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Assessment of Morphological Insights for Germplasm Conservation

Disturbs Translation Machinery Spark of First Life and Consciousness

Highlights

Discovering Thoughts, Inventing Future

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rpl-11.1 Knock-Down Disturbs Translation Machinery and Proteostasis in *Caenorhabditis elegans*

By Zishuo Sam Li, Jocelyne Mills, Dennis Bonal & Callie Millette

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Summary- An array of human chronic diseases, such as Alzheimer's disease, Huntington's disease, and Parkinson's disease, are related to defects in cellular proteostasis and the formation of protein aggregates. Using Caenorhabditis elegans PP563, a model organism developed for studying proteostasis stress, we can elucidate the biological role of specific genes and proteins involved in translation and proteostasis, advancing our understanding of relevant pathologies and therapeutics. Here, we report the knockdown of rpl-11.1 in C. elegans leads to disturbance in protein translation and proteostasis pathways, including the Ubiquitin-Proteosome System (UPS) and selective autophagy. We confirmed the importance of rpl-11.1 in ensuring correct ribosome biogenesis and translation accuracy.

Keywords: rpl-11.1, caenorhabditis elegans, RNAi, ubiquitin-proteosome system, selective autophagy, germline apoptosis.

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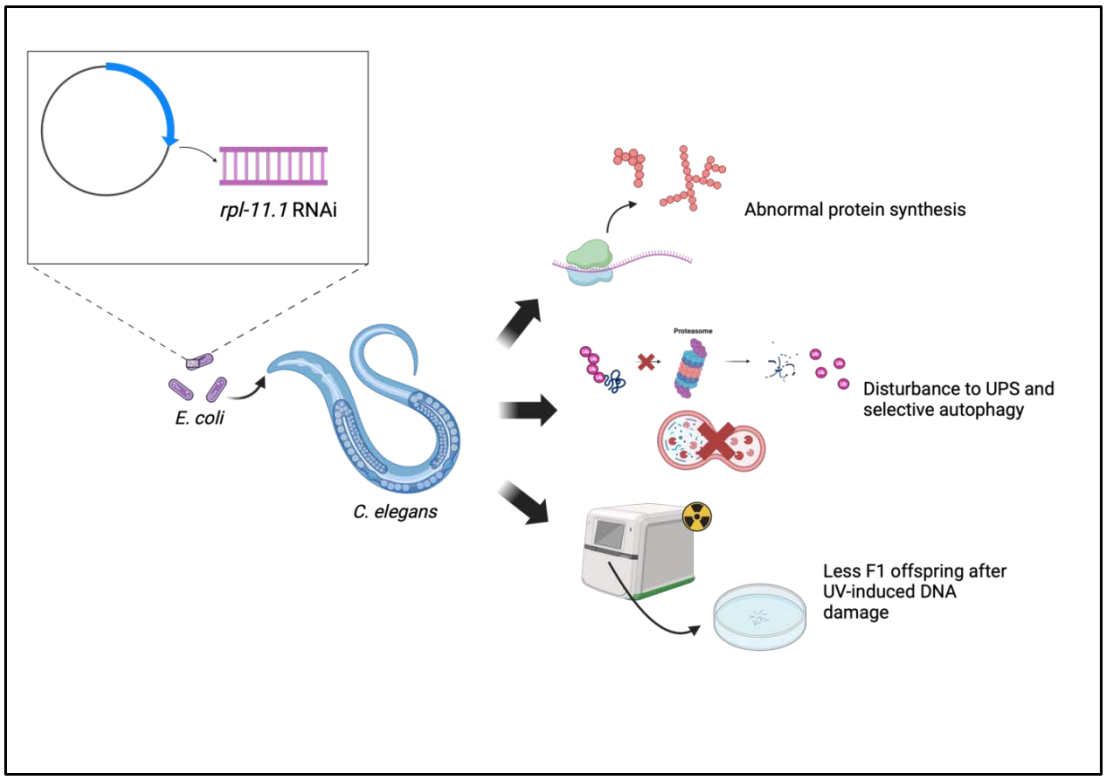
Keywords: *rpl-11.1, caenorhabditis elegans, RNAi, ubiquitin-proteosome system, selective autophagy, germline apoptosis.*

Graphical Abstract



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I. Introduction

Protein degradation is a major cellular process that maintains proteostasis and cellular physiology (Papaevgeniou & Chondrogianni, 2014). It primarily consists of degrading normal proteins in excessive amounts or eliminating damaged proteins, which might be a result of disturbances to normal

protein synthesis in the ribosomes. A major proteostasis pathway in charge of this role is the Ubiquitin-Proteosome System (UPS), which tags damaged or misfolded proteins with ubiquitin and digests them with the 26S proteosome complex (Kipreos, 2005).

Since the genes involved in UPS are generally evolutionarily conserved, their biological roles can be investigated in simple model organisms such as *Caenorhabditis elegans*, a well-studied nematode for understanding fundamental biological mechanisms. A specific strain of *C. elegans*, PP563, has been developed to specifically investigate the UPS pathway of proteostasis. Having a GFP linked to the ubiquitin protein, *C. elegans* PP563 provides a direct approach to monitoring UPS activity in nematodes through fluorescence signals (McCue et al., 2015). In a genetic screen that uses RNAi to silence 35 genes at the post-transcriptional level in *C. elegans* PP563, we discovered a particular gene, *rpl-11.1*, whose knockdown causes a marked increase in fluorescence signal in both intensity and localization. Such phenotype led us to deduce that *rpl-11.1* knockdown causes excessive UPS activity by increasing the number of active ubiquitin tags, which in turn disrupts proteostasis at the organismal level.

The biological role of *rpl-11.1*, a gene primarily expressed in the germline cells and pharyngeal muscle cells of *C. elegans* (Bgee, 2024), is still not completely elucidated. It was predicted to encode the 60S ribonucleoprotein L11-1 (RPL-11.1) in *C. elegans*, a homolog of ribosomal protein L11 (RPL-11) in humans (WormBase, 2022). RPL-11.1 constitutes a part of the large ribosomal subunit, which contains the peptidyl transferase center that catalyzes the formation of peptide bonds during mRNA translation (UniProt, 2024). Thereby, *rpl-11.1* knockdown might lead to the absence of RPL-11.1 in the ribonucleoprotein complex, affecting the biogenesis of ribosomes and forming a defect in translation machinery. The incorrectly assembled ribosomes will produce misfolded proteins with altered thermodynamic stability that may be functionally damaged or prone to aggregation after exposing hydrophobic regions. An excess of misfolded proteins and protein aggregates can be the source of proteostasis disturbance observed previously: a plethora of proteins are tagged with active ubiquitin and hence emit a substantial amount of fluorescence signal.

We thereby hypothesize that knocking down *rpl-11.1* via RNAi in *C. elegans* results in structurally incomplete ribosomes, which might produce misfolded proteins, recruit excessive ubiquitin for tagging and clearing through the UPS pathway, and ultimately overwhelm the proteostasis. Answering this hypothesis would reveal the biological role of *rpl-11.1* more clearly and possibly shed light on its relationship with translation efficiency and proteostasis regulation. To verify the hypothesis, two aims have been formulated: first, to determine if *rpl-11.1* has a role in ensuring correct protein synthesis. This will be achieved by conducting protein assays such as SDS-PAGE to compare the pattern of protein expression in *rpl-11.1* knockdown and that in *C. elegans* PP563 on an empty vector (L4440). We will further examine whether the other pathway of proteostasis that also makes use of ubiquitin tagging, selective autophagy, is involved in the clearance of *rpl-11.1* knockdown-induced protein aggregates and misfolded proteins as well. This would require knocking down *rpl-11.1* in *C. elegans* MAH215, which is built for studying autophagy (Chang et al., 2017), and conducting a double-gene RNAi knockdown in *C. elegans* PP563 for comparison with *rpl-11.1* single knockdown. Another minor aim of this study would be to determine the role of *rpl-11.1* in protecting germline cells. The human orthologue of *C. elegans* RPL-11.1 is involved in p53 pathway regulation through RPL11-MDM2 antagonism and acts as a tumor suppressor(Chène, 2003). Similarly, *C. elegans* also has a p53-like pathway (*cep-1*) that regulates DNA damage-induced apoptosis in germline cells(Derry, 2001). We hypothesize that knocking down *rpl-11.1* will inhibit cep-1 stabilization and hence germline apoptosis after UV-induced DNA damage, allowing increased germline cell proliferation and more egg deposition. This will be investigated through a F1 progeny count following parent generation exposure to UV.

As mentioned, the functionality and mechanism of *rpl-11.1* in *C. elegans* is not fully clarified in literature. Some of the of the earliest genome-wide screens indicate that *rpl-11.1* knockdown resulted in phenotypes such as life-span extension(Hsin & Kenyon, 1999) and gigantism(Patel et al., 2002). More recent studies have focused on the germline proliferation aspect of the gene as well as its evolutionary history in the *C. elegans* genome(Maciejowski et al., 2005). A more interesting study, without any experimental evidence, suggested the potential involvement of the gene in mitochondrial activity.(Sun et al., 2019). These various studies seemingly covered the role of *rpl-11.1* in *C. elegans* from a broad perspective, but none of them point out the specific mechanism by which this gene works in terms of its fundamental role: encoding a ribosomal protein. Additionally, none of the prior research exclusively focused on studying *rpl-11.1*. Here, we solely investigated *rpl-11.1* knockdown from the perspective of translation and proteostasis disturbance and germline proliferation. Elucidating the biological role of *rpl-11.1* in nematodes is of great importance as it can give insight into similar mechanisms in the translation machinery and UPS pathway of humans. A substantial homogeneity exists between humans and *C. elegans(Lai, 2000)*, so confirming the gene's role inthe nematode can potentially pave the way to developing therapeutic strategies for diseases related to protein aggregation and irregular proteostasis, such as Alzheimer's disease,

Huntington's disease, Parkinson's disease, and prion disorders(Papaevgeniou & Chondrogianni, 2014).

II. Methods

Culture of C. elegans. C. elegans PP563 and *C. elegans* MAH215 were used in this study and obtained from the *Caenorhabditis* Genetics Center (University of Minnesota, St. Pau, MN, USA). They were maintained at 25˚C under standard conditions on nematode growth media (NGM; 2% (w/v) agar, 0.3% (w/v) NaCl, 0.25% (w/v) peptone, 1 mM $CaCl_2$, 5 µg ml^{-1} cholesterol, 25 mM KH_2PO_4, 1 mM $MgSO_4$) agar plates(Sun et al., 2019). Nematodes were fed on *E. coli* OP50.

PP563 RNAi screen. The following screen protocol was followed:

Day 1. 100 uL of cultures of the Htt115 strain of *E. coli* containing the L4440 plasmid alone or containing an RNAi sequence targeting a specific gene were seeded onto NGM + carbenicillin p6 plates and allowed to dry overnight. Each plate has one targeting RNAi. (Refer to these plates as RNAi p6s.)

Day 2. All RNAi p6s had 50 uL of IPTG added to enhance expression of the RNAi. This was allowed to dry (~1 hour). Gravid worms were bleached to obtain a synchronized population of eggs. Approximately 50 eggs were plated onto the RNAi p6s and were allowed to develop to adulthood (~3 days) at 20˚C.

Day 5. About 10 worms were randomly selected from each RNAi p6, immobilized with sodium azide, and aligned for imaging. Micrographs were imaged at 150x magnification and 20ms exposure with LED at 16 and white-balanced. For the GFP signal, *C. elegans* PP563 was imaged at 800 ms exposure, and *C. elegans* MAH215 was imaged at 500 ms exposure. For the mCherry signal, *C. elegans* MAH215 was imaged at 500 ms exposure.

We conducted a preliminary screen for *C. elegans* PP563 with 36 different RNAi knockdowns. Of all the knockdowns that showed some degree of change in fluorescence signal in comparison to the L4440 genetic control, *the rpl-11.1* knockdown displayed the most drastic increase in both signal intensity and localization. We therefore decided to follow up on our investigation on *the RPL-11.1* knockdown exclusively.

The knockdown efficiency of *rpl-11.1* RNAi was confirmed using RT qPCR, where the mRNA level of the housekeeping gene *ama* was used as the internal reference. Primers for the target genes were designed by primer-BLAST (NCBI, 2019) and synthesized by a commercial company. The RT qPCR result showed that *rpl-11.1* RNAi achieved a near-complete (0.0025 fold change) knockdown of *rpl-11.1* in *C. elegans* PP563. It should be noted that the same gene knockdown in *C. elegans* MAH215 was not verified by RT qPCR.

Nematode population maintenance: Nematodes were synchronized by hypochlorite bleaching (2% sodium hypochlorite and 0.5 mol/L NaOH) according to standard protocols (Stiernagle, 2006). Approximately 1,000 extracted worm eggs were cultured on one NGM plate for protein isolation and RNA isolation (for RT qPCR cDNA synthesis).

Protein isolation: Proteins from *C. elegans* PP563 *rpl-11.1* knockdown and L4440 are isolated by washing the worms off the plate following standard protocols (Stiernagle, 2006), lysing through RIPA (50mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 1% or 5% SDS, tablet ofprotease inhibitor), centrifugation and incubation on ice. Proteins treated with 1% SDS are generally small-sized and soluble, while the 5% SDS aims to separate out proteins that are large-sized or aggregated.

SDS-PAGE: RIPA-treated proteins (including 1% SDS-treated and 5% SDS-treated) were loaded onto protein gel to run SDS-PAGE at 170V for the first 10 minutes and then at 200V for 1 hour. The gel was destained and visualized in imaging system.

Rpl-11.1 RNAi knockdown in C. elegans MAH215: C. elegans MAH215 were fed with *rpl-11.1* RNAi-expressing bacteria during development. Procedure similar to PP563 RNAi screen was followed. Micrographs were imaged at 31.6 ms exposure and 2x gain. GFP and mCherry fluorescence signal are later overlayed to create merged fluorescence micrographs.

Double-gene RNAi knockdown: Two genes (*rpn-6* and *lgg-1*, *rpl-11.1* and *lgg-1*, *rpn-6* and *rpl-11.1*) were knocked down via RNAi at once in *C. elegans* PP563 to evaluate the involvement of UPS and autophagy pathways of proteostasis in translation-defect cells' protein degradation. Bacteria expressing both types of RNAi are used as food source for worms and the procedure is similar to PP563 RNAi screen. Micrographs were imaged at 31.6 ms exposure and 2x gain.

UV radiation exposure: Two plates of *rpl-11.1* knockdown *C. elegans* PP563 and two plates of L4440 *C. elegans* PP563 were placed under a UV source (imaging system), with one plate exposed to UV for 30 sec and the other for 120 sec. Then, 5 adult worms from each plate is transferred to new plates seeded with *E. coli* OP50 and cultured for 3 days at 25˚C till the F1 progeny reaches adulthood. The number of worms on each plate was then counted manually. After worm (population) count, 10 worms from each group were transferred to new plate, immobilized and imaged under fluorescent microscope.

III. Results

a) 5% SDS-PAGE Reveals Potential Protein Aggregation

In *Figure 1*, it is observed that under 1% SDS treatment, which separates out smaller proteins, there is

no clear difference between the bands of *rpl-11.1* knockdown and the control (L4440). Nonetheless, under 5% SDS treatment that targets larger proteins, *rpl-11.1* knockdown exhibits markedly more bands and higher intensity in comparison with the control. These extra bands might be protein aggregates accumulated in the cells.

b) Rpl-11.1 Knockdown in MAH215 Induces More Merged Fluorescence Signal

In *Figure 2,* merged micrographs generally reveal more stand-alone m Cherry fluorescence in the control (L4440) and more merged fluorescence (mCherry and GFP) in *rpl-11.1* knockdown. One exception is that there are two nematodes in *rpl-11.1* knockdown that exhibit GFP fluorescence unaccompanied by any mCherry fluorescence. These two nematodes were believed to be dead by the time of imaging and were displaying auto-fluorescence because the GFP signal must be accompanied by the mCherry signal in *C. elegans* MAH215. Other than the two abnormalities, *rpl-11.1* knockdown displays mostly merged fluorescence.

It should be noted that in an attempt to verify if the autophagy pathway of proteostasis is involved in misfolded protein clearance, a double-gene knockdown experiment was also performed (refer to *Methods*). However, no valid results were obtained because there is minimal difference between the control and experiment groups (*Figure 3*).

c) Rpl-11.1 Knockdown Produces Less F1 Progeny after UV Exposure

Figure 4A reveals that there is no obvious difference between UV-treated *rpl-11.1* knockdown and the control (L4440) in terms of F1 progeny phenotype (fluorescence, size, mobility, etc.). However, when a worm count was conducted, it turned out that in both the 30s and 120s UV exposure groups, there are more nematodes surviving in the control group, with a 6.4-fold increase in the 30s exposure and a 2.5-fold increase in the 120s exposure. The knockdown of *rpl-11.1* inhibits nematode proliferation after UV exposure.

IV. Discussion

a) Protein Aggregation as a Result of Error in Translation Machinery

Our SDS-PAGE experiment substantiated that there are protein aggregates formed in *C. elegans* PP563 cells after *rpl-11.1* knockdown, as indicated by extra bands of proteins that are not seen in the control (*Figure 1*). Moreover, although the expression of smaller proteins appears to be similar in both the control and the *rpl-11.1* knockdown, we observed an overexpression of larger proteins (5% SDS-treated) in the *rpl-11.1* knockdown, suggesting a difference in protein expression pattern. This aligns with our hypothesis that abnormal protein synthesis will arise due to the absence of RPL-11.1 ribonucleoprotein in ribosome biogenesis. The abnormality can take the form of producing misfolded polypeptides that have altered thermodynamic stability, which are prone to exposing their hydrophobic regions and hence become aggregated (Berrill et al., 2011). Alternatively, the abnormal protein synthesis might also simply result in an overexpression of a group of large, insoluble proteins. Both outcomes will cause severe disruption to the UPS because excessive amounts of ubiquitin will be tagged to abnormal proteins, overwhelming the proteostasis pathway.

It is confirmed that knocking down *rpl-11.1* makes a difference in cellular protein synthesis in *C. elegans*. Next, we will attempt to identify which specific proteins are affected (overexpressed, misfolded, or become prone to aggregation) by such errors in translation machinery. Our preliminary RNAi screen indicated that most proteostasis defects occur in C. elegans intestinal cells after knocking down *rpl-11.1.* We will select a set of proteins whose expressions may be disturbed by the knockdown and use immunoblotting to confirm their identity.In addition, we will evaluate the degree of impact on ribosome biogenesis and translation efficiency caused by knocking down *rpl-11.1* through RT qPCR on rRNAs and polysome profiling, respectively.

b) Autophagy Pathway is Disturbed by Rpl-11.1 Knockdown Alongside UPS

Alongside UPS, the selective autophagy pathway of proteostasis also makes use of ubiquitin tagging to achieve protein degradation (Kocaturk & Gozuacik, 2018). We indeed observed a disturbance to the autophagy pathway in the *C. elegans* MAH215 *rpl-11.1* knockdown (*Figure 2*). The abundant mCherry signal in the control suggests that most GFP tagged to the autophagosomes has been quenched after fusing with the lysosome in the autophagy pathway. Conversely, in *rpl-11.1* knockdown, an increase in merged fluorescence signal indicates that autophagosomes are not efficiently undergoing lysosomal fusion, suggesting a delay in protein degradation and a buildup of protein waste. This is likely attributable to the protein aggregates and other large misfolded proteins generated by the translation error. Therefore, the hypothesis that selective autophagy is also involved in this knockdown-induced proteostasis disturbance is supported. Interestingly, autophagy differs from the UPS in that it primarily degrades long-lived proteins, insoluble protein aggregates, and organelles, whereas the UPS pathway targets short-lived proteins and soluble misfolded proteins (Kocaturk & Gozuacik, 2018). This aligns with our findings from the protein assay, which show that knocking down *rpl-11.1* produces protein aggregates and other insoluble large proteins,

necessitating the involvement of selective autophagy in maintaining proteostasis.

Our hypothesis could be better supported with results from the double-gene knockdown experiment. If simultaneously knocking down *rpl-11.1* and *lgg-1*, a key gene involved in autophagy (Romane Leboutet et al., 2023), produces a result that is no different from knocking down *rpl-11.1* alone, we could conclude that the autophagy pathway of proteostasis is already disturbed by knocking down *rpl-11.1* in addition to the UPS. However, unfortunately, the experiment did not yield valid data to draw any conclusions. We suspect that the RNAi knockdown efficiency might be problematic, resulting in an incomplete silencing of the targeted gene. To ensure validity in re-performing the double-gene knockdown experiment, we will include a confirmation of RNAi knockdown efficiency using RT qPCR.

c) Rpl-11.1 Might Be Integral to Germline Proliferation

We previously predicted that knocking down *rpl-11.1* will lead to more F1 offspring as germline apoptosis is inhibited by the deactivation of cep-1, which is under RPL-11.1 regulation(Schumacher et al., 2001). However, the experiment result indicates that *rpl-11.1* knockdown produces much less F1 progeny in comparison with the control after UV exposure for either 30s or 120s. The original hypothesis is hence rejected. We need to reconsider the role of *rpl-11.1* in maintaining germline proliferation.

According to Chang et al. (2017), cep-1, while activating DNA damage-induced germline apoptosis, is also required for meiotic chromosome segregation in the germline. Hence, it is reasonable that knocking down *rpl-11.1*, the stabilizer of *cep-1*, leads to less progeny after UV-induced DNA damage in the parent generation. Also, the mechanism by which cep-1 is stabilized by RPL-11.1 in *C. elegans* might be different from how p53 (the human homolog of cep-1) is stabilized by RPL-11 (the human homolog of RPL-11.1) in humans, requiring us to figure out the specific mechanism of cep-1 activation before making predictions. It can be concluded that *rpl-11.1* might be integral to germline proliferation in *C. elegans* in the face of radiation stress, based on the UV exposure experiment result.

In further investigation, we attempt to monitor the process of meiotic chromosome segregation in the germline of *rpl-11.1* knockdown *C. elegans* using live microscopy imaging. We will observe the gonads of the nematodes under fluorescent microscopes after their chromosomes are stained with fluorescent markers. This will allow us to evaluate our new hypothesis.

In conclusion, our original hypothesis was partially supported. The role of *rpl-11.1* in ensuring correct protein synthesis in *C. elegans* is confirmed. We believe the selective autophagy pathway of proteostasis is involved in the clearance of misfolded proteins alongside UPS, but evidence from the double-gene RNAi knockdown experiment is lacking. On the other hand, the role of *rpl-11.1* in protecting germline cells requires re-consideration and further investigation. It appears that the gene may be a necessity for meiotic chromosome segregation, as it plays an important role in nematode reproduction after radiation-induced DNA damage.

Due to the substantial homogeneity between humans and *C. elegans* (Lai, 2000), it is of utter importance to continue elucidating the biological role of *rpl-11.1* and its protein product in translation and cellular proteostasis, which relates closely to an array of human diseases involving proteomic defects. We would like to further evaluate the disturbance caused by RPL-11.1 dysfunction to translation efficiency by conducting polysome profiling and rRNA qPCR. The immunoblotting of specific proteins involved in the disturbance is equally crucial for developing therapeutic strategies toward relevant proteomic diseases.

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Abbreviations

UPS	Ubiquitin-Proteosome System
RNAi	Ribonucleic acid interference
mRNA	Messenger ribonucleic acid
DNA	Deoxyribonucleic acid
GFP	Green fluorescent protein
UV	Ultraviolet
SDS-PAGE	Sodium dodecyl-sulfate polyacrylamide gel electrophoresis
RT qPCR	Quantitative reverse transcription polymerase chain reaction
NGM	Nematode growth medium

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GJSFR-C Classification: *ASFA: Q1 01403*

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Results and implications: Overall, high morphological diversity was observed among the cultivars for all the traits evaluated. The most diverse traits included petiole color, leaf color, leaf vein color, flowering and seed set abilities, branching levels, end branch color, stem epidermis color, lobe margins, and growth habit of stem. Seven morphotypes with interesting features were identified through cluster analysis. Morphotype 1 is made of unflowering and unbranching cultivars with greenish-red petioles. Morphotype 2 is composed of varieties exhibiting purple petioles, three levels of branching, dichotomous branching habit, good flowering and seed set ability. Morphotype 3 made of only one cultivar was considered as outlier. Morphotype 4 cultivars are characterized by red petioles, compact plants, white root pulp and bad seed set hability; whereas cultivars belonging to morphotype 5 exhibited green petiole, good flowering and seed set abilities. Morphotype 6 is made of cultivars with sessile peduncle root, conical cylindrical root, orange root pulp, short distance between leaf scars, good flowering and seed set ability. Morphotype 7 genotypes exhibited dark green apical leaves, cream stem epidermis, two levels of branching, good flowering and seed set ability. The most diverse traits identified in this study could be used for genetic resources identification. Parent cultivars could be selected from morphotypes harbouring good flowering and seed set habilities.

Keywords: *phenotypic variability, core collection, qualitative trait, germplasm conservation, breeding programs, cassava, Togo.*

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I. Introduction

Cassava belongs to the family of Euphorbiaceae and includes 98 species. The crop is native to the American continent, being distributed from the USA to Africa. The main diversity center of cassava (Brazil) posses at least 78 species, approximately 80% of the total number of species. *M. esculenta* is its only domesticated species (Rogers and Appan, 1973).

Cassava plays an essential part in the food security of millions of families in tropical and subtropical regions of Africa. It is one of the main sources of carbohydrates, especially in developing regions, where it is grown as subsistence crop (FAO, 2023).Cassava has a wide range of uses in the so-called '4Fs' of: (i) food for human consumption, (ii) feed for animals, (iii) fuel, which in the form of ethanol is produced from cassava, and (iv) factories, where it is used to make alcohol, citric acid, clothing, medicines, paper, and chemicals. For many years, global demand for cassava has grown strongly due to its many industrial uses and the fact that it has often been cheaper than other starchy crops. This has then lifted it to the status of being the world's 5th most important crop, after corn, wheat, rice, and potatoes.

In 2022, 303 million tons of cassava were produced globally worlwide, grown on 23.87 million hectares, with an average yield of 11.24 t ha^{-1} (FAO, 2023). In Togo, cassava used to be a crop of the poor for a long time, but of late it is becoming more of a staple crop especially in the areas of production (Sogbedji et al., 2015). Across the country, 10,297 hectares were occupied by cassava plantations, and 38,542 tons of cassava root were harvested in 2022, with an average yield of 4 t ha^{-1}, 75% lower than the global productivity (DSID, 2022).

Cassava is a diploid (2n=36 chromosomes) and monoicous species, with predominantly allogamous fertilization, making it highly heterozygotic (Pootakhan et al. 2014) and giving it high genetic diversity, even though it propagates vegetatively (Costa et al. 2013). Cassava can adapt to different edapho climatic conditions, such as drought and low-fertility soils (Vidal et al. 2015). Because of these characteristics, cassava cultivation is attractive to farmers with limited resources in Togo.

Despite the importance of cassava as a staple crop (Sogbedji et al., 2015), its genetic diversity is poorly documented and consequently the genetic improvement of this crop is limited in Togo (Kombaté et al., 2017). The study of Kombaté et al. (2017) using ethnobotanical survey and morphological descriptors revealed the existence of high diversity. However, ethnobiological studies involving farmers' knowledge in varietal classification have shown large variations according to Agre et al.(2017). Also, there is no consistency in the naming of varieties by farmers. This results in the possibilities of duplicates and mislabelling within the local varieties collected from farmers' fields. Additionally, the number of local varieties with different features and names, most often planted together in a single field, suggest the existence of high diversity within this crop (Siqueira et al., 2009; Rabbi et al., 2015b), which is important for plant breeding and genetic resources programs. This substantial genetic variability is due to the high heterozygosity of the crop, ease of natural cross pollination, fruit dehiscence, and to the volunteer seedlings in farmers fields (Rabbi et al., 2015b; Ceballos et al., 2016). Besides, the informal plant material exchange between farmers promotes a large number of new cultivars and expand cassava genetic diversity (Peprah et al., 2020).

From a point of breeding, small-farm cultivation of cassava is of great importance to the conservation of genetic resources. Exploring the morphological diversity of a given germplasm is fundamental to guide its conservation, management and use in conventional breeding programs (Ceballos et al., 2016).

In West Africa, genetic diversity studies have been carried out for cassava germplasm management and breeding using both morphological descriptors (Adjebeng-Danquah et al., 2016; Agre et al., 2017; Kamanda et al., 2020) and molecular markers (Rabbi et al., 2014; Soro et al., 2024). In addition, multivariate analyses allows for the simultaneous integration of data for multiple traits and has been widely used to quantify the phenotypic diversity in several crops (Kamanda et al., 2020, Soro et al., 2024).

Morphological descriptors are inexpensive and easy to record for most breeders compare to molecular markers. They are strongest determinants of taxonomic classification and agronomic value of varieties (Soyode and Oyetundi, 2009; Rabbi et al., 2015b).

The objective of this study was to explore the phenotypic diversity in a *core* collection of cassava cultivars based on thirty two (32) morphological traits.

II. Material and Methods

a) Plant Material

A *core* collection (Table 1) made of: i.one hundred (100) cultivars obtained from major cassava growing areas across the country, ii. thirty five (35) improved varieties introduced from IITA cassava breeding program, iii. seven (7) cultivars sourced from the gene bank of the Laboratory of Virology and Biotechnology of the University of Lome and iv. two (2) varieties obtained from the cassava gene bank of Embrapa Mandioca Fruticultura (Cruz das Almas, BA, Brazil) was used in this study. Five improved varieties (high yielding and CMD resistant) namely Gbazekoute, TMS 96_0409, TMS 96_0166, CRI Sika Bankye and CRI Ampong bankye, recently released by the national cassava breeding unit were used as checks.

b) Experimental Site

The experiment was run at the Togolese Agronomic Research Institute (ITRA) station of Davié (latitude: 6° 23' 5" North; longitude: 1° 12' 18" East; altitude: 76 meters) located in the cassava production belt. This site is representative of typical cassava-growing conditions in Togo and is characterized by a bimodal rainfall pattern. During the experimentation, a total rainfall of 1231.5 mm was recorded for 80 rainy days. July was the highest monthly rainfall with 207.8 mm for 14 rainy days, while November was the lowest monthly rainfall with 8.7 mm for 4 days rainy days. The annual average temperature was 28.5°C. The egetation is characterized by herbaceous vegetation (Banito et al., 2010). The site's soil, suitable for cassava cultivation (Ezui, 2017) and known as 'Terres de Barre,' is characterized as sandy-clay with 70% sand, 3.8% silt, 8.1% clay, acid pH (H_2O 1:1) 5.5, 1.05% organic matter, 0.41% total nitrogen (N), 10 ppm available phosphorus (P), and cation exchange capacity (CEC) of 2.89 milli-equivalents (meq)/100g of soil in the top 15 cm samples (Sogbedji et al. 2015).

c) Experimental Design, Field Layout and Maintenance

The experiment was laid out in an augmented block design with one hundred fourty four (144) cultivars as tested genotypes and five (5) checks varieties, distributed in twelve (12) blocks. Each block was delimited after ploughing and harrowing of the site. Distance of 1.5 m separated adjacent blocks and plots. The experimental unit was composed of four rows of 4 m with 16 plants of a genotype. A spacing of 1 m between plants and rows was adopted. The experiment was carried under rainfed conditions. Neither herbicide nor fertilizers were applied. The experiment was kept weed free by regular hand weeding. The trial was harvested twelve months after planting.

Table 1: List of Togo's Cassava Germplasm Cultivars Characterized

N°	Cultivar	Type	Origin	N°	Cultivar	Type	Origin
1	CRI Sika Bankye	Improved	Ghana	27	TMS 92_0326	Improved	Togo
2	CRI Ampong Bankye	Improved	Ghana	28	TMS 96_1708	Improved	Togo
3	TMS 95_0166	Improved	IITA	29	TMS 98_2132	Improved	Togo
4	TMS 96_0409	Improved	IITA	30	TMS 99_0554	Improved	Togo
5	Gbazekoute	Landrace	Togo	31	Agbede	Landrace	Togo
6	TMS 01_0006	Improved	IITA	32	Agou	Landrace	Togo
7	TMS 00_0354	Improved	IITA	33	Aguidagba	Landrace	Togo
8	TMS 00_0364	Improved	IITA	34	Akaleyo	Landrace	Togo
9	TMS 01_0034	Improved	IITA	35	Akebou	Landrace	Togo
10	TMS 01_0046	Improved	IITA	36	Akoss	Landrace	Togo
11	TMS 01_0093	Improved	IITA	37	Ankra atihe	Landrace	Togo
12	TMS 01_0098	Improved	IITA	38	Akpadjin Feto	Landrace	Togo
13	TMS 01_0131	Improved	IITA	39	Alagno	Landrace	Togo
14	TMS 01_0379	Improved	IITA	40	Ankra 3	Landrace	Togo
15	TMS 01_1085	Improved	IITA	41	Ankra Atiyibo	Landrace	Togo
16	TMS 01_1086	Improved	IITA	42	Assiatoe	Landrace	Togo
17	TMS 01_1097	Improved	IITA	43	Atidjin1	Landrace	Togo
18	TMS 01_1206	Improved	IITA	44	Atidjin 2	Landrace	Togo
19	TMS 01_1224	Improved	IITA	45	Atidjin Poli	Landrace	Togo
20	TMS 01_1368	Improved	IITA	46	Atidokpo	Landrace	Togo
21	TMS 01_1368(2)	Improved	IITA	47	Atihe1	Landrace	Togo
22	TMS 01_1371	Improved	IITA	48	Atiyibo 1	Landrace	Togo
23	TMS 01_1610	Improved	IITA	49	Atiyobo2	Landrace	Togo
24	TMS 01_1662	Improved	IITA	50	Awou	Landrace	Togo
25	TMS 01_1797	Improved	IITA	51	Awouye	Landrace	Togo
N°	Cultivar	Type	Origin	N°	Cultivar	Type	Origin
53	Badjogou	Landrace	Togo	79	Kanbom Bantchi	Landrace	Togo
54	Bazoka	Landrace	Togo	80	Kanigbeli 1	Landrace	Togo
55	Bob	Landrace	Togo	81	Kanigbeli 2	Landrace	Togo
56	Bob Assou	Landrace	Togo	82	Kataoli	Landrace	Togo
57	Bob Yegue	Landrace	Togo	83	Katawole	Landrace	Togo
58	BRS Caipira	Landrace	Brazil	84	Kidirondi	Landrace	Togo
59	Degaule	Landrace	Togo	85	Kisseimou Koutowou	Landrace	Togo
60	Djakoagni	Landrace	Togo	86	Kola	Landrace	Togo
61	Djeble	Landrace	Togo	87	Kolaoung	Landrace	Togo
62	Djolaoba	Landrace	Togo	88	Kolmon kamkam	Landrace	Togo
63	Djoliba	Landrace	Togo	89	Kossikouma	Landrace	Togo
64	Donmoyibo	Landrace	Togo	90	Koutowou 2	Landrace	Togo
65	Fetonegbodji	Landrace	Togo	91	Kperoung Felgou	Landrace	Togo
66	Flawavi	Landrace	Togo	92	Kperoung Mamougue	Landrace	Togo
67	Gabonvi-ESA	Landrace	Togo	93	Kpla	Landrace	Togo
68	Gbadovi	Landrace	Togo	94	Loki	Landrace	Togo
69	Gbaze- ESA	Landrace	Togo	95	M'beou	Landrace	Togo
70	Vivigbaze	Landrace	Togo	96	MM96/5280	Improved	Togo
71	Ghana spana	Landrace	Togo	97	MM96/JW2	Improved	Togo
72	Gnidou	Landrac‹	Togo	98	Nigeria Fleur	Landrace	Togo

73	Hogninvo 1	Landrace	Togo	99	Nigeria Kikpaou	Landrace	Togo
74	Hogninvo 2	Landrace	Togo	100	Nigeria Kissaimon	Landrace	Togo
75	Inconnu	Landrace	Togo	101	N'tossou	Landrace	Togo
76	IRAT- Davie	Landrace	Togo	102	Ankra atihe	Landrace	Togo
77	Jhonson	Landrace	Togo	103	Okpoli	Landrace	Togo
78	Kalba	Landrace	Togo	104	Pela	Landrace	Togo
N°	**Cultivar**	**Type**	**Origin**	**N°**	**Cultivar**	**Type**	**Origin**
105	Peloumkoute	Landrace	Togo	131	D00_126	Improved	IITA
106	Penivi	Landrace	Togo	132	D00_54	Improved	IITA
107	Sabe	Landrace	Togo	133	D00_166	Improved	IITA
108	Sankara	Landrace	Togo	134	Toma 9	Landrace	Togo
109	Sassakawa	Landrace	Togo	135	CVTM4	Landrace	Togo
110	Sorad	Landrace	Togo	136	Toma 162	Landrace	Togo
111	Sawa	Landrace	Togo	137	Unknown 02	Landrace	Togo
112	Spana Assou	Landrace	Togo	138	TMS 96_1317	Improved	Togo
113	Spana Yegue	Landrace	Togo	139	TMS 96_0304	Improved	Togo
114	BRS Tapioqueira	Landrace	Brazil	140	TMS 96_0102	Improved	Togo
115	Tassiodo	Landrace	Togo	141	TMS 96_0869	Improved	Togo
116	Tchigouevi	Landrace	Togo	142	TMS 96_1642	Improved	Togo
117	Tetetidadjin	Landrace	Togo	143	TMS 96_0590	Improved	Togo
118	TME 419	Improved	Togo	144	TMS 96_539	Improved	Togo
119	TM1	Improved	Togo	145	TMS 96_1565	Improved	Togo
120	TME1	Improved	Togo	146	TMS 96_0603	Improved	Togo
121	TME 696	Improved	Togo	147	TMS 30572	Improved	IITA
122	Touwevi	Landrace	Togo	148	KPEM_10_03	Improved	Togo
123	Tuaka Atsu	Landrace	Togo	149	TMS 4(2) 1425	Improved	IITA
124	Tuaka komi Mami	Landrace	Togo	129	D00_208	Improved	IITA
125	Yabaka	Landrace	Togo	130	D00_14	Improved	IITA
126	D00_8300	Improved	IITA	52	Unknown	Landrace	Togo
127	M94_0583	Improved	IITA				
128	D00_137	Improved	IITA				

d) Phenotypic Data Collection

Thirty two (32) morphological traits were recorded using the cassava descriptor (Guevara et al., 2010) at three (3), six (6), nine (9) and twelve (12) months after planting (MAP). Data were recorded from the plants within the whole plot, and the most frequent occurence variant was noted. At 12 MAP, the inner eight (8) plants within each plot were uprooted and observations on roots were taken. The traits assesment date, and method of assessment are summarized in Table 2.

Table 2: List of Morphological Traits recorded in Togo's Cassava Germplasm

N°	Trait	Code	Assessment date	[1]Assessment scale
1	Colour of apical leaves	ColApLea	3 MAP	3, 5, 7 or 9
2	Pubescence on apical leaves	PubApLea	3 MAP	0 or 1
3	Lobe margins	LoMar	6 MAP	3 or 5
4	Colour of leaf vein	ColLeaVe	6 MAP	3, 5, 7 or 9
5	Petiole Colour	PetCol	6 MAP	1, 2, 3, 5, 7 or 9
6	Leaf color	LeaCol	6 MAP	3, 5, 7 or 9
7	Number of leaf lobes	NLeaLo	6 MAP	3, 5, 7, 9 or 11
8	Shape of central leaflet	ShaCeLea	6 MAP	1-10
9	Orientation of petiole	OriPet	6 MAP	1, 3, 5 or 7
10	Flowering hability	FlHa	6 MAP	0 or 1
11	Pollen	Pol	6 MAP	0 or 1
12	Leaf retention	LeaRet	6 MAP	1-5
13	Stipule margin	StiMar	9 MAP	1 or 2
14	Length of stipule	LenSti	9 MAP	3 or 5
15	Color of stem cortex	ColStCor	9 MAP	1-3
16	Colour of stem epidermis	ColStEpi	9 MAP	1, 2, 3, or 4
17	Colour of stem exterior	ColStExt	9 MAP	3, 4, 5, 6, 7, 8 or 9
18	Colour of end branches of adult plant	CoEBran	9 MAP	3, 5, or 7
19	Growth habit of stem	GrHaSt	9 MAP	1 or 2
20	Distance between leaf scars	DisLeaSca	9 MAP	3, 5, or 7
21	Prominence of foliar scars	ProFoSca	9 MAP	3 or 5
22	Fruit	Frt	9 MAP	0 or 1
23	Levels of branching	LeBran	12 MAP	0, 1, 2 or 3
24	Branching habit	BranHab	12 MAP	1, 2, 3 or 4
25	Root constrictions	RoCons	12 MAP	1-3
26	Colour of root cortex	ColRoCor	12 MAP	1-4
27	Colour of root pulp	ColRoPu	12 MAP	1-5
28	External colour of storage root	ExColRo	12 MAP	1-4
29	Extent of root peduncle	ExRoPed	12 MAP	0, 3 or 5
30	Shape of plant	ShaPl	12 MAP	1-4
31	Root shape	RoSha	12 MAP	1-4
32	Texture of root epidermis	TexRoEpi	12 MAP	3, 5, or 7

[1] *Each phenotypic trait had distinct phenotypes which were depicted by the values ranging from 0 to 9. Images associated with these scale values can be found in Fukuda et al. (2010). MAP = months after planting*

e) Phenotypic Diversity Analyses

The morphological diversity of the *core* collection was assessed following two approaches. Traits distribution was determined using Microsoft Excel (2016) in the first approach. In the second approach, morphological data were subjected to Multiple Correspondence Analysis (MCA) for identification of relevant traits contributing mostly to the germplasm diversity (Giles et al. 2018). From the MCA results, traits that presented the highest variability were used as active variables to perform cluster analysis for morphotypes identification within the germplasm using the Ward's method (Kawuki et al. 2011). The optimal number of clusters was determined using the distribution of the variance function methods. The morphological diversity of the germplasm was visualized by plotting the factors scores for individual genotype in the first factorial plan in order to assess the relationship among cultivars (Selamawit Abebe et al. 2021). Analyses were run in SAS version 9.4.

III. Results

a) Descriptive Analysis of Morphological Traits

The variability observed for qualitative traits among cassava cultivars is given in figure 1. In all 37.58% cultivars showed purplish green colour, 22.82% had purple, 3.36% showed dark green and 36.24% had purplish green colour. About 45% of the cultivars had pubescence on apical leaves, while 55% had not (Figure 1). Approximately 25% of cultivars had yellowish green petioles, 22.82% purple petioles, 16.78% red petioles, 15.54% reddish-green petioles, 11.41% green petioles and 8.72% accessions showed greenish-red petioles (Figure 1). Nearly half of the cultivars (47.65%) had green leaf vein, 28.19% cultivars showed reddish-green leaf vein in less than half of the lobe, 13.42% had reddish-green leaf vein in more than half of the lobe and 10.74% had red leaf vein. Four morphotypes were observed in the germplasm based on the leaves colour. The first morphotype with dark green leaves was

represented by 51.68% of the cultivars, the second morphotype had light green leaves and was represented by 22.82% of the cultivars, the third morphotype exhibited purple green leaves and was represented by 14.76% of the cultivars and the fourth one had purple leaves was represented by 10.74% of the cultivars. The petioles of most cultivars in the collection were horizontal (50.34%), the irregular type was observed in 29.53% cultuvars. 12.75% cultivars showed petioles inclined downwards while 7.38% cultivars had petioles inclined upward. About 40.27% accessions had lanceolate central leaflet, 32.88% had elliptic-lanceolate, 10.07% accessions had oblong-lanceolate central leaflet, 10.74% had obovate-lanceolate, 4.03% had ovoid, 0.67% had linear, 0.67% had pandurate and 0.67% had linear pandurate central leaflet (Figure 1). Most cultivars (66.44%) showed a winding lobe margin and 33.56% cultivars in the germplasm had a smooth lobe margin. In the germplasm, 61.07% cultivars had nine leaf lobes, 28.19% had seven leaf lobes, 8.05% had eleven and 2.69% five leaf lobes. For the leaves retention trait, 31.54% cultivars exhibited very poor leaf retention, 33.56% cultivars showed less than the average leaf retention, 19.46% exhibited average leaf retention while 15.44% cultivars exhibited better than average leaf retention (Figure 1).

With regards to stem related traits, 31.54% cultivars showed an upright growth habit of the stems, while the cultivars exhibiting a zigzag growth habit were observed in 68.46% cultivars. About 44% of the characterized cultivars had green end branches, 42.95% cultivars had green-purple and 12.75% had purple end branches. Four morphotypes were observed in the germplasm with regard to the stem epidermis colour (Figure 1). About 33.56% cultivars had light brown stem epidermis, 25.5% had dark brown, 24.83% had orange and 16.11% showed light-green stem epidermis. The colours of stem cortex observed were dark green (61.07% cultivars), orange (22.82% cultivars) and light green (16.11% cultivars). Approximately 30% cultivars had silver stem exterior, 17.45% had greenish-yellowish stem exterior, 14.17% showed gray stem, 12.08% orange stem, 10.74% dark brown stem, 9.39% light brown stem and 6.04% golden stem exterior. Majority of the cultivars (63.09%) exhibited prominent foliars scars while 36.91% had semi- prominent foliar scars. The distance between leaf scars was short for 92.63% cultivars characterized, medium for 6.04% cultivars and long for 1.34% cultivars. About 69.80% of the cultivars had long stipules while 30.20% cultivars had short stipules. The stipule margin of 61.74% cultivars was split or forked and entire for 38.26% cultivars (Figure 1).

Differences in the flowering ability among cultivars were observed. About 50% of the cultivars produced flowers while remaining did not flower. At harvest, seeds were observed on 42.28% of cultivars. Cultivars exhibiting zero level of branching (47%) and three level of branching (34.23%) were predominant. About 60.40% cultivars showed trichotomous branching habit, 26.85% cultivars showed dichotomous type, 8 cultivars had tetrachotomous type while only 4 cultivars showed an erect type. Cassava cultivars examined phenotypically based on their plant shape exhibited variation with umbrella (39.60% cultivars), compact (33.56%), open (12.64%) and cylindrical (6.04%) shapes. (Figure 1)

At harvest, the external colour of storage root also exhibited variation with dark brown (40.82%), light brown (35.37%), yellow (12.93%), and white or cream (10.88%) colours. The root cortex colour showed high variability among cultivars and four morphotypes were observed. Majority (49.0%) had white or cream root cortex colour, followed by yellow (22.2%), pink (11.1%) and purple (8.9%) colours. Root epidermis was white or cream for 47.62% cultivars, yellow (18%), pink (20.41%) and purple (6.52%). Based on the colour of root pulp cultivars exhibited variation with cream (48.98%), yellow (30.61%), white (14.29%), and pink (6.12%) colours. In the germplasm, 69% cultivars had conical cylindrical root shape, 18.37% cylindrical, 9.52% conical and 3.4% had irregular root shape. Cultivars with few (50.34%) and some (30.61%) root constrictions were predominant. Majority of the cultivars (77.55%) had sessile roots, whereas 14.29% had pedunculate roots. The mixed type was recorded on 8.16% of the cultivars. The texture of root epidermis exhibited variation with rough (33.33%), smooth (34%), and intermediate (32.65%) textures. (Figure 1).

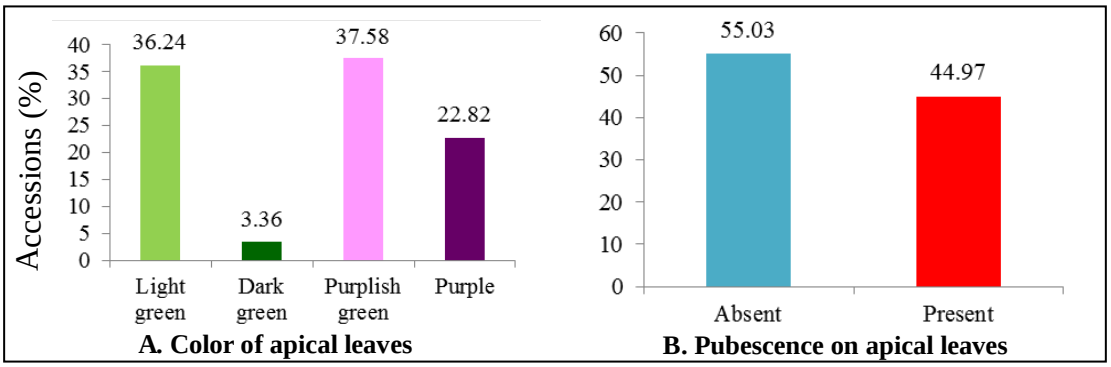
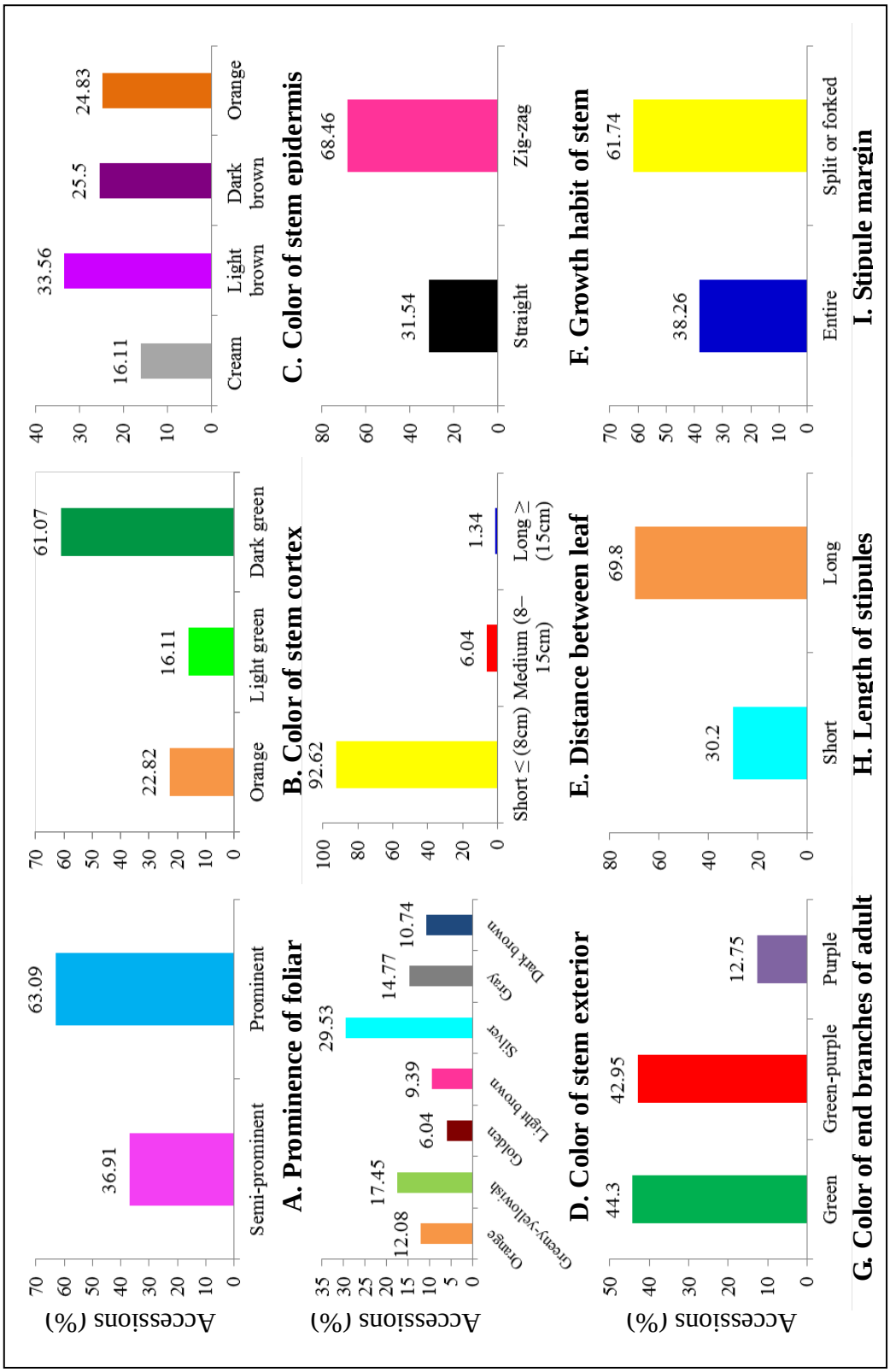


Figure 1: Frequency Distribution of 149 Cassava Cultivars based on Morphological Traits



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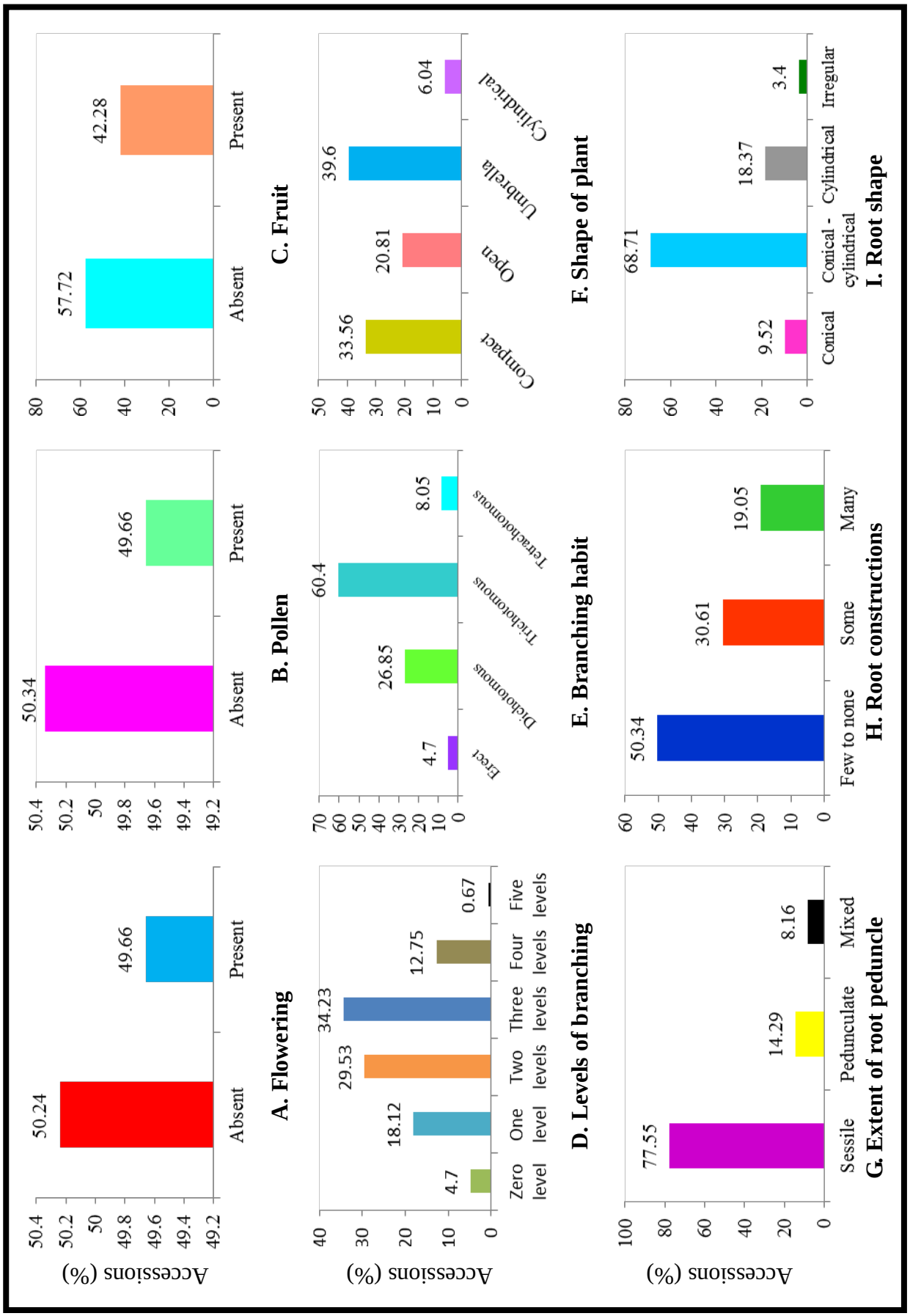

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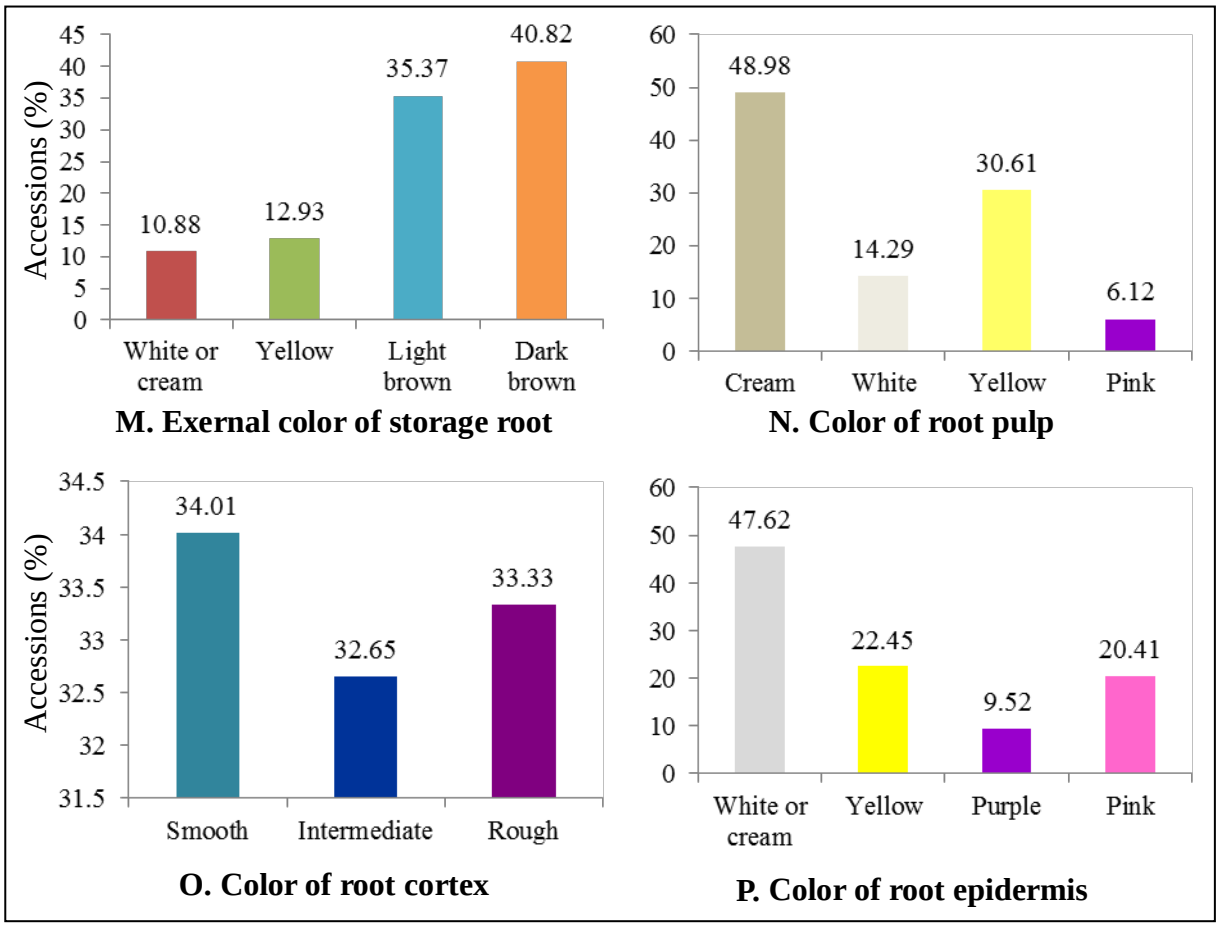

Figure 1: Frequency Distribution of 149 Cassava Cultivars based on Morphological Traits

b) Diversity Among Cultivars and Differentiation based on Morphological Traits

i. *Significant traits describing the germplasm diversity*

The objective of Multiple Correspondence Analysis (MCA) is to provide interpretable visualization of complex variable space. The meaning given to the axes and analysis of proximities between traits and conditions are usually made from the factorial planes. Thus, the first factorial plan and the factors having eigenvalue greater than one were retained. On the basis of this criterion, the first 11 factors with an eigenvalue greater than one were significant and therefore retained for the subsequent analyses (Table 3). These first eleven factors (Fs) explained 68.14% of the morphological variability among cultivars.

Factor 1 with an eigenvalue of 5.17 and accounted for 16.16% of the morphological variability. This factor was strongly correlated with petiole colour, leaf colour, colour of leaf vein, flowering and seed set ability and the levels of branching. Factor 2 with an eigenvalue of 3, explained 9.40% of the total variation, and was positively defined by leaves colour of end branches, color of stem epidermis, leaf lobe margin and the growth habit of stem. Factor 3 represented by traits such the colour of stem exterior and color of stem cortex, had an eigenvalue of 2.5, and explained 7.81% of the divergence among cultivars. Factor 4 with an eigenvalue of 1.73 correlated with leaf retention and color of apical leaf. Factor 5 with an eigenvalue of 1.71 was related mainly to the distance between leaf scars. In Factor 6, shape of central leaflet and pubescence on apical leaves were the main traits, while the extent of root peduncle was most important trait in factor 7. In factor 8, the most important traits describing the germplasm variability were the stipule length and the prominence of foliar scars. Factor 9 with an eigenvalue of 1.26, contribute 3.95% of the total variability and was mainly related to the stipule margin. Factor 10 was mainly represented by the orientation of petioles, plante shape and the color of root cortex while factor 11 was represented by the colour of root pulp (Table 3).

Table 3: Eigen values, proportion of variation and contribution associated with the axes of the MCA of 32 qualitative traits

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
Eigenvalue	5.172	3.000	2.502	1.734	1.718	1.508	1.394	1.362	1.265	1.148	1.003
Variability (%)	16.161	9.375	7.817	5.419	5.369	4.714	4.356	4.256	3.953	3.586	3.134
Cumulative %	16.161	25.536	33.353	38.773	44.142	48.855	53.211	57.467	61.420	65.007	68.141
ColApLea[1]	0.053	0.122	0.001	**0.287**	0.006	0.010	0.016	0.040	0.037	0.051	0.068
PubApLea[2]	0.001	0.019	0.091	0.040	0.001	**0.180**	0.047	0.146	0.016	0.003	0.009
LeaRet[3]	0.024	0.122	0.000	**0.298**	0.028	0.019	0.117	0.018	0.029	0.012	0.027
ShaCeLea[4]	0.001	0.000	0.049	0.047	0.020	**0.230**	0.034	0.053	0.052	0.025	0.021
PetCol[5]	**0.494**	0.235	0.028	0.002	0.005	0.003	0.039	0.001	0.001	0.012	0.002
LeaCol[6]	**0.426**	0.282	0.003	0.000	0.001	0.043	0.013	0.010	0.015	0.031	0.000
NuLeaLob[7]	**0.352**	0.005	0.036	0.001	0.018	0.159	0.038	0.012	0.058	0.000	0.006
LobMar[8]	0.003	**0.187**	0.000	0.016	0.142	0.023	0.007	0.021	0.149	0.003	0.001
ColLeaVei[9]	**0.409**	0.352	0.002	0.000	0.015	0.027	0.012	0.008	0.017	0.016	0.008
OrPet[10]	0.014	0.142	0.061	0.023	0.001	0.027	0.146	0.008	0.000	**0.215**	0.013
Flow[11]	**0.714**	0.095	0.013	0.042	0.000	0.004	0.003	0.013	0.000	0.007	0.017
Pol[12]	**0.714**	0.095	0.013	0.042	0.000	0.004	0.003	0.013	0.000	0.007	0.017
Fru[13]	**0.635**	0.082	0.012	0.065	0.002	0.001	0.002	0.022	0.000	0.001	0.007
ProFoSca[14]	0.104	0.001	0.001	0.000	0.072	0.148	0.000	**0.224**	0.036	0.065	0.003
ColSteCor[15]	0.113	0.084	**0.232**	0.028	0.101	0.107	0.047	0.047	0.034	0.001	0.000
ColSteEpi[16]	0.014	**0.187**	0.018	0.021	0.086	0.173	0.001	0.005	0.009	0.057	0.013
ColSteExt[17]	0.016	0.079	**0.301**	0.039	0.154	0.038	0.017	0.036	0.012	0.010	0.001

[1]Colour of apical leaves, [2]Pubescence on apical leaves, [3]Leaf retention, [4]Shape of central leaflet, [5]Petiole Colour, [6]Leaf color, [7]Number of leaf lobes, [8]Lobe margin, [9]Colour of leaf vein, [10]Orientation of petiole, [11]Flowering hability, [12]Pollen, [13]Fruit, [14]Prominence of foliar scars,[15]Color of stem cortex,[16] Colour of stem epidermis, [17]Colour of stem exterior. Traits that contributed most to the morphological variation of a particular factor are in bold and underlined

Table 3: Continued: Eigen values, proportion of variation and contribution associated with the axes of the MCA of 32 qualitative traits

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
Eigenvalue	5.172	3	2.502	1.734	1.718	1.508	1.394	1.362	1.265	1.148	1.003
Variability (%)	16.161	9.375	7.817	5.419	5.369	4.714	4.356	4.256	3.953	3.586	3.134
Cumulative %	16.161	25.536	33.353	38.773	44.142	48.855	53.211	57.467	61.420	65.007	68.141
DisLeaSca[18]	0.105	0.001	0.010	0.061	**0.189**	0.009	0.023	0.052	0.085	0.052	0.042
GroHaSte[19]	0.036	**0.179**	0.025	0.002	0.028	0.011	0.105	0.070	0.031	0.000	0.031
ColBrAdPl[20]	0.275	**0.319**	0.006	0.074	0.045	0.011	0.048	0.007	0.002	0.001	0.012
LenSti[21]	0.058	0.020	0.061	0.096	0.005	0.002	0.019	**0.243**	0.011	0.002	0.081
StiMar[22]	0.007	0.000	0.021	0.031	0.018	0.005	0.110	0.001	**0.489**	0.055	0.016
LevBran[23]	**0.211**	0.193	0.022	0.073	0.143	0.000	0.029	0.002	0.039	0.000	0.002
BraHab[24]	0.048	0.015	**0.179**	0.052	0.054	0.139	0.008	0.007	0.001	0.017	0.076
ShaPl[25]	0.085	0.087	0.044	0.161	0.016	0.011	0.004	0.018	0.000	**0.198**	0.044
ExRoPed[26]	0.004	0.002	0.121	0.000	0.093	0.044	**0.233**	0.046	0.000	0.003	0.028
RoConst[27]	0.003	0.035	0.082	**0.179**	0.016	0.009	0.048	0.054	0.000	0.025	0.103
RoSha[28]	0.003	0.008	**0.244**	0.011	0.000	0.025	0.013	0.008	0.054	0.026	0.000
ExtColRo[29]	0.001	0.022	**0.428**	0.000	0.135	0.003	0.081	0.032	0.009	0.049	0.001

ColRoPul[30]	0.120	0.001	0.051	0.036	0.006	0.038	0.008	0.017	0.038	0.051	**0.239**
ColRoCor[31]	0.117	0.020	0.041	0.005	0.148	0.004	0.111	0.009	0.036	**0.151**	0.092
TexRoEpi[32]	0.014	0.009	**0.308**	0.001	0.168	0.000	0.009	0.117	0.005	0.003	0.021

[18]Distance between leaf scars, [19]Growth habit of stem, [20]Colour of end branches of adult plant, [21]Length of stipule, [22]Stipule margin, [23]Levels of branching, [24]Branching habit, [25]Shape of plant, [26]Extent of root peduncle, [27]Root constrictions, [28]Root shape, [29]External colour of storage root, [30]Colour of root pulp, [31]Colour of root cortex, [32]Texture of root epidermis. Traits that contributed most to the morphological variation of a particular component are in bold and underlined.

c) *Structure of the Germplasm Diversity*

Factor 1 is positively correlated to the flowering and seed set ability and negatively correlated to the leaf colour, while factor 2 is positively correlated to the end branches colour, stem epidermis colour, leaf lobe margin, and the growth habit of stem (Figure 2). With regard to figure 2, four morphotypes were distinguished. Morphotypes 1 and 2 exhibited good flowering and seed set ability but differred in terms of the end branches colour, stem epidermis colour, and growth habit of stem. The varieties belonging to morphotype 3 and 4 did not flower and differred in terms of the colour of the end branches, stem epidermis and the leaf lobe margin.

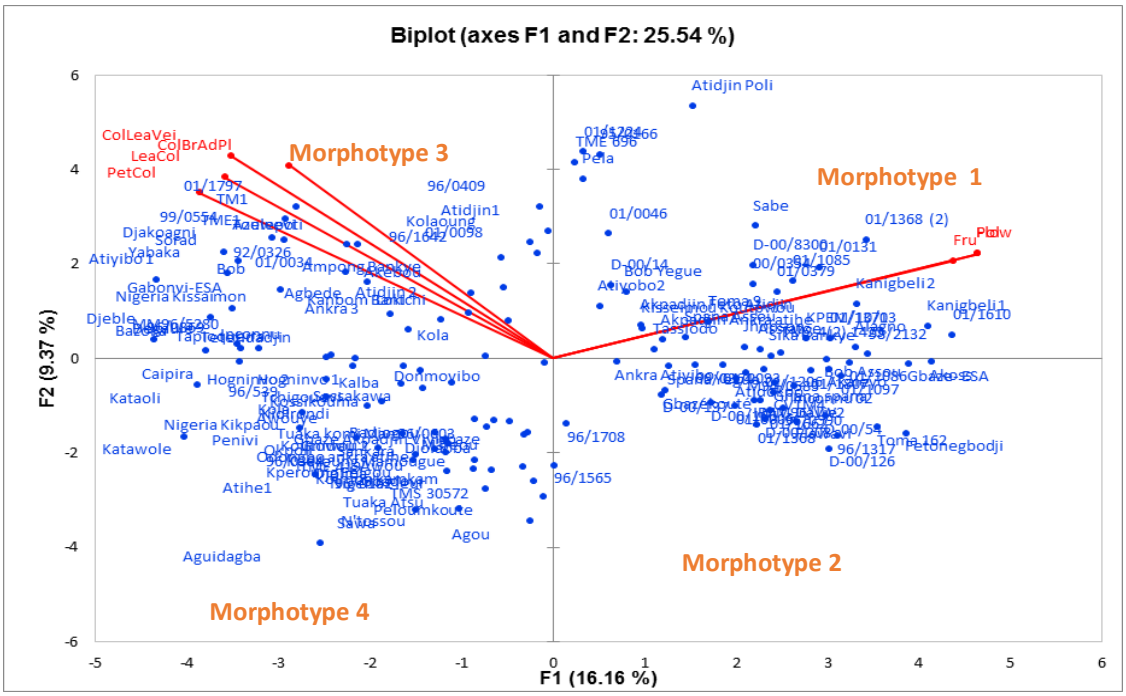

Figure 2: Component patterns of the MCA based upon factor 1 and 2 using significant phenotypic traits observed on 149 cassava cultivars of Togo

From the cluster analysis, the variance distribution function revealed that the optimal number of clusters was seven, with within class variance of 53.7 (Figure 3). Thus, the varieties were clustered into seven morphotypes (Figure 4). Morphotype 1 was composed of 137 varieties among which 53 were improved varieties, while remaining were landraces. Morphotype 1 is made of unflowering and unbranching cultivars with greenish-red petioles (Figure 4 and 5). Morphotype 2 was composed of 3 varieties (TMS 01_0379, Akoss, Kolaoung) exhibiting ovoid central leaflet, purple leaves, reddish green leaf vein, light green stem cortex, greeny-yellowish stem exterior, three levels of branching, dichotomous branching habit, conical root, cream root pulp, good flowering and seed set ability. Morphotype 3 composed of cultivar Akaleyo was considered as outlier. The fourth morphotype comprised of cultivars Akebou, Akpadjin and Tassiodo is characterized by red petioles, obovate-lanceolate central leaflet, compact plants, pink root cortex, white root pulp and bad seed set hability; whereas cultivars belonging to morphotype 5 (Alagno, Pela) exhibited good leaf retention, irregularly shaped roots, many root constrictions, good flowering and seed set abilities (Figure 4 and 5). In morphotype 6, there were 8 cultivars (D00_126, Inconnu 2, D00_137, D00_208, D00_14, D00_54, D00_166 and D00_8300) with sessile peduncle root, conical cylindrical root, orange root pulp, short distance between leaf scars, good flowering and seed set ability. The morphotype 7

composed of cultivar TMS 96_0590 exhibited dark green apical leaves, cream stem epidermis, two levels of branching, good flowering and seed set ability (Figure 4 and 5).

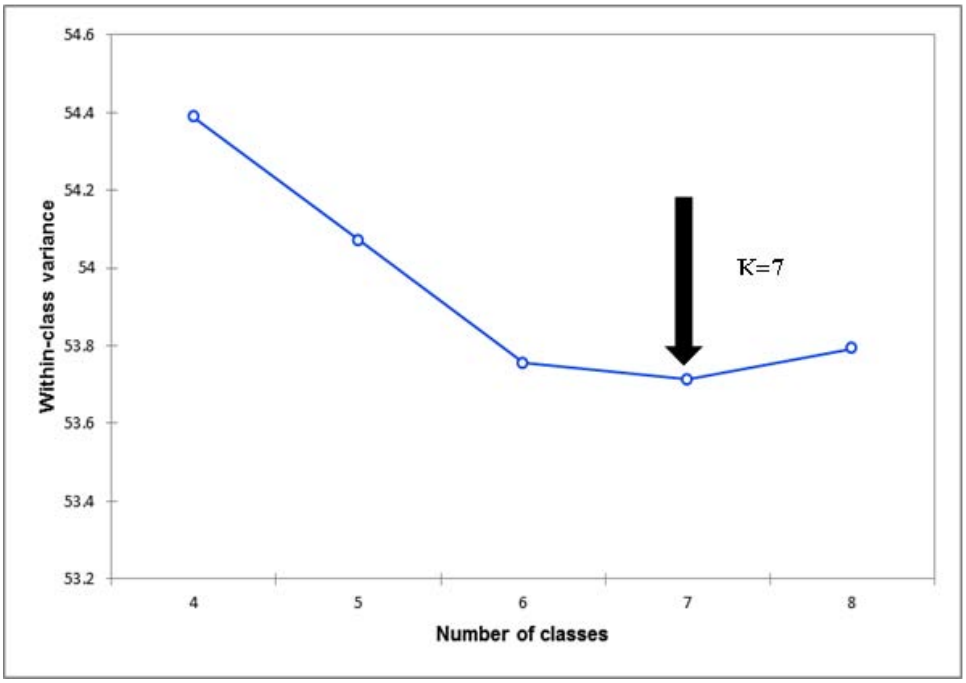

Figure 3: Distribution of the variance function according to the number of clusters obtained from cluster analysis based on significant morphological traits

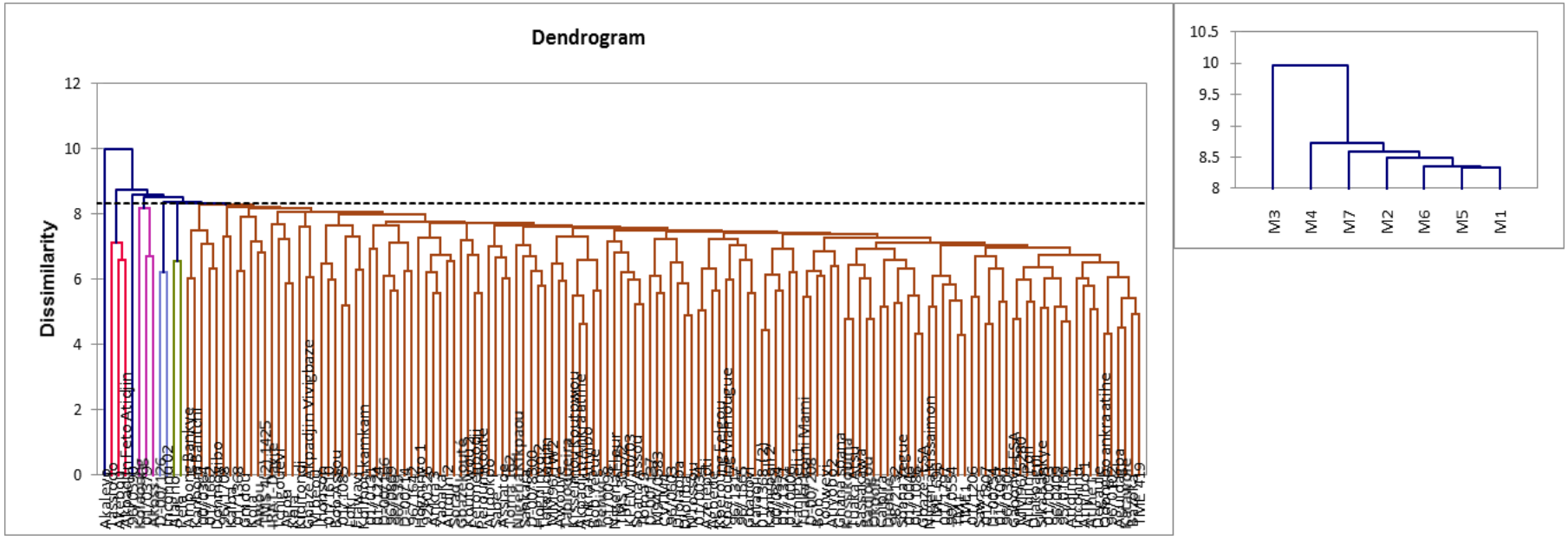

Figure 4: Dendrogram of 149 cassava genotypes revealed by the Wards method based on significant morphological traits

From left to right of the dendrogram the Morphotype 3, Morphotype 4, Morphotype 7, Morphotype 2, Morphotype 6, and Morphotype 5 are respectively represented.

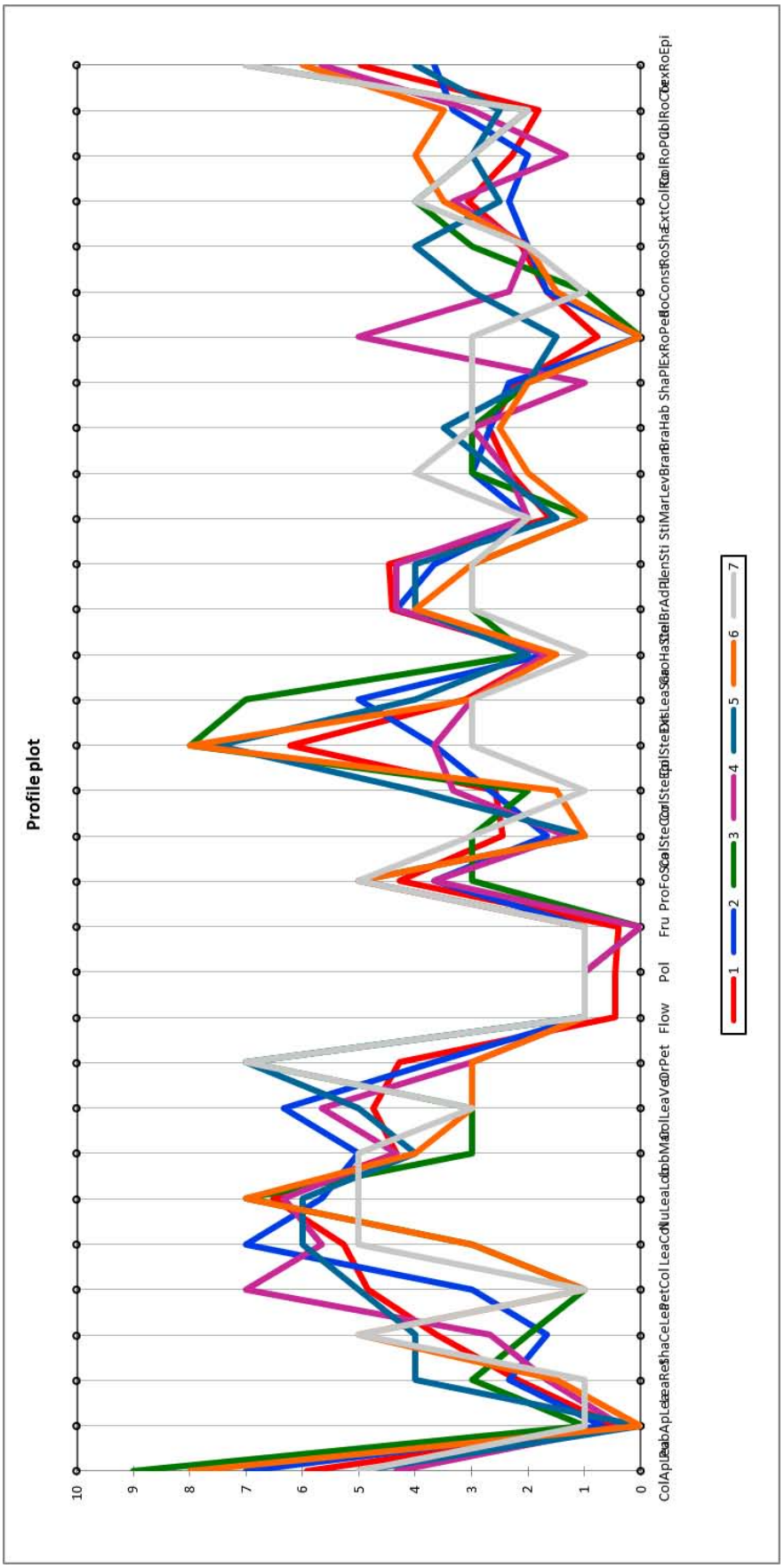

Figure 5: Profile plot describing the seven morphotypes obtained from the cluster analysis based on 32 morphological traits evaluated in 149 cassava cultivars of Togo

IV. Discussion

a) Morphological Diversity of the Germplasm

Phenotyping of plant materials based on morphological traits has been used to determine the phenotypic variability among cultivars (Avijala et al., 2015; Agre et al., 2015; Adjebeng-Danquah & Gracen, 2020). The use of these traits allows rapid identification of cultivars. In addition, morphological traits are found to be stable, highly heritable and independant from the environment (Fukuda et al., 2010). However, molecular characterization may allow a more accurate detection of differences between germplasm bank cultivars than morphological characterization.

In our study, substantial variation was observed within the germplasm. Traits such as petiole colour, leaf colour, colour of leaf vein, flowering ability, seed set ability, levels of branching, colour of end branches, colour of stem epidermis, leaf lobe margins, growth habit of stem, and root flesh colour were underscored as the most relevant traits for cultivars discrimination. Genetic variability for morphological traits has been reported in different studies in Ghana (Asare et al., 2011; Adjebeng-Danquah & Gracen, 2017); in Benin (Agre et al.,2017), in Burkina Faso (Gmakouba et al., 2018) and in Brazil (Oliveira et al., 2015).

Root flesh colour is a trait with great importance for cassava because of dietary habits of Togolese. Moreover, this trait is directly related to the presence of vitamin. Orange varieties are riched carotenoids (provitamin A) (Kamanda et al., 2020). Low occurrence was found for yellow root colour and pink root colour varieties, which possibly have lycopene in their roots.

b) Structure of the Germplasm Diversity

Cluster analysis classified the varieties into seven morphotypes, showing random distribution of the varieties. The fact that cassava is an outcrossing crop which can propagate vegetatively could explain this result. This facilitates the dispersion of varieties the exchange among farmers and, consequently, the exchange of genes (Agre et al., 2017). The main factor involved in the high diversity found may be gene flow promoted by farmers, who have acted as a dispersing agents for the species. An intense exchange system of varieties has been documented among farmers growing cassava.

The germplasm bank cultivars were not grouped based on the geographical origin distribution. Cultivars collected from place such as Vogan, Wetrope, Akebou, Danyi, Aouda, Davié, and Assoukoko were clustered in morphotype 1. Likewise cultivars from Bafilo, Assoukoko, Danyi, and Bourondè were also clustered together in morphotype 2. The remaining clusters also included cultivars from different collection regions. The informal farmers to farmers seed supply system practiced in the country could explained this result. This agrees with earlier studies on cassava (Ojulong et al., 2010; Sing et al., 2015; Adjebeng-Danquah & Gracen, 2017, Gmakouba et al., 2018). In addition, the cassava cultivars collected from the same region were clustered into different morphotypes which suggest a high genetic diversity within each collection area. Similar findings were also reported by Agre et al. (2017) in Bénin. Moreover, there was no clear differentiation and real structuring between local and improved varieties in this study as also reported by Kombo et al. (2012).

Morphotypes identified may be valuable in cassava germplasm management and cultivars identification. Especially, the varieties belonging to morphotypes 5, 6 and morphotype 7 might be most desirable for breeding due to their good flowering and seed set ability and adaptability to environmental conditions. These cultivars could be used to set up crossing blocks in order to develop segregating breeding lines with farmers desired traits.

V. Conclusions

The study revealed that the cassava germplasm of Togo exhibited high phenotypic diversity. Morphological traits such as petiole colour, leaf colour, colour of leaf vein, flowering ability, seed set ability, levels of branching, colour of end branches, colour of stem epidermis, leaf lobe margins and the growth habit of stem were the most diverse and could be used for cultivar identification in the field. The varieties of morphotypes 5, 6 and 7 harboured interesting features such as flowering and seed set ability and may be usefull for the national breeding programme. For breeding purpose, superior parental clones could be selected from these morphotypes for crossing and generating a breeding population.

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Consent for Publication: The authors declare that they obtained an informed consent for publication from people involved in this study.

Availability of Data and Materials: Data are within the paper and its supporting information files. The datasets are fully available without restriction on reasonable request from the corresponding author.

Competing Interests: The authors declare that they have no competing interests.

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Spark of First Life and Consciousness

By Chandra Prakash Trivedi

Abstract- The electrostatic force is the force that governs the motion of the elementary particles, which caused them to aggregate or collide in various ways with oxidation and reduction with the transfer of electrons in the primordial soup. The vibratory movement of the charged ions with equal and opposite wavelength developed a dynamo with streaming in extreme anaerobic condition.

It has been observed in the ultra-resolution image that one purine and one pyrimidine base differing only in Nitrogen are complimentary to each other shed with cosmology. The elementary particles adhered to space, the sound of vibration touched, press the mark, and rebound. The colliding protons, decaying into hadron jets, the electrons converted them into electric vibrations to join the purine and pyrimidine base in series with mass.

The electrostatic interaction between the charged ions of the water with dehydration separated the hydrogen bond. It has formed a covalent Hydrogen bond between the purine and pyrimidine complementary base. The complementary wavelength of hydrogen bond activated the nucleotide pair with transfer of electron.

Keywords: *spark of life, phonon, slime soup.*

GJSFR-C Classification: *DDC Code: 843.914*

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Spark of First Life and Consciousness

Chandra Prakash Trivedi

Abstract- The electrostatic force is the force that governs the motion of the elementary particles, which caused them to aggregate or collide in various ways with oxidation and reduction with the transfer of electrons in the primordial soup. The vibratory movement of the charged ions with equal and opposite wavelength developed a dynamo with streaming in extreme anaerobic condition.

It has been observed in the ultra-resolution image that one purine and one pyrimidine base differing only in Nitrogen are complimentary to each other shed with cosmology. The elementary particles adhered to space, the sound of vibration touched, press the mark, and rebound. The colliding protons, decaying into hadron jets, the electrons converted them into electric vibrations to join the purine and pyrimidine base in series with mass.

The electrostatic interaction between the charged ions of the water with dehydration separated the hydrogen bond. It has formed a covalent Hydrogen bond between the purine and pyrimidine complementary base. The complementary wavelength of hydrogen bond activated the nucleotide pair with transfer of electron. The hydrogen triple bond converts into the double bond, and reunited on the opposite side with change in the electron with oxidation and reduction in chain with the first genetic code and amino acid in series. The synthesized chromosomes divided into four with the first prokaryotic cell. Life appears with the streaming of the protoplasm and disappears with aging of the cell. The complementary wavelength of hydrogen triple bond of the nucleotide pair led the development from generation to generation with new life.

Keywords: *spark of life, phonon, slime soup*.

I. Introduction

Origin of life consciousness is a great puzzle, life appears with the streaming of the protoplasm and disappears with aging of the cell body. I have traced the roots of life consciousness in pre-cosmic condition. The phonon wave appeared first and activated the dark matter with blast and light. The phonon and photon run parallel with equal and opposite wavelength. The purine and pyrimidine base differing only in Nitrogen shed like bullet with incandescent gaseous clouds with phonon photon interaction with the vibrations. The electrostatic force governs the motion of the elementary particles, which caused them to aggregate or collide in various ways with oxidation and reduction with the transfer of electrons in the primordial soup. It has activated the purine and the pyrimidine complementary base pair with resonance.

The electrostatic interaction between the charged ions of the water with dehydration separated the hydrogen bond. It has formed a covalent Hydrogen bond between the purine and pyrimidine complementary base. The complementary wavelength of hydrogen bond activated the nucleotide pair with transfer of electron. The hydrogen triple bond converts into the double bond, and reunited on the opposite side with change in the electron with oxidation and reduction in chain with the first genetic code and amino acid in series. The synthesized chromosomes divided into four with the first prokaryotic cell. Life appears with the streaming of the protoplasm and disappears with aging of the cell. The complementary wavelength of hydrogen triple bond of the nucleotide pair led the development from generation to generation with new life.

II. Early Work

The Russian Chemist A.I. Oparin 1922 and English Geneticist J.B.S. Haldane 1928 first conceived of the theory of the pre-biotic origin of life. DNA Watson and Crick 1953, Darwin Origin of Species 1859, Life evolved from the single DNA with Genetic recombination and cell division. How did the first Life begin? NASA researchers noticed polycyclic aromatic hydrocarbons (PAHs) in meteorites. Extra hydrogen or oxygen called Quinone has the potential for the origin of life.

Higgs field 1914, phonon scattered the photon in a crystal Lie et al 2014, Einstein 1923 there must be two equal and opposite forces. The photon is the smallest unit of light, and immortal phonon is smallest unit of the sound wave vibration are connected at the molecular level with equal and opposite wavelength.

The DNA with photon-phonon interaction is universally present. Hence its complimentary resonant wave blackouts radio communications on the earth and the protons damage human beings in space if not protected properly. Because the entry of radiation rays with protons checked by the magnetosphere and ozone layer and complimentary resonance finds its counterpart protons astronaut human in space.

a) Life on the Earth

The incandescent gaseous cloud cooled down with time and the movement of the molten mass generated the geomagnetic field and magnetosphere around the earth has given the place for the ionization of the solar flares trapped by the magnetosphere and interacts with the sun's magnetic field. The ions flow down and filled the earth with the water.

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b) Ozone Layer

Stratospheric ozone is formed naturally through the interaction of solar ultraviolet (UV) radiation with molecular oxygen (O2). Ozone absorbs the toxic UV rays with the entry of visible light, it has given the way for the origin of life on the earth.

III. Theory

I have traced its root in pre-cosmic cosmology and the sun.

The earth is a part of our solar system, which is one unit of the cosmos. The human body is a microcosm inside a macrocosm. All can be searched just like a drop of water in the sea can reveal the character of the ocean I have studied the sun with the naked eyes with my yogic practice otherwise it is impossible to face the sun even for a second with confirmation from Egypt Rosetta granite stone, pyramids of the Egypt, Gold plate Grand Canyon North. America, NASA pictures & Veda,

I have observed the nuclear reactions on the sun's surface with blast and light. The photon and phonon run in a straight way in concentric circles. It has been confirmed from the Sun disc gold plate Grand Canyon and Veda.

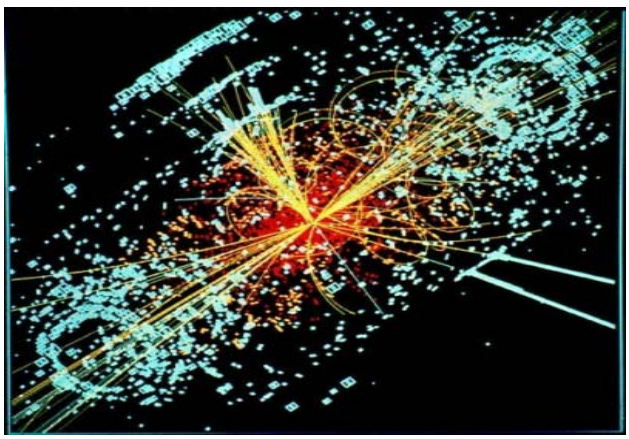
The digitally stacked sequence reveals that the photon and phonon running in concentric circles from the sun Grand Canyon Star Trails NASA - March 3, 2013. The Scientists are searching the Dark matter, is not a matter. The dark atmosphere is hidden in the interior of the sun, black caters and sunspots, which explode with blast and light.

The shock waves are antimagnetic, white, and travel with supersonic speed, and dark matter is an inactive condensed zone of magnetism without movement, just like a waste. The shock waves are 'anti-matter of dark matter, immortal with opposite character. It appears first in the pre-cosmic darkness like the shock waves appear before the earthquake, and activated the dark matter with resonance with blast and light. The photon and photon are complementary to each other.

The activation of dark matter is activation of inherent magnetism in cosmos, with formation of charged elementary particles. The electrostatic force is the force which governs the motion of the elementary particles, which caused them to aggregate or collide in various ways with oxidation and reduction with the transfer of electron.

The photon and phonon have broad complementary spectrum from gamma rays to radio waves with equal and opposite wavelength. The immortal phonon stimulate the event with electron configuration and half-spin change in the opposite wavelength and photon undergoes the synthesis and degradation with time Einstein's Equation$E=Mc^2$.

The flow of the photon and photon has been halted with the Higgs field underlying space imparted mass to the elementary particles.



Higgs Field and Mass to the Elementary Particles

All elementary particles are vibrating with the resonance of vibration and their respective charge. They are complementary to each other from gamma rays to radio waves. They find their resonant with resonance. The resonant vibrations of electromagnetic rays, touch, press-mark, and rebound. The colliding protons, decaying into hadron jets and electrons, converted them into electric vibrations to join them in series with phonic compression electromagnetic force. It has maintained its continuity in the molecules and the matter with Higgs field 2013 with asteroids and planets.

IV. Discussion

All elementary particles are vibrating with the resonance of vibration and their respective charge. They are complementary to each other from gamma rays to radio waves. They find their resonant with resonance. The phonon touch press mark and rebound with electron configuration and the half spin change in the opposite wavelength, and the photon undergoes the synthesis and degradation with time Einstein's

equation $E=Mc^2$

The first life arose in the primordial soup with the streaming movement of the charged ions in the colloidal solution. The respective complementary wavelength of the charged ions caused them to vibrate with streaming

The vibratory movement of the ions with streaming developed a dynamo in the center with actions and interactions in series with electron transfer and photon undergoes synthesis and degradation with time.

The electron transfer is associated with the oxidation loss of an electron and reduction gain of electron in anaerobic condition. The electrostatic interaction between the charged ions developed dynamo in the center with the electromagnetic field. The vibration waves activated the equal and opposite wavelengths of purine and pyrimidine base differing only in Nitrogen The elementary particles adhered to space, the sound of vibration, touched, press the mark, and rebound. The colliding protons, decaying into hadron jets and electrons, converted them into electric vibrations to join them in series with electron configuration and half spin change in the opposite wavelength.

The electrostatic interaction between the charged ions of the water with dehydration separated the hydrogen bond. It has formed a covalent Hydrogen bond between the purine and the pyrimidine base.

Ultra resolution image of DNA with Electron transfer

The phonon wave strike and rebound with a press mark with the electron configuration in the opposite direction of hydrogen triple bond, it triggered off the chain of oxidation and reduction reaction, and the hydrogen triple bond converted into double bond and Nitrogen reunite it on the other side simultaneously.

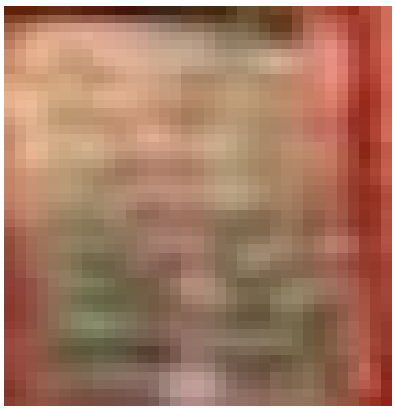
The equal and opposite wavelength of hydrogen triple bond led the development with electron transfer. With the first genetic code and amino acid in series synthesized the chromosomes. The chromosomes divided into four with first prokaryotic cell. Life appears with the streaming of protoplasm with the food metabolism as source of life and disappears with aging and death of the cell body. The complementary wavelength of hydrogen triple bond of the nucleotide pair led the development from generation to generation with new life.

T Nucleotides Divide in air Like Image in the Mirror with Electron Transfer

The equal and the opposite wavelength of hydrogen triple bond led the development with electron transfer in series and the hydrogen triple converted into double bond and reunited in the opposite direction simultaneously with Nitrogen in series as identity of the individual cell with equal and opposite wavelength. Hence, even the time twins have different genetic identity and fate in life.

The prokaryotes evolved into the eukaryotic autotrophic cell with the entry of the red wavelength of light made apparent the three places of nucleotide pair with the photosynthesis and generation of immortal chemical energy. The immortal phonon wave follows the immortal DNA from generation to generation with new life and cell division.

At the Point of Two Different DNA the Complementary Phonon Wave Strike and Rebound with Generation of Triplet code in air with Electron Configuration and Half Spin Change in the Opposite Wavelength



The Complementary Wavelength Led the Development Vigorously

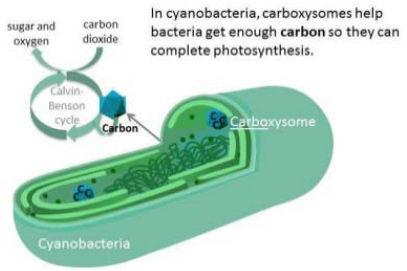
The Triplet Genetic Code of DNA Divides in Chain with Never Breaking Nitrogen

The complementary phonon wavelength acts as antennae and speaker to execute the functions of life, It led the development with the synthesis of amino acids and proteins in chain to synthesize the chromosome pair with the first prokaryote.

The Hydrogen triple bond Nitrogen triple bond with oxidation and reduction separate and unite simultaneously on other side, with oxidation the hydrogen bond break and Nitrogen reunite it on other side, due to this the double helix chain never break.

V. Result and Conclusion

The entry of the Red wavelength of light through the plasma membrane activated the place of the chlorophyll pigment on the DNA.



The first prokaryotic cell with an incipient nucleus maintained its continuity with cell division, and immortal phonon follow it from generation to generation with new life.

The entry of the Red wavelength of light through the plasma membrane activated the place of the chlorophyll pigment on the DNA as source of life with food metabolism.

It has given double horsepower to the developing cells and the prokaryotic autotrophic cell evolved into the eukaryotic cell and moved on the path of evolution with genetic recombination and cell division with the hereditary characters and the complementary phonon wave follow it from the generation to generation with new life as hereditary life principle.

Life appears with the streaming of the protoplasmic vibrations with food metabolism and disappears with the aging of the cell body.

It is like this that all the rotating astronomical bodies rotate at their axis with the generation of the dynamo in the centre with the magnetic field and the magnetosphere around them. In the same fashion, the streaming of the protoplasm with the nucleus in the centre generates dynamo in the centre with a magnetic field and magnetosphere but is hard to detect, which disappears with death, aging of the cell body.

The purine and the pyrimidine base pair of DNA differing only in Nitrogen have shed from the Nebula with the cosmological event. It divides in the air just like the image in the mirror. The Purine and pyrimidine base pair of the DNA has an inbuilt mechanism for the transcription and translation with time, with three immortal and three stages of life. The three immortals are, 1- the Higgs field ensign of the existence, 2- the immortal chemical energy of photosynthesis, with food metabolism is the source of life. 3. The immortal DNA with resonant vibrations light of life for all.

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- Electronic material
- Any other original work

Authorship Policies

Global Journals follows the definition of authorship set up by the Open Association of Research Society, USA. According to its guidelines, authorship criteria must be based on:

1. Substantial contributions to the conception and acquisition of data, analysis, and interpretation of findings.
2. Drafting the paper and revising it critically regarding important academic content.
3. Final approval of the version of the paper to be published.

Changes in Authorship

The corresponding author should mention the name and complete details of all co-authors during submission and in manuscript. We support addition, rearrangement, manipulation, and deletions in authors list till the early view publication of the journal. We expect that corresponding author will notify all co-authors of submission. We follow COPE guidelines for changes in authorship.

Copyright

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Appealing Decisions

Unless specified in the notification, the Editorial Board's decision on publication of the paper is final and cannot be appealed before making the major change in the manuscript.

Acknowledgments

Contributors to the research other than authors credited should be mentioned in Acknowledgments. The source of funding for the research can be included. Suppliers of resources may be mentioned along with their addresses.

Declaration of funding sources

Global Journals is in partnership with various universities, laboratories, and other institutions worldwide in the research domain. Authors are requested to disclose their source of funding during every stage of their research, such as making analysis, performing laboratory operations, computing data, and using institutional resources, from writing an article to its submission. This will also help authors to get reimbursements by requesting an open access publication letter from Global Journals and submitting to the respective funding source.

Preparing your Manuscript

Authors can submit papers and articles in an acceptable file format: MS Word (doc, docx), LaTeX (.tex, .zip or .rar including all of your files), Adobe PDF (.pdf), rich text format (.rtf), simple text document (.txt), Open Document Text (.odt), and Apple Pages (.pages). Our professional layout editors will format the entire paper according to our official guidelines. This is one of the highlights of publishing with Global Journals—authors should not be concerned about the formatting of their paper. Global Journals accepts articles and manuscripts in every major language, be it Spanish, Chinese, Japanese, Portuguese, Russian, French, German, Dutch, Italian, Greek, or any other national language, but the title, subtitle, and abstract should be in English. This will facilitate indexing and the pre-peer review process.

The following is the official style and template developed for publication of a research paper. Authors are not required to follow this style during the submission of the paper. It is just for reference purposes.

Manuscript Style Instruction (Optional)

- Microsoft Word Document Setting Instructions.
- Font type of all text should be Swis721 Lt BT.
- Page size: 8.27" x 11'", left margin: 0.65, right margin: 0.65, bottom margin: 0.75.
- Paper title should be in one column of font size 24.
- Author name in font size of 11 in one column.
- Abstract: font size 9 with the word "Abstract" in bold italics.
- Main text: font size 10 with two justified columns.
- Two columns with equal column width of 3.38 and spacing of 0.2.
- First character must be three lines drop-capped.
- The paragraph before spacing of 1 pt and after of 0 pt.
- Line spacing of 1 pt.
- Large images must be in one column.
- The names of first main headings (Heading 1) must be in Roman font, capital letters, and font size of 10.
- The names of second main headings (Heading 2) must not include numbers and must be in italics with a font size of 10.

Structure and Format of Manuscript

The recommended size of an original research paper is under 15,000 words and review papers under 7,000 words. Research articles should be less than 10,000 words. Research papers are usually longer than review papers. Review papers are reports of significant research (typically less than 7,000 words, including tables, figures, and references)

A research paper must include:

a) A title which should be relevant to the theme of the paper.
b) A summary, known as an abstract (less than 150 words), containing the major results and conclusions.
c) Up to 10 keywords that precisely identify the paper's subject, purpose, and focus.
d) An introduction, giving fundamental background objectives.
e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition, sources of information must be given, and numerical methods must be specified by reference.
f) Results which should be presented concisely by well-designed tables and figures.
g) Suitable statistical data should also be given.
h) All data must have been gathered with attention to numerical detail in the planning stage.

Design has been recognized to be essential to experiments for a considerable time, and the editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned unrefereed.

i) Discussion should cover implications and consequences and not just recapitulate the results; conclusions should also be summarized.
j) There should be brief acknowledgments.
k) There ought to be references in the conventional format. Global Journals recommends APA format.

Authors should carefully consider the preparation of papers to ensure that they communicate effectively. Papers are much more likely to be accepted if they are carefully designed and laid out, contain few or no errors, are summarizing, and follow instructions. They will also be published with much fewer delays than those that require much technical and editorial correction.

The Editorial Board reserves the right to make literary corrections and suggestions to improve brevity.

Format Structure

It is necessary that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.

All manuscripts submitted to Global Journals should include:

Title

The title page must carry an informative title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) where the work was carried out.

Author details

The full postal address of any related author(s) must be specified.

Abstract

The abstract is the foundation of the research paper. It should be clear and concise and must contain the objective of the paper and inferences drawn. It is advised to not include big mathematical equations or complicated jargon.

Many researchers searching for information online will use search engines such as Google, Yahoo or others. By optimizing your paper for search engines, you will amplify the chance of someone finding it. In turn, this will make it more likely to be viewed and cited in further works. Global Journals has compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Keywords

A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy: planning of a list of possible keywords and phrases to try.

Choice of the main keywords is the first tool of writing a research paper. Research paper writing is an art. Keyword search should be as strategic as possible.

One should start brainstorming lists of potential keywords before even beginning searching. Think about the most important concepts related to research work. Ask, “What words would a source have to include to be truly valuable in a research paper?” Then consider synonyms for the important words.

It may take the discovery of only one important paper to steer in the right keyword direction because, in most databases, the keywords under which a research paper is abstracted are listed with the paper.

Numerical Methods

Numerical methods used should be transparent and, where appropriate, supported by references.

Abbreviations

Authors must list all the abbreviations used in the paper at the end of the paper or in a separate table before using them.

Formulas and equations

Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

Tables, Figures, and Figure Legends

Tables: Tables should be cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g., Table 4, a self-explanatory caption, and be on a separate sheet. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.

Figures

Figures are supposed to be submitted as separate files. Always include a citation in the text for each figure using Arabic numbers, e.g., Fig. 4. Artwork must be submitted online in vector electronic form or by emailing it.

Preparation of Eletronic Figures for Publication

Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/ photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). Please give the data for figures in black and white or submit a Color Work Agreement form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

For scanned images, the scanning resolution at final image size ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs): >350 dpi; figures containing both halftone and line images: >650 dpi.

Color charges: Authors are advised to pay the full cost for the reproduction of their color artwork. Hence, please note that if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a Color Work Agreement form before your paper can be published. Also, you can email your editor to remove the color fee after acceptance of the paper.

Tips for Writing a Good Quality Science Frontier Research Paper

Techniques for writing a good quality Science Frontier Research paper:

1. Choosing the topic: In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

2. Think like evaluators: If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

3. Ask your guides: If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

4. Use of computer is recommended: As you are doing research in the field of science frontier then this point is quite obvious. Use right software: Always use good quality software packages. If you are not capable of judging good software, then you can lose the quality of your paper unknowingly. There are various programs available to help you which you can get through the internet.

5. Use the internet for help: An excellent start for your paper is using Google. It is a wondrous search engine, where you can have your doubts resolved. You may also read some answers for the frequent question of how to write your research paper or find a model research paper. You can download books from the internet. If you have all the required books, place importance on reading, selecting, and analyzing the specified information. Then sketch out your research paper. Use big pictures: You may use encyclopedias like Wikipedia to get pictures with the best resolution. At Global Journals, you should strictly follow here.

6. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right? It is a good habit which helps to not lose your continuity. You should always use bookmarks while searching on the internet also, which will make your search easier.

7. Revise what you wrote: When you write anything, always read it, summarize it, and then finalize it.

8. Make every effort: Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

9. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several unnecessary diagrams will degrade the quality of your paper by creating a hodgepodge. So always try to include diagrams which were made by you to improve the readability of your paper. Use of direct quotes: When you do research relevant to literature, history, or current affairs, then use of quotes becomes essential, but if the study is relevant to science, use of quotes is not preferable.

10. Use proper verb tense: Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

11. Pick a good study spot: Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

12. Know what you know: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. Use good grammar: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

14. Arrangement of information: Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

16. Multitasking in research is not good: Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

17. Never copy others' work: Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

18. Go to seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.

20. Think technically: Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

21. Adding unnecessary information: Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

22. Report concluded results: Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

23. Upon conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear: Adhere to recommended page limits.

Mistakes to avoid:

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:

Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

Approach:

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

Procedures (methods and materials):

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

What to keep away from:

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

What to stay away from:

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

Figures and tables:

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."

Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

The Administration Rules

Administration Rules to Be Strictly Followed before Submitting Your Research Paper to Global Journals Inc.

Please read the following rules and regulations carefully before submitting your research paper to Global Journals Inc. to avoid rejection.

Segment draft and final research paper: You have to strictly follow the template of a research paper, failing which your paper may get rejected. You are expected to write each part of the paper wholly on your own. The peer reviewers need to identify your own perspective of the concepts in your own terms. Please do not extract straight from any other source, and do not rephrase someone else's analysis. Do not allow anyone else to proofread your manuscript.

Written material: You may discuss this with your guides and key sources. Do not copy anyone else's paper, even if this is only imitation, otherwise it will be rejected on the grounds of plagiarism, which is illegal. Various methods to avoid plagiarism are strictly applied by us to every paper, and, if found guilty, you may be blacklisted, which could affect your career adversely. To guard yourself and others from possible illegal use, please do not permit anyone to use or even read your paper and file.

Criterion for Grading a Research Paper (Compilation) by Global Journals

Please note that following table is only a Grading of "Paper Compilation" and not on "Performed/Stated Research" whose grading solely depends on Individual Assigned Peer Reviewer and Editorial Board Member. These can be available only on request and after decision of Paper. This report will be the property of Global Journals.

Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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