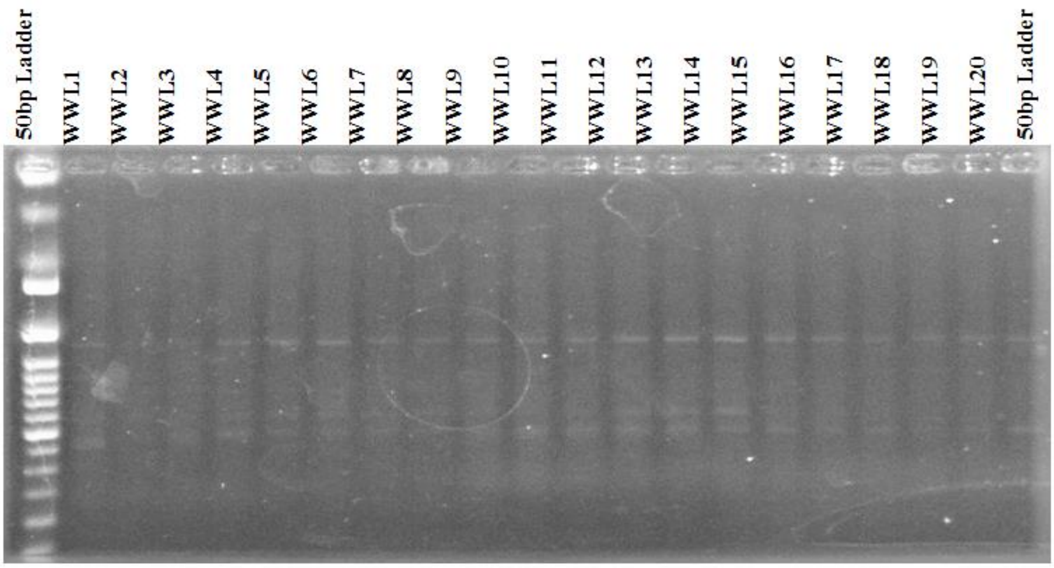

Fig. 9. PCR Amplification of Twenty Talinum accession using EST-SSR primer 41 with a (50bp ladder) on Agarose gel.

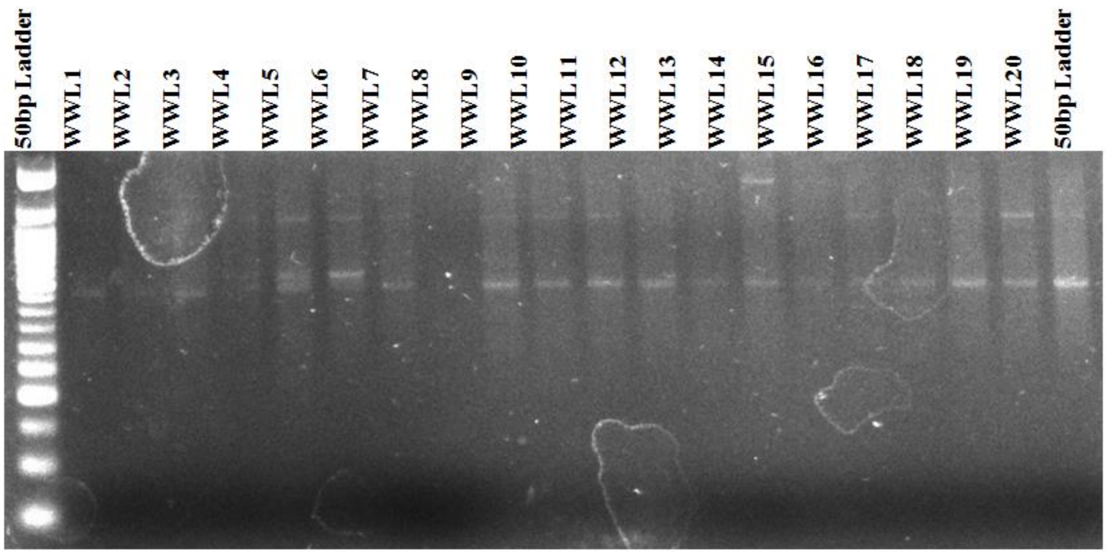

Fig. 10. PCR Amplification of Twenty *Talinum* accession using EST-SSR primer 46 with a (50bp ladder) on Agarose gel.

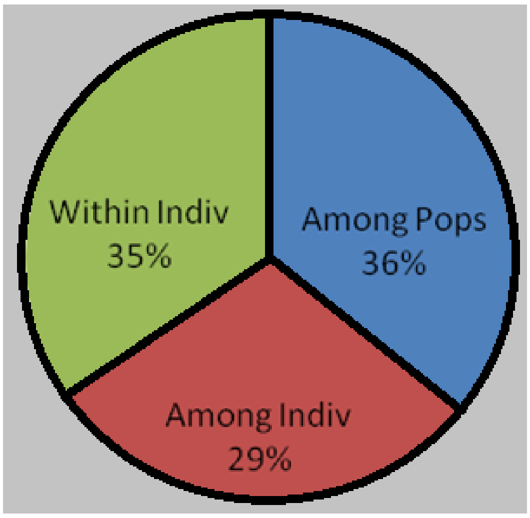

Fig. 11. Percentage molecular variance among water leaf population and among individual species within each accessional population.

Discussion

This research provided comprehensive insights into the morphological and genetic characteristics of *Talinum triangulare*, underscoring its significance as a valuable regional vegetable crop. By integrating morphological analysis with SSR-based genotyping, the study generated critical data on cultivation practices, stakeholder perspectives, and genetic variation. Morphological markers played a crucial role in assessing plant genetic diversity, contributing to advancements in crop productivity, conservation, and global food security. The identification of distinct morphological traits enabled researchers to differentiate among accessions, uncover hidden genetic variation, and inform targeted breeding programs aimed at improving yield, disease resistance, and environmental adaptability. Conservation strategies benefited from the ability to identify specific morphological features, supporting the preservation of genetically diverse populations. This multifaceted approach also clarified taxonomic relationships and phylogenetic histories among accessions. Moreover, the examination of morphological variation revealed mechanisms of plant adaptation, offering insights into responses to various environmental pressures. The demographic survey of 50 participants provided valuable socio-economic context surrounding waterleaf cultivation. The findings revealed a predominantly female-driven industry, with over half of the participants between the ages of 21 and 40, an age group representing a key stakeholder demographic in local food systems. The widespread consumption of waterleaf, along with the substantial presence of traders (36%), indicated a vibrant market. Furthermore, the unanimous acknowledgment of its culinary and medicinal importance aligned with existing research on the plant's nutritional and health benefits (Aronu et al., 2019; Nya et al., 2023).

Morphological analysis of *Talinum triangulare* accessions revealed remarkable diversity, highlighting the species' adaptability to varied environmental conditions. Notable variations were observed in plant height (36.24–82.64 cm) and leaf length (4.6–9.1 cm). This diversity aligned with previous findings on indigenous Nigerian vegetables (Nya et al., 2023; Swarna et al., 2015), demonstrating the plant's resilience across different ecosystems. Statistical analysis showed significant differences in plant height ($P = 0.027$) and leaf area ($P < 0.001$), suggesting complex interactions between genetic and environmental factors. These findings underscored the importance of conservation in preserving the species' morphological diversity, which supports ecological balance and agricultural sustainability. Conservation strategies could help safeguard the genetic diversity of *T. triangulare*, ensuring its adaptability and resilience for future generations. Genetic diversity analysis of 20 *T. triangulare* accessions using SSR markers resulted in successful amplification with five primers, indicating moderate genetic diversity (genetic diversity index: 0.43). This finding supported earlier research emphasizing the critical role of genetic variation in crop resilience (Nya et al., 2023; Rajpoot et al., 2020). Analysis of genetic variation revealed substantial differences both within and among accessions, 35% within individuals, 29% among individuals, and 36% among populations, underscoring the importance of genetic information in guiding targeted breeding efforts. Phylogenetic analysis identified distinct genetic profiles and clusters, reflecting shared ancestry and evolutionary adaptation. These genetic insights were essential for informing conservation strategies and developing breeding programs focused on enhancing resilience and promoting sustainable agriculture.

Conclusions

This research provided valuable insights into the morphological and genetic diversity of *Talinum triangulare* in Mid-Western Nigeria. By integrating morphological analysis with SSR-based genotyping, the study emphasized the importance of conservation strategies in preserving genetic diversity, promoting ecological balance, and supporting agricultural sustainability. The findings not only underscored the vegetable's significance in local diets and economies but also reinforced the critical role of genetic conservation in shaping strategies for sustainable agriculture, food security, and biodiversity protection. Moving forward, future research should explore the genetic mechanisms underlying the observed diversity and investigate how this variability can be harnessed in crop improvement programs.

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Author Contributions
Conceptualization, CEO and BI; methodology, WOI, WAO, CEO, and BI; software, WAO and BI; validation, WAO, CEO, and BI; formal analysis, WAO and BI; investigation, WOI and GOO; resources, all authors (private); data curation, BI; writing—original draft preparation, WOI and GOO; writing—review and editing, WOI, WAO, GOO, CEO, BI; supervision, CEO and BI; project administration, CEO and BI. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest
The authors indicate no conflict of interest in this work.

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