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AGRONOMIC PERFORMANCE AND MUTATION ANALYSIS OF PLASTID GENE OF CLONES OF TEA PLANTS UNDER CULTIVATION IN KAKARA TEA PLANTATION MAMBILA, SARDAUNA LOCAL GOVERNMENT AREA TARABA STATE – NIGERIA

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ABSTRACT

Tea is one of the most consumed non-alcoholic beverages globally and it is made from tea plant using the leaf. Morphological and molecular analysis are important for characterizing the plant. There is no documented information on agronomic performance and current mutation analysis of tea clones under cultivation in Kakara tea plantation. Therefore, this research seeks to determine the agronomic performances and mutation analysis of tea clones under cultivation in Kakara tea plantation using Mat K gene. Six original tea clones and 40 tea samples were randomly collected germplasm, Kusuku, Bangoba and Kasalasa. Morphological parameters measured, genomic DNA extracted, PCR amplifications and sequencing of Mat K gene were performed. Morphological data were analysed using Predictive Analytic Software. Dendrograms were constructed using Ward's method. Chromatograms were converted into nucleotide sequences using ChromasPro software, Multiple sequence alignment was performed using MEGA X software for SNPs analysis. The highest leaf length was 24.530±3.036cm from Kasalasa (KA2) and the least was 10.030±0.044cm for clone 236. The highest and least value of leaf width was 7.560±0.044cm (KA9) and 3.803±0.028cm (clone 236) respectively. The highest leaf area was 104.900±16.627cm^2 (KA2) and the least was 28.200±0.235cm^2 for clone 236. The leaf area-based dendrogram revealed two main clusters (Cluster I and Cluster II) with sub-clusters I and II. The mutation analysis of Mat K gene sequences from *C. sinensis* samples showed that KU3 (Kusuku 3) was more genetically diverse based on amino acids sequences when compared with all original clones. Morphological characters were used in this study. The mutation analysis of Mat K gene sequence revealed greater conserved region (homogenous population); depicting narrow genetic diversity in the tea population in Kakara Tea Plantation, Mambilla plateau, Taraba State, Nigeria. There is need to introduce new gene pool.

Keywords: Tea Plant, Morphological Traits, Chloroplast Gene, Diversity, Clones

1 INTRODUCTION

Tea is one of the mostly consumed non-alcoholic beverages globally [1] and it is an infusion of leaves of tea plant (*Camellia sinensis* (L.) O. Kuntze) with therapeutic benefits and pharmacologically important compounds [2]. This plant is grown primarily in the tropical and subtropical regions of Africa like Nigeria [2-4]; Asia and Europe [5-7]. *Camellia sinensis* phenotypic study are mostly based on visually accessible traits, such as leaf colour, leaf shape, and growth habits [7-9]. The research methods based on morphological analysis are easy to implement and are commonly used in the studies of genetic diversity in plants [7-9]. The maturase K (Mat K) gene encodes maturase that produces the Mat K protein**;** an organelle intron maturase responsible in chloroplast function; and important for correct plant cell function and photosynthesis [10].

Recently, the most effective markers for the detection of the genetic diversity of plants are DNA molecular markers [7,11-13]. Unlike morphological data, molecular approaches provide extensive information of plant species [9,12,13]. Molecular markers have been proved to be effective tools in the study of genetic variability and assessment of interrelationships between different genotypes [9-15]. Molecular analysis, genome size determination is also an important tool for characterizing germplasm to understand taxonomic relationships and track evolutionary changes and domestication events [11,16]. To date, there is no documented information on agronomic performance and current mutation analysis of tea clones under cultivation in Kakara tea plantation, Mambila Plateau, Sardauna Local Government Area of Taraba State, Nigeria to the best of our knowledge. Therefore, this research seeks to determine the agronomic performances and mutation analysis of tea clones under cultivation in Kakara tea plantation, Mambila Plateau, Sardauna Local Government Area of Taraba State, Nigeria using Mat K gene; an example of plastid gene

2 MATERIAL AND METHODS

2.1 Location and sample collection

Tea plant samples were collected in Mambilla Plateau, Taraba State; located at coordinates range of $7^{0}20'$N and $11^{0}43'$E [3]. A total of forty-six (46) tea clone samples were used for the study. Six (6) samples were collected from the six original clones (CBB36, C68, C143, C236, C318 and C1212). Also, forty (40) samples were randomly collected; comprising ten (10) from germplasm (G1-G10), ten (10) from Kusuku (KU1-KU10), ten (10) from Bangoba (BA1-BA10) and ten (10) from Kasalasa (KA1-KA10). The commercial tea plant plantation in Kakara, Taraba State are of different tea plants clones cultivated for production of both green tea and black tea by the Mambila Beverages Limited Nigeria as displayed (Figure 1).

2.2 Morphological characterization of tea plant

Agronomic characters of the tea plant clones were measure and recorded immediately after collecting of plant parts. All data sets were collected by a single person to minimize the visual errors for certain phenotypic or agronomic characters. The morphological descriptors used for data analyses was based on the outcomes of previous studies conducted on tea plants [9,16]. The parametric descriptors include: leaf length, leaf width, leaf area, shoot length and internode length and leaf area. To authenticate the data collected and to ensure uniformity of each measurement, shoot length was measured from the stalk to the 6^{th} leaf of each tea plant while the internode length was measured from the 5^{th} and 6^{th} leaves of each tea plant as displayed (Figure 2).

Figure 1: Commercial tea plant plantation in Kakara, Mamilla Plateau, Taraba State

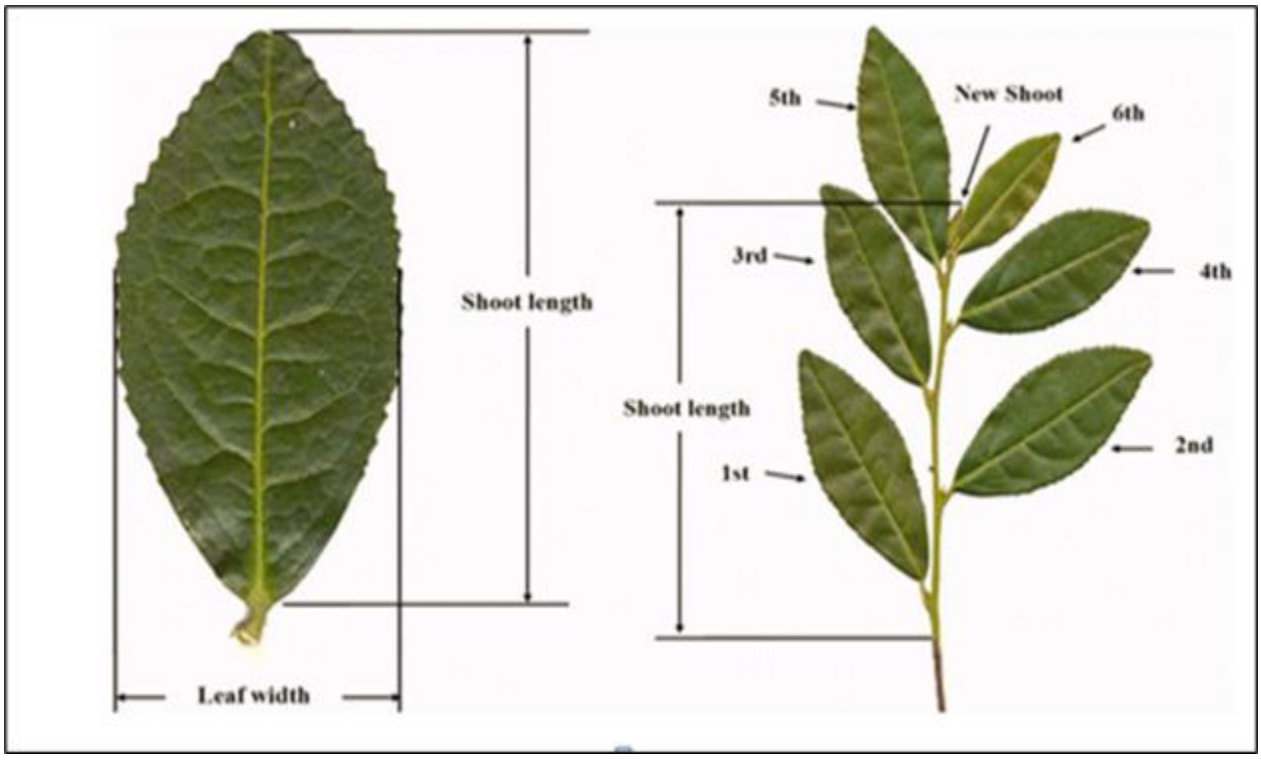

Figure 2: Measurement of the leaf parameters in tea plant [16,17]

The leaf area was calculated using the following formula according to Kim *et al*. [16] and Yong *et al*. [17] as follows:

Leaf area (cm^2) = Leaf length X Leaf width X (0.74). As for the leaf width, the longest and widest part was measured accordingly [16,17].

2.3 Genomic DNA extraction and primer design

Tea plant leaves were collected from the 46 tea clones. The leaves were dried, packaged, neatly labelled and forwarded to University of Free State, South Africa. Total genomic DNA of the ancient tea plant germplasm was extracted using a modified Cetyl-trimethylammonium bromide (CTAB) method [18]. Primers were designed using Integrated DNA

Technologies (IDT) primer tool (www.eu.idtdna. com). The 'Oligonucleotide Properties Calculator' online tool was used to determine the appropriate melting Temperature (Tm), Guanine-Cytosine (GC) content and any possible formation of Hairpin's for all primers. The primers were designed according to the following standards; a Tm closest to 60°C, a guanine-cytosine (G-C) contents of approximately 45%, a primer length of not less than 20 nucleotides. All primers had a preferable length of between 19 to 22 nucleotide bases. Once the primers are delivered in a pellet form, concentrated stock solutions of 100 µM (100 pmol/ µl) was prepared by dissolving each lyophilized pellet in Tris Ethylenediamine Tetra-Acetic acid (EDTA) buffer (10 mMTris, pH 7.5; 1 mM EDTA, pH 8.0). 10 µM working solution was prepared for future use during PCR [13].

2.4 Polymerase chain reaction (PCR) amplification

The compounds in the PCR mixture for amplification comprised 1.5 µL DNA (2 ng/µL), 10.72 µL ddH2O, 1.5 µL 10× buffer (Mg2+), 0.6 µL dNTP (2.5 mmol/L), 0.08 µL Easy Taq (5 U/µL), 0.3 µL primer of forward (10 µmol/L), and 0.3 µL primer Reverse (10 µmol/L). The PCR program had the following two stages: in the first stage, pre-denaturation was performed at 94 °C for 4 mins, denaturation at 94 °C for 40 seconds, annealing at Tx + 8 °C for 30 seconds (Tx is the optimal annealing temperature for the primer), and extension at 72 ◦ C for 90 seconds, for 16 cycles (temperature in each cycle was reduced by 0.5 °C). In the second stage, the annealing temperature was reduced to Tx, denaturation was performed at 94 ◦ C for 40 seconds, annealing at Tx for 30 seconds, extension at 72 °C for 90 seconds, for 24 cycles, and final extension was performed at 72 ◦ C for 12 mins and stored at 12 °C. The PCR products were detected by gel electrophoresis [19]. The capillary electrophoresis apparatus was developed and produced by AATI Corporation in America.

2.5 Sequencing of amplified gene

The bidirectional sequencing of purified PCR amplicon was carried out at Inqaba Biotech Laboratory, South Africa using the same primer pair that was used for amplification of Mat K gene. The reactions contained 1x Big Dye Terminator Premix, 3.2 µM PCR primer, 1x Sequencing buffer, and 3 µl PCR amplicon, in a total volume of 10 µl. The conditions for the thermal cycler consisted of an initial denaturation step at 96°C for one min, followed by 25 cycles of 96°C for 10 secs, 50°C for five secs, and 60°C for four mins.

2.6 Statistical and bioinformatics analysis

Morphological data (leaf length, leaf width, leaf area, shoot length and internode length and leaf area) were analysed using Predictive Analytic Software (PASW) version 20.0 and least significant differences (LSD) were determined. The p-value of ≤ 0.05 was considered statistically significant. Dendrogram of leaf area in tea plant was also constructed using Ward's method. Mat K gene sequences were confirmed from the National Centre for Biotechnology Information (NCBI) data base using BLASTN. The chromatograms were converted into nucleotide sequences using ChromasPro software (www.technelysium.com.au). Multiple sequences and pairwise alignments were carried out using Clustal W in MEGA X software according to Kumar *et al* [20] and excluding all gaps. Codon-Code Aligner version 6.06 was used to analysis SNPs in the aligned sequences.

3 RESULTS

Table 1 shows the morphological parameters (leaf length, leaf width, leaf area, shoot length and internode length) of tea plants in Mambilla Plateau, Taraba State, Nigeria. There were significant differences ($P<0.05$) in all the leaf parameters investigated. The highest value of leaf length was 24.530±3.036cm from Kasalasa community (KA2), followed by 19.66±0.047cm from Bangoba community (BA8) and the least was 10.030±0.044cm for clone 236. The highest and least value of leaf width was 7.560±0.044cm (KA9) and 3.803±0.028cm (clone 236) respectively. The internode length of 5.060±0.026cm was observed for *C. sinensis* from Kasalasa (KA10), followed by 5.010±0.017cm from Kusuku community (KU7 and KU8) and 2.960±0.024cm was the least value from from Kasalasa community (KA2). The highest value of leaf area was 104.900±16.627cm^2 (KA2), followed by 110.300±0.611cm^2 (BA8) and the least was 28.200±0.235cm^2 for clone 236 (Table 1).

The dendrogram of leaf area clustering pattern for 46 *C. sinensis* samples is illustrated in Figure 3. The leaf area-based dendrogram using Ward's method revealed two main clusters (Cluster I and Cluster II) with sub-clusters I and II. Though there were no specify pattern in the clustering of tea plant clones, sub-cluster I in the main cluster I comprised mainly of the original cultivated tea plant clones namely C1212, BB35, C318, C143 and C236. This notwithstanding, there were however traces of location-specific clustering, especially in the cultivated tea plant clones. We however, decided to cluster the leaf area of tea plant clones based on location/site of collection. It was observed that two clusters were generated; and the cultivated original clones were clustered with those from Bangoba while those collected from the germplasm were clustered with those of Kusuku and Kasalasa (Figure 4). Figure 5 displayed the dendrogram of internode length clustering pattern for 46 *C. sinensis* samples consisting of two main clusters (cluster I and cluster II). The dendrogram generated from internode length shows no specific trend or pattern of clustering as tea plant clones

collected from locations were mixed in the respective clusters. Cluster I further divided into two sub-clusters (sub-cluster I and sub-cluster II). The sub-cluster I in main cluster I generated 17 clones of *C. sinensis* samples; comprising four (4) from Kusuku community (KU6, KU7, KU8, KU9), four (4) from Kasalasa community (KA7, KA8, KA9, KA10), one (1) from Bangoba community (BA10), four (4) from germplasm (G3, G4, G7, G10) and two (2) from original clones (C318, C143) (Figure 5). The mutation analysis of Mat K gene sequences from *C. sinensis* samples are displayed on Figure 6 and the KU3 (tea sample from Kusuku 3) was more genetically diverse based on amino acids sequences when compared with all original clones (CBB36, C68, C143, C236, C318 and C1212).

4 DISCUSSIONS

The phenotype (morphological character) is one of the commonly method used in evaluating plants owing to the fact that it is simply based on the morphological traits in order to analyse the genetic diversity [7,9, 15, 21]. Interestingly, clonal identification and characterization is traditionally based on morphological descriptors such as plant shape, stem width, leaf shape, etc. [7,9, 21]. As has been mentioned that tea plant is an out-crossing species [1, 8, 22], as such it is highly heterozygous with most of its morphological, physiological and biochemical descriptors showing continuous variations and high plasticity [7, 9, 21]. In our current study, the highest value of leaf length was 24.530±3.036cm from Kasalasa community (KA2), followed by 19.66±0.047cm from Bangoba community (BA8) and the least was 10.030±0.044cm for clone 236. The highest and least value of leaf width was 7.560±0.044 cm (KA9) and 3.803±0.028cm respectively. The internode length of 5.060±0.026cm was observe

Table 1: Morphological parameters of tea plants in Mambilla Plateau

Clones	Leaf length (cm) X ± SE	Leaf width (cm) X ± SE	Shoot length (cm) X ± SE	Internode length (cm) X ± SE	Leaf area (cm^2) X ± SE
CBB36	11.090 ± 0.050	4.950 ± 0.020	13.140 ± 0.064	4.030 ± 0.025	40.800 ± 0.286
C68	10.850 ± 0.028	4.400 ± 0.027	14.330 ± 0.090	3.730 ± 0.033	35.300 ± 0.245
C143	12.060 ± 0.081	4.980 ± 0.053	16.170 ± 0.049	4.850 ± 0.027	44.500 ± 0.617
C236	10.030 ± 0.044	3.803 ± 0.028	11.650 ± 0.068	3.610 ± 0.039	28.200 ± 0.235
C318	12.770 ± 0.024	4.880 ± 0.046	11.730 ± 0.060	4.940 ± 0.035	46.100 ± 0.465
C1212	14.770 ± 0.047	4.860 ± 0.020	10.080 ± 0.076	2.960 ± 0.024	43.500 ± 0.237
G1	14.350 ± 0.085	6.600 ± 0.043	11.200 ± 0.019	4.950 ± 0.020	70.000 ± 0.485
G2	15.540 ± 0.046	6.260 ± 0.054	13.960 ± 0.062	4.060 ± 0.032	71.800 ± 0.586
G3	13.950 ± 0.028	5.850 ± 0.043	13.360 ± 0.046	4.940 ± 0.023	60.300 ± 0.417
G4	14.310 ± 0.043	6.440 ± 0.048	11.710 ± 0.068	4.870 ± 0.022	68.300 ± 0.596
G5	13.970 ± 0.031	6.020 ± 0.075	11.510 ± 0.054	3.970 ± 0.019	62.200 ± 0.691
G6	14.720 ± 0.036	6.520 ± 0.075	11.710 ± 0.067	3.960 ± 0.016	71.100 ± 0.876
G7	14.520 ± 0.039	6.370 ± 0.046	12.280 ± 0.075	4.960 ± 0.020	68.500 ± 0.544
G8	12.050 ± 0.032	5.030 ± 0.041	10.600 ± 0.060	4.540 ± 0.046	44.700 ± 0.455
G9	14.530 ± 0.054	6.42 ± 0.047	11.150 ± 0.093	3.950 ± 0.024	69.100 ± 0.713
G10	11.880 ± 0.052	6.070 ± 0.053	10.230 ± 0.061	4.860 ± 0.036	53.400 ± 0.474
KU1	14.110 ± 0.015	6.250 ± 0.067	11.360 ± 0.061	4.280 ± 0.042	65.300 ± 0.687
KU2	12.920 ± 0.048	6.040 ± 0.066	11.730 ± 0.053	3.760 ± 0.028	57.800 ± 0.707
KU3	12.570 ± 0.048	6.310 ± 0.066	11.200 ± 0.051	3.680 ± 0.041	58.700 ± 0.558
KU4	17.130 ± 0.037	6.950 ± 0.024	14.120 ± 0.058	3.800 ± 0.030	88.300 ± 0.437
KU5	15.930 ± 0.018	6.930 ± 0.045	13.550 ± 0.068	3.970 ± 0.020	81.600 ± 0.497
KU6	12.970 ± 0.023	6.480 ± 0.044	10.430 ± 0.060	4.790 ± 0.032	62.200 ± 0.402
KU7	14.050 ± 0.019	6.930 ± 0.043	11.630 ± 0.053	5.010 ± 0.017	72.000 ± 0.478

KU8	15.820 ± 0.094	6.690 ± 0.035	13.650 ± 0.051	5.010 ± 0.017	78.500 ± 0.698
KU9	15.630 ± 0.042	6.430 ± 0.049	13.600 ± 0.053	4.840 ± 0.031	74.200 ± 0.527
KU10	12.310 ± 0.042	6.910 ± 0.021	10.620 ± 0.050	3.890 ± 0.019	45.000 ± 0.279
BA1	11.650 ± 0.006	6.570 ± 0.048	9.700 ± 0.041	3.950 ± 0.019	56.700 ± 0.447
BA2	11.760 ± 0.066	5.770 ± 0.042	10.370 ± 0.042	3.740 ± 0.038	50.300 ± 0.492
BA3	15. 280 ± 0.040	6.490 ± 0.036	14. 200 ± 0.080	3.830 ± 0.028	73.300 ± 0.486
BA4	11.810 ± 0.071	6.010 ± 0.045	10.270 ± 0.116	4.020 ± 0.031	52.600 ± 0.515
BA5	12.890 ± 0.041	5.620 ± 0.047	11.060 ± 0.048	3.890 ± 0.024	53.700 ± 0.455
BA6	12.240 ± 0.042	5.680 ± 0.051	10.920 ± 0.054	3.860 ± 0.023	51.400 ± 0.477
BA7	15.150 ± 0.037	5.880 ± 0.061	11.550 ± 0.053	3.960 ± 0.022	66.000 ± 0.727
BA8	19.660 ± 0.047	7.590 ± 0.045	14.390 ± 0.077	4.040 ± 0.023	110.300 ± 0.611
BA9	15.970 ± 0.013	5.970 ± 0.057	13.520 ± 0.097	4.870 ± 0.028	75.500 ± 0.643
BA10	12.270 ± 0.042	5.540 ± 0.041	11.130 ± 0.042	4.670 ± 0.033	50.400 ± 0.467
KA1	13.960 ± 0.021	6.120 ± 0.036	11.590 ± 0.058	3.920 ± 0.021	63.100 ± 0.384
KA2	24.530 ± 3.036	5.490 ± 0.037	10.900 ± 0.043	3.160 ± 0.031	104.900 ±16.627
KA3	14.410 ± 0.050	6.620 ± 0.035	11.780 ± 0.058	3.980 ± 0.017	70.300 ± 0.414
KA4	12.480 ± 0.055	6.400 ± 0.038	10.750 ± 0.038	4.000 ± 0.023	59.300 ± 0.581
KA5	13.950 ± 0.020	6.690 ± 0.032	11.570 ± 0.043	4.120 ± 0.026	69.000 ± 0.383
KA6	12.970 ± 0.284	7.200 ± 0.045	12.950 ± 0.058	3.910 ± 0.022	68.800 ± 1.516
KA7	13.120 ± 0.035	5.810 ± 0.027	10.580 ± 0.052	4.960 ± 0.016	56.500 ± 0.303
KA8	14.580 ± 0.045	6.590 ± 0.040	11.730 ± 0.053	4.700 ± 0.033	71.100 ± 0.517
KA9	16.970 ± 0.360	7.560 ± 0.044	14.800 ± 0.045	4.810 ± 0.027	94.800 ± 2.017
KA10	15.420 ± 0.043	5.710 ± 0.028	11.860 ± 0.063	5.060 ± 0.026	65.200 ± 0.330
LSD	**4.917**	**0.412**	**0.620**	**0.252**	**1.860**

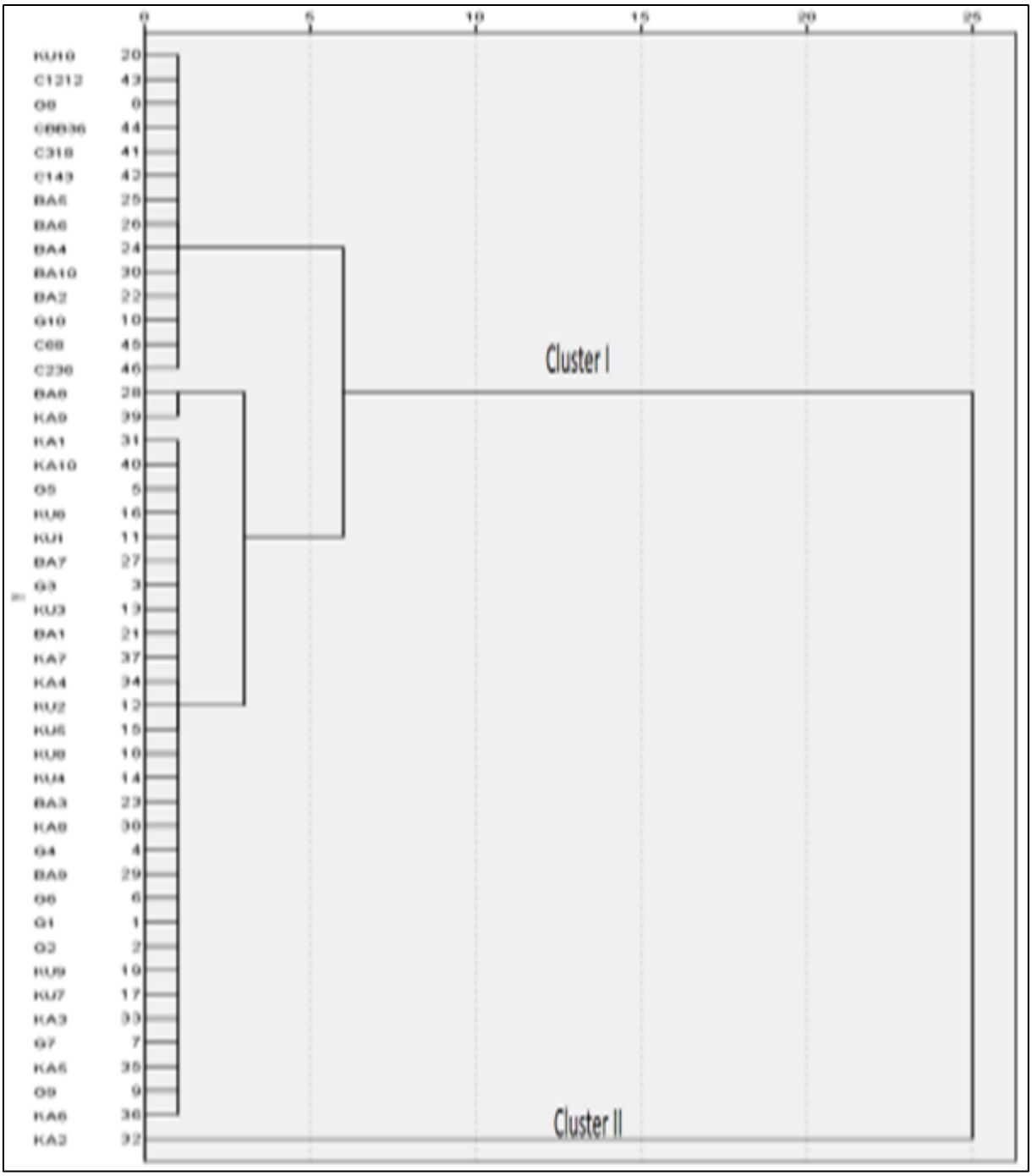

Figure 3: The dendrogram of leaf area clustering pattern of 46 samples

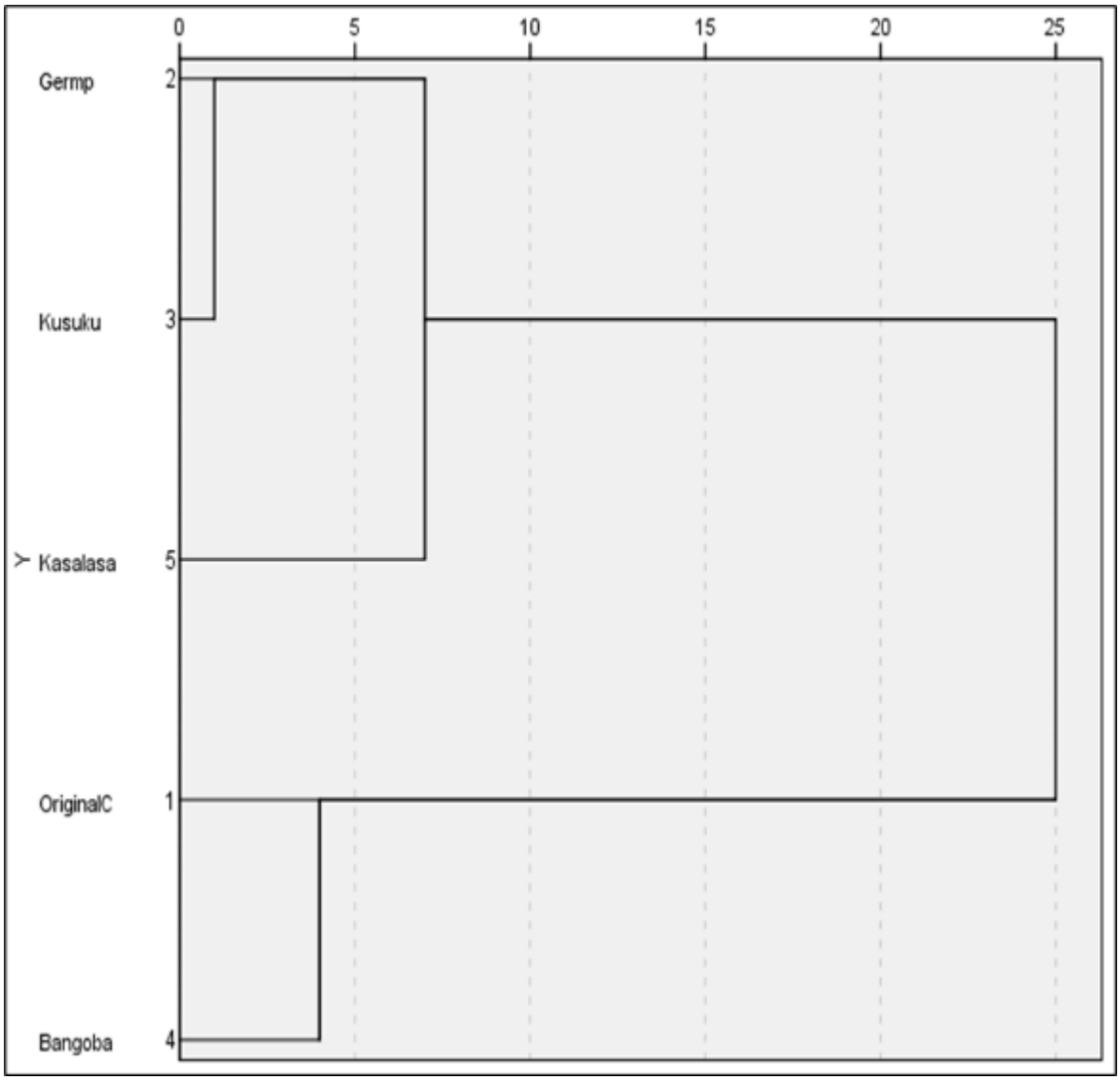

Figure 4: Dendrogram of mean leaf area in *C. sinensis* from different populations

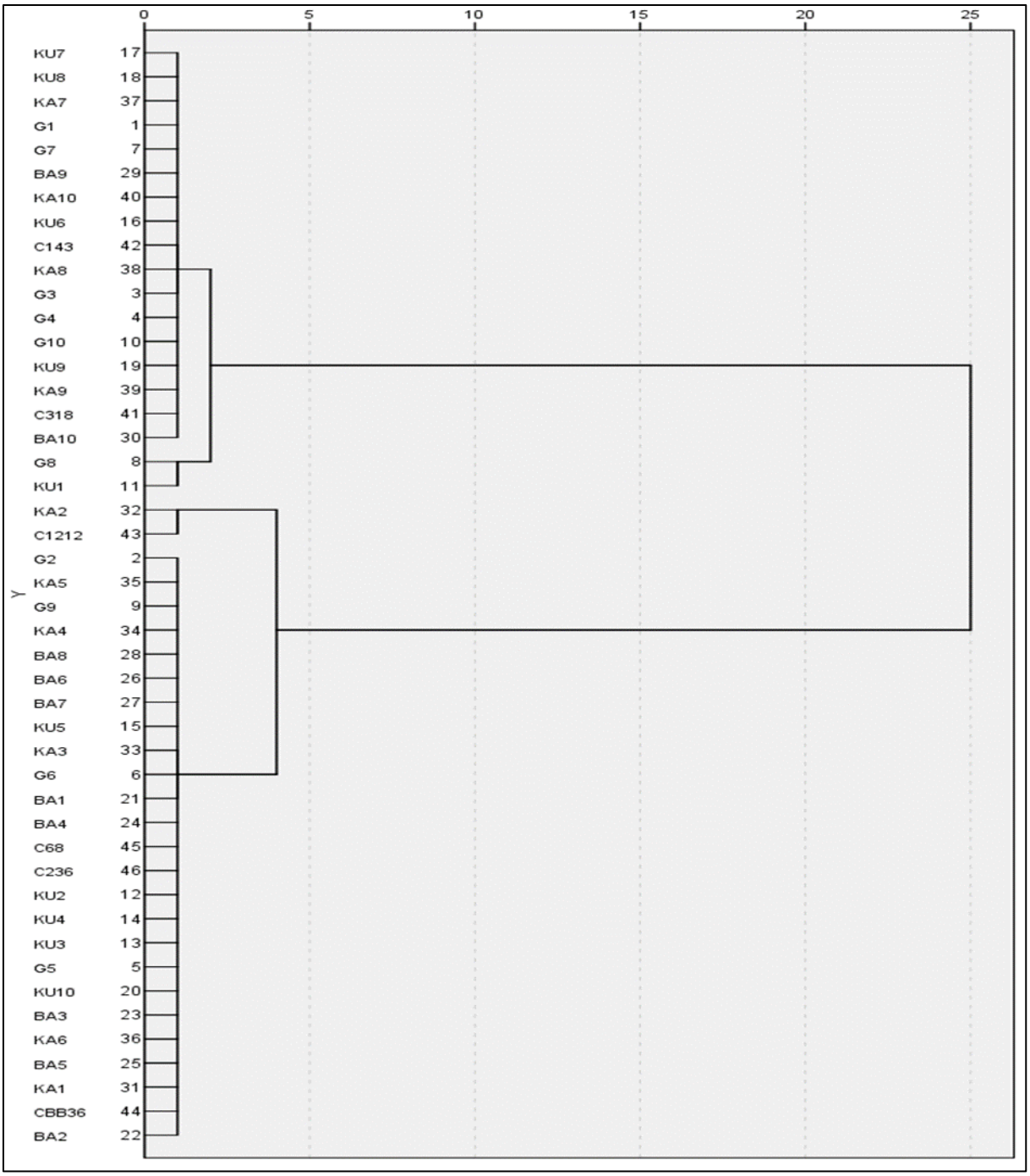

Figure 5: The dendrogram of internode length clustering pattern for 46 *Camellia sinensis* samples

Key: CBB36, C68, C143, C236, C318 and C1212 are original tea clones; G1-G10: Tea plant from germplasm; KU1-KU10 ten tea samples from Kusuku, BA1-BA10: ten tea samples from Bangoba and KA1-KA10: ten tea samples from from Kasalasa

Figure 6: Multiple alignment of Mat K amino acid sequences for 46 *Camellia sinensis* samples

for *C. sinensis* from Kasalasa (KA10) and 2.960±0.024cm was the least value from from Kasalasa community (KA2). The highest value of leaf area was 104.900±16.627cm2 (KA2), followed by 110.300±0.611cm2 (BA8) and the least was 28.200±0.235cm2 for clone 236. All tea plants were approximately 35 years old in the Kakara germplasm and Kakara commercially tea plantation. Samarina *et al*. [7] reported higher leaf area (34.3 – 149.1cm2) and leaf width (9.8 – 19.7 cm) in Russian germplasm tea plant clones that were approximately 31 - 33 years old when compared with the leaf width (lowest and highest value: 7.560±0.044cm and 104.900±16.627cm2 respectively) and leaf area (lowest and highest value: 28.200±0.235cm2 and 104.900±16.627cm2 respectively) obtained in this study that have approximately survived for about 35 years in Mambilla Plateau, Taraba State. These differences in morphological traits in our populations and other populations may be due to the variations in genetic architecture of different tea cultivars and prevailing environmental conditions; although, this study did not focus on partitioning of the genetic and environmental components of tea clones. Researchers had established that phenotypic differences may also be the reflection of environmental effects [9, 23]. Proper growth and functions of plants increased yield and improve the quality in tea crop [24]. Researchers [7-9, 21. 22] reported significant variations in morphological parameters among different *C. sinensis* clones and other plants. Also, Samarina *et al*. [7] previously reported significant moderate correlation between the genetic make-up and leaf area size variations among the different tea clones investigated in Russian germplasm. Therefore, similar reasons may be attributed to the variations in morphological parameters observed in this present study.

Tea plant is an out-crossing species and selected elite genotypes are propagated through vegetative means and released as clonal varieties [1, 22, 25]. The local farmers in Kusuku, Bangoba and Kasalasa communities cultivating tea plants took their parenating materials (vegetative part for planting) from the main Kakara commercial tea plantation, Mambilla Plateau, Taraba State; agreeing with other researchers [22, 25] in which tea plants were propagated through vegetative parts. It is worthy to mention relatively low amount of genetic variation between countries and the lack of geographic structure indicated that trans-national germplasm exchange of tea germplasm has been common practice among countries in Africa and Asia [25-27]. The dendrogram of leaf area clustering pattern for 46 *C. sinensis* samples using Ward's method revealed two main clusters; cluster I and cluster II with sub-clusters I and II. Though there was no specify pattern in the clustering of tea plant clones. However, sub-cluster I in the main cluster I comprised mainly of original cultivated tea plant clones (namely C1212, BB35, C318, C143 and C236). This notwithstanding, there were however traces of location-specific clustering, especially in the cultivated tea plant clones. We however decided to

cluster the tea plant clones based on location/site of collection. We observed that there were two clusters; where the cultivated original clones were clustered with those from Bangoba community while those collected from the germplasm clustered with those of Kusuku and Kasalasa communities. No wonder that parenating materials (vegetative part for planting) are often collected from the main Kakara tea plantation, Mambilla Plateau, Taraba State for planting by local communities' farmers to maintain better gene pool.

Single nucleotide polymorphisms (SNPs) are valuable for characterizing genetic diversity [1,13, 26-28]. Numerous molecular markers served as excellent markers in genetic analyses of *C. sinensis* [7, 13, 19, 26-28]. This study is one of the current molecular-based approach to determining the genetic variability of tea plant in Taraba State, Nigeria using Mat K gene (plastid gene); in which variation was observed on tea plant collected from Kusuku community when compared with others. This study revealed that genetic analysis can be used to track genetic admixtures. The implication of such finding means that the tea population in Kakara Tea Plantation originated from the same source; thus, having low genetic diversity among clones.

5 CONCLUSION

Morphological character is one of the traditional methods employed in evaluating diversity of tea clones and morphological descriptors such as leaf length, leaf width, leaf area, shoot length, internode length and leaf area were used in this study. The highest value of leaf length was 24.530±3.036cm from Kasalasa community (KA2) and the least was 10.030±0.044 cm for clone 236 (one of the original clones). The mutation analysis of Mat K gene sequence from *C. sinensis* samples revealed greater conserved region (homogenous population); depicting narrow genetic diversity in the tea population in Kakara Tea Plantation, Mambilla plateau, Taraba State, Nigeria.

Limitation

A single plastid gene (Mat K gene) was used for this study; revealing only maternal/organellar lineage and complementary nuclear markers could be considered in future research.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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