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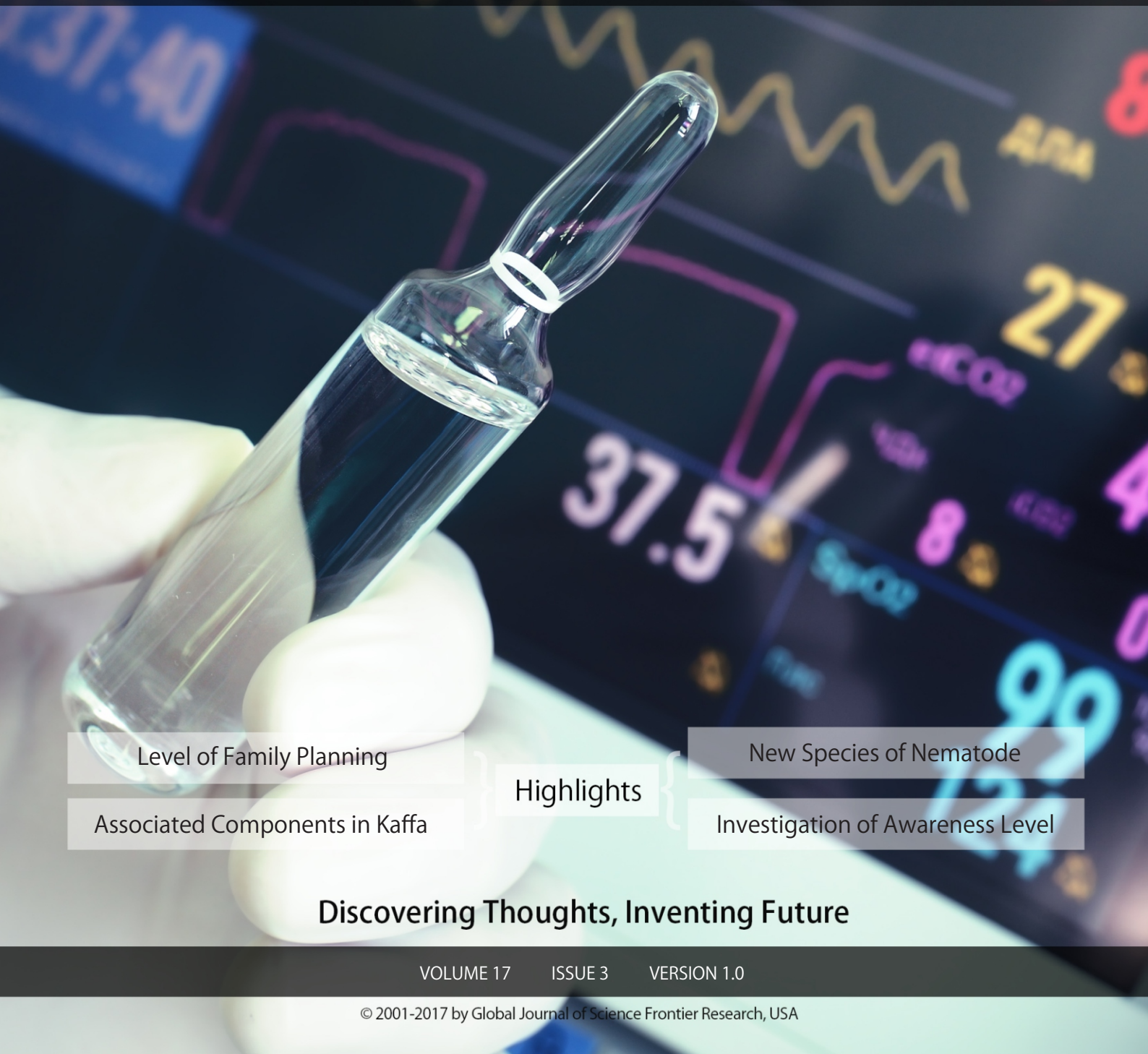
# Global Journal

## of Science Frontier Research: C

# Biological Science

Botany & Zoology



Highlights

- Level of Family Planning
- Associated Components in Kaffa
- New Species of Nematode
- Investigation of Awareness Level

**Discovering Thoughts, Inventing Future**

VOLUME 17 ISSUE 3 VERSION 1.0

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# Characterization of a Classical W14 Albino Mutation and a Putative New Robertson's Mutator-Induced Allele in Maize (Zea Mays)

By Ngoc (Josie) Huynh
*Midwestern State University*

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## I. Introduction

Albino mutations are well known in plant and animal species. These mutations are caused by various recessive alleles which, when homozygous, lead directly or indirectly to a reduction in normal pigmentation in the organism. Genes that cause albinism in maize are identified by a number of different designations (Vancetovic et al., 2010): w1, w2, w3 represent three white-albino loci; wd1 the white deficiency locus; lw1, lw2, lw3, lw4 four lemon white loci; vp2, vp5, vp9 three viviparous loci; and y10 the yellow endosperm locus (Neuffer et al., 1997).

A common feature of these genetic defects is that among their phenotypic effects is a depletion of carotenoid pigmentation in some or all tissues. The concomitant loss of chlorophyll and other biomolecules in affected tissues is due to the loss of photo- protective carotenoids. The first comprehensive review of albino maize mutants was by Robertson (1971). Since then, studies of these mutants have focused on developmental and metabolic features. Most studies have only described the mutations phenotypically, there are no molecular assays for the different genes that produce albino mutants in maize (Vančetović, et al., 2004; Vančetović, et al., 2010).

Robertson divided albino mutations into two classes. Class I albino mutations produce white-endosperm and white seedlings, whereas class II albino mutants produce both white and yellow endosperm and off-white seedlings (Robertson, 1975). In the albino mutant that is the focus of this research, designated w*-5200, trace amounts of chlorophyll are formed under dim light conditions. Since these albino seedlings grow from both white kernels and yellow kernels, they bear a Class II albino mutation, most likely in the w14 locus (Stinard, 2013, and personal communication). Based on chromosome mapping of previously characterized w14 alleles (chromosome 6L), and putative gene identification within the B73 draft genomic sequence, the likely identity of the w14 locus is Dxs1, which encodes 1-deoxy-D-xylulose-5-phosphate synthase.

The newly identified mutation was derived from a population carrying Robertson's Mutator transposable elements. To determine whether the new albino mutation was due to a lesion at the w14 locus, it was compared with another genetically confirmed w14 allele: 616B w14-N335 (generated by EMS mutagensis). Since most studies on albinism in maize have only described these mutations phenotypically, there was no comprehensive molecular assay for the potential genes that produce these albino mutants or to identify the mutation causing the new w*-5200 phenotype. The DNA sequences of w*-5200 and w14 allele 616B w14-N335 were compared following amplification of gene segments by Polymerase Chain Reaction (PCR). We hypothesized that the new albino phenotype was caused by failure of the mutant to produce normal levels of carotenoids due to a mutation that affects the w14 gene product. Specifically, we pursued the hypothesis that the defect was due to the insertion of a Roberton's Mutator transposable element into the w14 locus.

Typically, allelism of a new lethal mutation is confirmed by field crosses between plants heterozygous for the new mutation and plants heterozygous for a mutation in the target locus. However, at the outset of this project municipal water restrictions, which continued for three successive summers, prohibited field work, which would have included allelism testing. Given the availability of a draft genome sequence, confirmed alleles of the putative target gene, and the facilities to perform the required molecular operations, the sequences of the new mutation and a known w14 allele were determined and compared to identify the lesions resulting in their mutant phenotypes.

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## II. Literature Review

### a) *Maize as a Model Organism*

Maize has historically been an important model organism for classical genetic research. However, it has a long reproductive cycle (four-months) and tall stature that are not easily accommodated in a greenhouse setting, as well as a large (2.3 x 109 bp) haploid genome. By comparison, Arabidopsis thaliana, which has become the preferred flowering plant model, boasts diminutive size, a short (~6-week) reproductive cycle, and a small (1.35 x 108 bp) haploid genome size (Johnston et al., 2007). Although Arabidopsis is superior for many studies, maize remains an attractive model organism for the study of mutations that result in seedling lethal phenotypes. Arabidopsis seeds provide too little stored energy to support the growth of homozygous photosynthesis- defective seedlings to a size useful for biochemical analysis, even when grown on sucrose-supplemented medium (Koornneef and Meinke, 2010). In contrast, typical maize kernels store adequate starch to produce 3- or 4-leaf seedlings, which provides sufficient tissue for many biochemical analyses. Furthermore, the mechanics of classical genetic manipulation of maize remain as straightforward as ever, including simple and reliable self- and outcrosses.

### b) *Transposable Elements in Maize*

In maize, transposable elements (TE) make a big contribution to the genome (about 85% of the genomic material). Although most of the transposable elements are silenced most of the time to maintain genome stability, TEs still play an important role in plant evolution and environmental adaptation (Tenaillon et al., 2010). Robertson's Mutator (Mu) has been frequently utilized for mutant induction (Vollbercht et al., 2010). The Mutator family of elements includes both an autonomous (master) element, Mu9/MuDR, and nonautonomous components, Mu1-Mu8 (Chomet, 1994). The elements routinely insert to unlinked sites in high numbers, making the family very mutagenic (McCarty et al., 2013). There are six classes within the Mu family, all of which share a comparable ~200 bp terminal inverted repeats (Bennetzen et al., 1993).

Miniature inverted-repeat transposable elements (MITE) are class II transposable elements. MITEs are short (80-500 bp) non-autonomous DNA transposons that are present near or within plant genes. Most MITEs are AT rich and produce target-site duplications of between 2 and 9 bp (Charrier et al., 1999; Patel et al., 2004).

### c) *Effect of Albino Mutations on Leaves*

Chloroplasts degenerate in sunlight if they lack photo-protective carotenoids. Besides their photo-synthetic role, chloroplasts perform a variety of other essential biochemical functions, such as biosynthesis of amino acids, vitamins, and storage proteins. In the absence of carotenoids, the variety of compounds that are synthesized by chloroplasts, are reduced or absent. Plants bearing such mutations are only able to grow to maturity as heterozygotes or with sugar in their growth media (Walles, 1963). In particular, carotenoids play an important role in protection of chlorophyll from photochemical degradation. Without carotenoids, chlorophyll is destroyed under normal light conditions resulting in a white (albino) leaf phenotype. Different albino mutations produce phenotypes that differ developmentally depending on the step in carotenoid biosynthesis affected and on the expression pattern of the gene that is mutated.

The w*-5200 mutation produces unpigmented white albino seedlings in ambient sunlight. In very dim light, mutant seedlings produce very low levels of chlorophyll. Furthermore, there is no linkage between the white seedling phenotype and white endosperm as is the case for certain carotenoid-deficient mutations. Based on this phenotype, w*-5200 is hypothesized to encode DXS (1-deoxy-D-xylulose-5- phosphate synthase), which catalyzes the first step in the 2-C-methyl-D-erythritol-4-P (MEP), isoprenoid biosynthetic pathway (Fig. 1). In maize, DXS is encoded by three different genes: Dxs1 on chromosome 6, Dxs2 on chromosome 7, and Dxs3 on chromosome 9 (Cordoba et al., 2011). Dxs1 is expressed primarily in young leaves and at lower levels in husks, tassel and mature leaves. Dxs2 is expressed primarily in mature leaves and at lower levels in yellow kernels and roots. Dxs3 is expressed in all vegetative and reproductive tissues but at the highest level in mature leaves. In addition to tissue-specific differences in expression, the three genes differ in the magnitude of their responses to light.

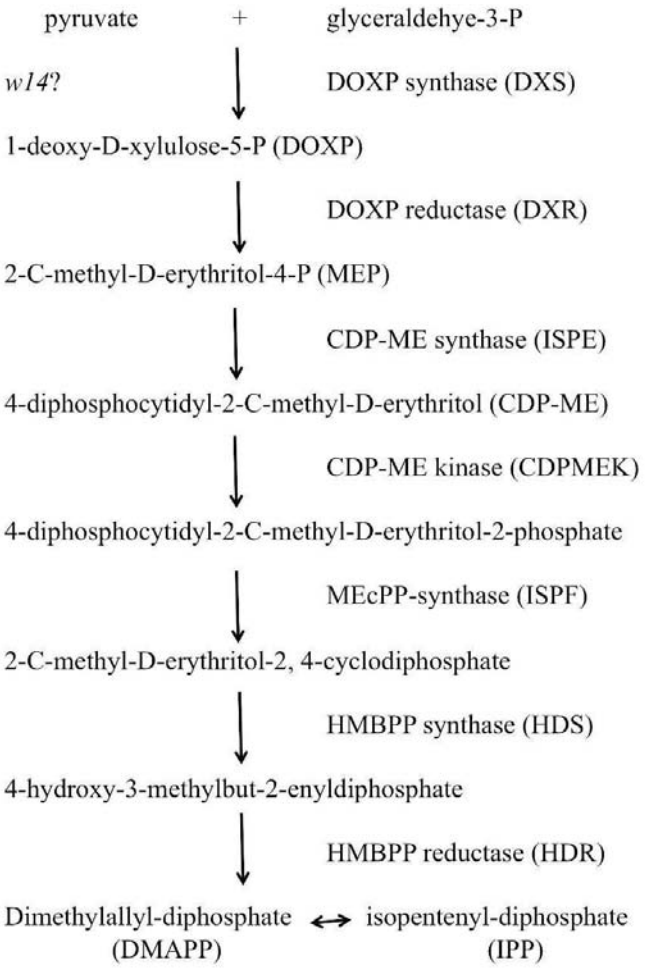


*Figure 1:* Chloroplast MEP Isoprenoid Biosynthetic Pathway (adapted from Stinard, 2013)

## III. Materials and Methods

*a) Genetic Seed Sources*

The maize (Zea mays) seed stock bearing w*-5200 arose from a self-pollination in a family that segregated another photosynthetic mutation independent of the w14 locus. The immediate source of experimental seed was ear 5200-a5 from the 2012 field season, which was the last field season before water use restrictions interrupted field production. The 616B w14-N335 EMS-induced stock was obtained from the Maize Genetics Cooperation Stock Center at the University of Illinois.

*b)* Growth Conditions

Zea mays kernels were grown in potting soil in the greenhouse at 21 °C - 25 °C (70 °F - 77 °F) under ambient light (360-650 $\mu$mol photons $m^{-2}$ $s^{-1}$) For phenotypic verification of low light chlorophyll production, seedlings were germinated at 22 °C - 23°C at a light intensity of approximately 6 $\mu$mol photons m-2 s-1

*c) Mutant Screening*

Mutant seedlings were screened visually and recognized by their paper-white leaf color under ambient natural light in the greenhouse. Sprouting w*-5200 mutant seedlings were screened for the w14 albino phenotype using cool-yellow fluorescent lamps (approximately 6 $\mu$mol photons $m^{-2}$ $s^{-1}$ 100-fold lower photon flux compared to the midday green house) in the dark. The use of a low photon flux permitted the light dependent conversion of barely detectable amounts of protochlorphyllide to chlorophyll without its subsequent destruction by photo-bleaching. The weakly pigmented leaves subsequently turned to paper-white under greenhouse lighting. To distinguish between homozygous and heterozygous phenotypically normal 616B seedlings with or without a MITE insertion in intron 3, genomic DNA was amplified using PCR primers A5F and A5R to produce products of one size in homozygotes (~800 bp) and products of two different sizes (~650 bp and ~800 bp) in heterozygotes (Table 1).

*d) DNA Extraction*

For Polymerase Chain Reaction (PCR) screens, genomic DNA was extracted from albino mutant seedlings and normal green seedlings (modified from Doyle and Doyle, 1987). Fresh, young leaves of albino and normal plants were collected. The tips (3 cm2 of fresh leaves were cut into small pieces. Leaf tissue was ground thoroughly with a plastic pestle in 1.5 ml microfuge tubes containing 250 $\mu$l of extraction buffer (2% cetyl trimethylammonium bromide [CTAB], 20 mM EDTA (pH 8), 280 mM Tris-HCl (pH 8), 1.4 M NaCl, 8.2 mM 2-mercaptoethanol and 10 $\mu$g ml-1 RNAse A). An additional 750 $\mu$l extraction buffer were added to the test tube and the mixture was heated at 55 °C in a shaking water bath for 10 min then left at room temperature (25 °C) for 5 min to cool. To the cooled homogenate, 400 $\mu$l chloroform/isoamyl alcohol (24:1) were added and shaken gently to form an emulsion. Samples were centrifuged at 13,000g for 10 min at 5°C. The aqueous phase was transferred to a new microfuge tube and 2/3 volume of isopropanol (-20 °C) was added. Samples were placed at -20 °C for 10 min then centrifuged (13,000g) for 10 min to precipitate the DNA. The supernatant was removed and the pellet was washed with 76% EtOH and centrifuged (13,000g) for 10 min at 4 °C. The supernatant was removed and the pellet was air dried at room temperature. The DNA pellet was resuspended in 100 $\mu$l deionized H2O (dH2O).

*e) Polymerase Chain Reaction (PCR) Amplification*

The quality of the DNA samples was verified by PCR amplification using a primer pair unrelated to the w14 locus. Screening PCR was performed using Phusion Master Mix with HF Buffer (NEB). The PCR reactions of 20 $\mu$l contained 50 ng template DNA, 1X

Phusion High-Fidelity buffer mix and forward and reverse primers, each at 500nM. PCR amplification was carried out in an Applied Biosystems thermal cycler (v.2.09) with the following profile: initial denaturation for 1 min at 98 °C, followed by 35 cycles each of 10 s at 98 °C (denaturation), 30 s at 66 °C (annealing), 2.0-3.0 min depending on product size at 72°C (extension), followed by a final extension of 5 min at 72 °C. Screening PCR was also performed using Stratagene 1X master mix. The thermal profile for using Stratagene master mix was 5 min at 94°C for initial denaturation, followed by 35 cycles each of 1 min at 94 °C (denaturation), 1 min at 60 °C (annealing), 3.0-4.0 min depending on product size at 72 °C (extension), followed by a final extension of 5 min at 72 °C.

*f) PCR Product Analyses*

The amplification products were electrophoresed in 1% agarose gels in 1× TAE and stained with ethidium bromide (0.5 $\mu$g ml$^{-1}$ for detection. PCR product sizes were determined by comparison to Hi-Lo DNA size markers (Minnesota Molecular). Selected PCR products were cloned in vector pHSG299 by blunt-end ligation in reactions containing 0.1 $\mu$g vector and 3 $\mu$l PCR product in 1x T4 DNA ligase reaction mix (New England Biolabs) incubated overnight in water at 10 °C and allowed to warm to room temperature through the night. Three microliters of the ligation reaction were added to NEB 5-alpha competent E. coli cells, mixed by flicking, and incubated on ice for 30 min. Cells were heat shocked at 42 °C for exactly 30 s, then incubated on ice for 5 min before addition of 950 $\mu$l of room temperature SOC. The mixture was incubated in a shaking incubator (260 rpm) at 37 °C for 60 min. The transformation was diluted 10-fold in SOC and 100 $\mu$l were plated on LB-Kanamycin plates supplemented with 50 $\mu$l X-gal (40 $\mu$g ml$^{-1}$) and 50$\mu$l IPTG(100 $\mu$g ml-1) and dried just before plating. Colonies with recombinant plasmids were selected by blue white screening, checked for insert size by PCR, and sequenced by Eurofins Genomics. Alternatively, some PCR products were sequenced directly by the same commercial lab. Sequences were analyzed manually using SerialCloner® software (SerialBasics) by comparison to the Dxs1 region of maize inbred line B73 chromosome 6, RefGen_v4, whole genome shotgun sequence (NCBI accession number: NC_024464) to identify potentially mutagenic lesions. Putative mutagenic regions were sequenced with 3X or better coverage.

*Table 1:* Tm for selected primer pairs at 500 nM in 1X Master Mix

| 1F4 | GCACACTCTCTCCCCGGC | Tm (°C) | |
|---|---|---|---|
| Primer | Primer Sequence | Phu | Stra |
| 1F4 | GCACACTCTCTCCCCGGC | | |
| 1R4 | CCACCGCCATCCCGA | 66 | 59 |
| 2F4 | GAGTACGACAGCTTCGGCACG | | |
| 2R4 | GAATGGGCCGGTCAAAACCTAG | 69 | 60 |
| 3F4 | GGATCTCAGGTCGCAGCAAGTT | | |
| 3R4 | ACGACGTCGATCTGCAGAAGCTA | 69 | 60 |
| 4F4 | GGTCCTCGACTGACGCCG | | |
| 4R4 | CGGTAACTGTTGTTCCGGCG | 67 | 60 |
| A5F | ATCCTCAACGACAACAAGCA | | |
| A5R | AGAGTCAACTTGCTGCGACC | 67 | 60 |
| 6BF | ACGTCGGGATCGCGGAGCAG | | |
| 6BR | CAACGGGACGCCAACGCCGT | 69 | 60 |
| 1Falig | CCAACATGGTCGTCATG | | |
| 1Ralig | AAGTTCAGACACTCTAG | 69 | 60 |
| 2Falig | GCACACTCTCTCCCCGG | | |
| 2Ralig | GGTGGTTAATTAGCTAG | 65 | 60 |
| 3Falig | ATGGCTCTGGGTAACGT | | |
| 3Ralig | ACAGTCTGGAAATTTGA | 69 | 60 |
| UpSF | CATGGGGCTTTAGGAGCATAGGTCT | | |

| | | | |
|---|---|---|---|
| UpSR | TGCGAGCAATGGGTGTCCTACCAAT | 69 | 60 |
| 1021UpSR | GTCAGCGGTGGCAAAGTGAAGATTA | | |
| UpSR | TGCGAGCAATGGGTGTCCTACCAAT | 68 | 60 |
| DSF | GCCAAACGCGTAGAACTTGTGCTGA | | |
| DSR | TTCCCAGAAATGGAGAAATTGGATCT | 69 | 60 |

*g) Allelism Test*

Three years into the project municipal outdoor watering restrictions were lifted permitting a return to field propagation and the opportunity for a genetic test of allelism between w*-5200 and 616B in the summer of 2016. Reciprocal crosses were performed between individuals heterozygous for the w*-5200 allele and one individual heterozygous for 616B. When present on cross participants, a second viable ear shoot was self-pollinated in order to distinguish between heterozygous and homozygous-normal individuals. Progeny of allelism crosses were screened for segregation of the albino phenotype indicating heterozygous parentage.

## IV. Results

*a) Seeding Screening*

Albino mutants were initially identified by their white leaf phenotypes. In subsequent screens albino seedlings were examined for low-light greening using 15W cool-yellow fluorescent lamps (approximately 6 $\mu$mol photons $m^{-2}$ $s^{-1}$ in a darkened room before transfer to the greenhouse for photo-bleaching (Fig. 2).


*Figure 2:* Pale green pigmentation in w14 mutant leaves. A Chlorophyll (black arrow), following low-light treatment; B Chlorophyll (white arrow) on day 4 of the greenhouse growth before sunrise; C No trace of chlorophyll following growth in full sunlight

*b) Allelism Test*

Allelism crosses confirmed that w*-5200 is allelic to 616B w14-N335 (Table 2). After a nearly complete crop failure, one individual of the 616B stock survived. This individual served as pollen donor for one cross to w*-5200 heterozygotes and as pollen recipient in addition to supporting a self-pollination to verify heterozygosity.

*Table 2:* Allelism test w*-5200 × 616B w14-N335

| Cross | Phenotype | | Ratio | Result |
|---|---|---|---|---|
| | Albino | Normal | | |
| *w*-5200⊗* | 82 | 247 | 1:3.01 | heterozygous |
| *w*-5200-sib × w*-5200-sib* | 37 | 102 | 1:2.76 | heterozygous |
| *616B⊗* | 17 | 53 | 1:3.12 | heterozygous |
| *w*-5200 × 616B* | 15 | 37 | 1:2.47 | allelic |

*⊗indicates self-pollination*

*c) Sequence Analysis of DXS from w*-5200 and 616B*

To identify the mutations causing the w*-5200 and 616B phenotype, their normal and mutant Dxs1 alleles were sequenced. The sequences of the two mutants were compared to each other, their normal siblings, and to the reference genome sequence of B73.

Figures 3 and 4 illustrate typical results from the amplification of the Dxs1 locus. Primers pairs 1F-2R, and 3F-4R gave similar results in that both amplified products of approximately the same sizes in mutant and normal samples.

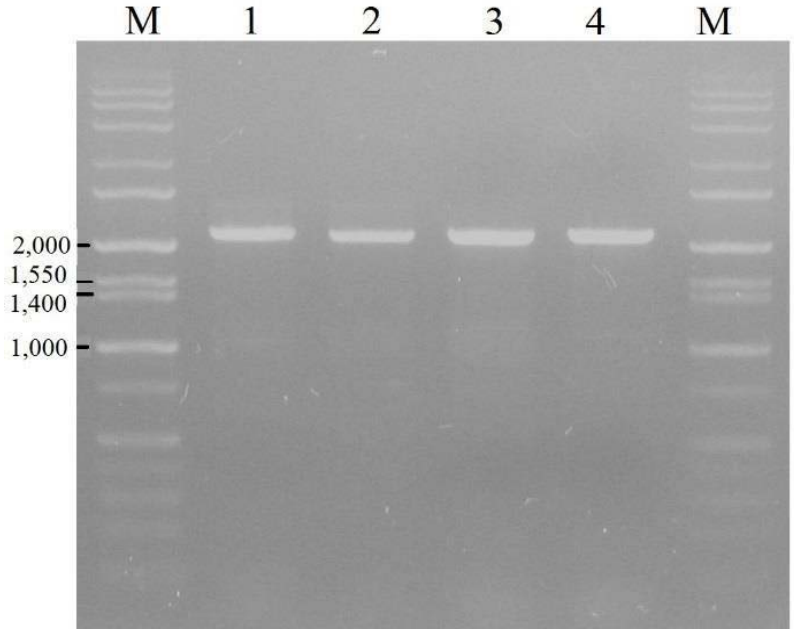


*Figure 3:* Agarose gel electrophoresis (1 %) of PCR products amplified with primers 1F and 2R. Lane 1: w*-5200 albino; lane 2: w*-5200 normal; lane 3: 616B albino; lane 4: 616B normal. M: Hi-Lo DNA markers (bp).

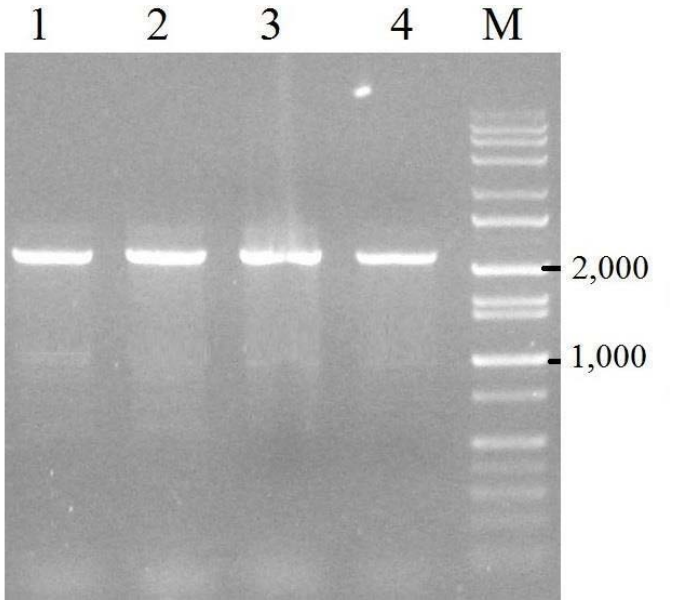


*Figure 4:* Agarose gel electrophoresis (1 %) of PCR products amplified with primers 3F and 4R. Lane 1: w*-5200 albino; lane 2: w*-5200 normal; lane 3: 616B albino; lane 4: 616B normal. M: Hi-Lo DNA markers (bp).

17 The sequence alignment of the DNA from the four w14 alleles is shown in Figure 5. The alignment identifies no consequential difference between w*-5200 and 616B w14.

There are a few difference between the Dxs1 reference sequence and w*-5200 and 616B w14 (Table 3). Most of sequence differences occur in non-coding regions: in 5′ or 3′ untranslated regions (UTR) (G15T and C50T) or within the introns (A1291C, C1322A, T1338A, A1396G) in locations unlikely to affect splicing. Among mutations within exons, most were silent or consevative mutations (G1183T).

*Table 3:* Consequential differences between B73 and 616B albino, w*-5200 albino, w*- 5200 normal, 616B normal and effects on protein sequence

| Difference Relative to B73 | Effect on Amino Acid Sequence | *616B* Albino | *w*-5200* Albino | *w*5200* Normal | *616B* Normal |
|---|---|---|---|---|---|
| A294C | N31H | √ | √ | √ | |
| G2769A | S436N | √ | √ | √ | √ |
| G3063A | D484N | √ | | √ | |
| G3314A | G568D | √ | √ | √ | |
| C3452T | A586V | √ | | √ | |

The sequences of the Dxs1 locus from the two mutants were more than 90% identical. In comparision to the Dxs1 sequence from B73, w*-5200 albino, w*-5200 normal and 616B normal, the 616B albino sequence includes a 140-bp deletion from intron 3 (Fig. 5). Where present, the 140-bp sequence is flanked by 3-bp direct repeats and contains 15-bp inverted repeats, each a signature of a minature inverted-repeat transposable element (MITE) of the PIF/Harbinger class (Wessler et al., 1995).


*Figure 5:* Map of maize Dxs1 transcribed region. Grey: 5′, 3′ UTR or intron; white: exon; black: MITE insert when present. Segment lengths drawn to scale. Scale bar= ~200 bp.

As the MITE was inserted into intron 3 of the 616B normal allele and in both w*- 5200 albino and normal alleles, the possibility that the deletion of the element from 616B was the cause of the albino phenotype was evaluated. The region surrounding the site of the MITE insertion was amplified in order to determine the presence or absence of the element in 616B and three other genetically confirmed w14 alleles: 612A, 612N, and 612M using A5F and A5R (Table 1). To determine whether the MITE elements, when present, were inserted in the same location in all cases, the amplification products were sequenced.

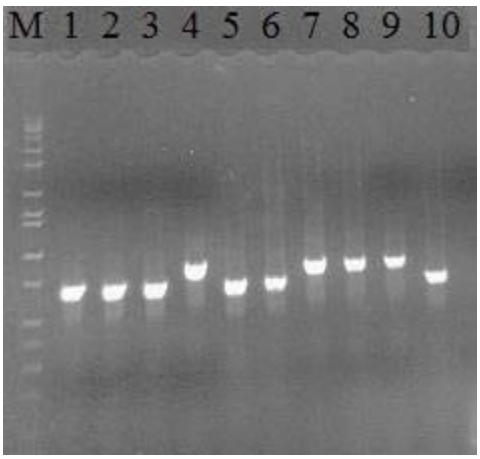


*Figure 6:* Agarose gel electrophoresis (1 %) of PCR products amplified with primers A5F and A5R. Lane 1: 612A normal; lane 2: 612A albino; lane 3: 612M normal; lane 4: 612M normal; lane 5: 612N normal; lane 6: 612N albino; lane 7: w*-5200 normal, lane 8: w*-5200 albino; lane 9: 616B normal; lane 10: 616B albino. M: Hi-Lo DNA markers (bp).

The results in both PCR products (Fig. 6) and sequences (appendix A3) provide evidence that the MITE is present in either or both normal and mutant DNAs among the five different allele-bearing stocks and that in each case where it is present, the MITE insertion is in the same location.

An additional mutation was considered as the possible cause of one or more of the albino phenotypes. A mutation that affects a critical upstream and downstream regulatory region could prevent expression of the gene and result in the observed phenotypes. To evaluate this possibility, the 2,000 bp regions immediately upstream and downstream of the coding regions were amplified and the products compared to detect any large-scale insertions, deletions or other rearrangement that might affect gene expression (Fig. 7, 8).

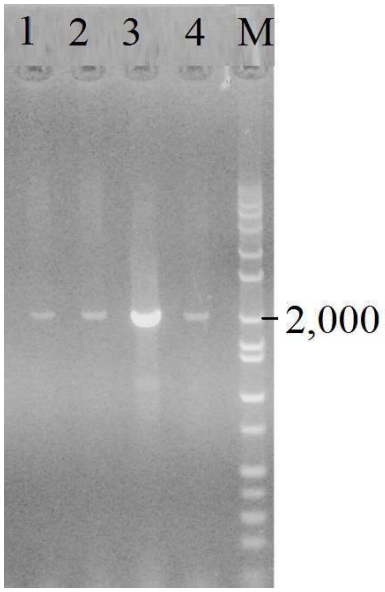


*Figure 7:* Agarose gel electrophoresis (1 %) of PCR products amplified downstream of Dxs1 with primers DSF and DSR. Lane 1: w*-5200 normal, lane 2: w*-5200 albino; lane 3: 616B normal; lane 4: 616B albino. M: Hi-Lo DNA markers (bp).

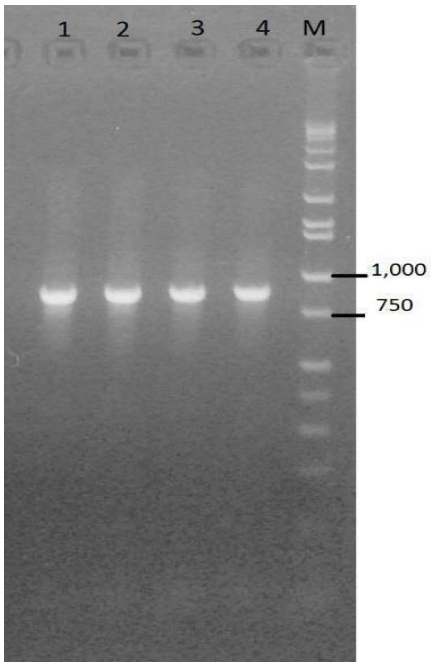


*Figure 8:* Agarose gel electrophoresis (1 %) of PCR products amplified upstream of Dxs1 with primers 1021UpSF and UpSR. Lane 1: w*-5200 normal, lane 2: w*- 5200 albino; lane 3: 616B normal; lane 4: 616B albino. M: Hi-Lo DNA markers (bp).

## V. Discussion and Conclusion

The allelism test confirmed that the newly isolated albino mutation affects the w14 locus. The mutations in w*-5200 and 616B were determined to affect the same locus because the segregation ratio among progeny of the allelism crosses approximated the 1 (albino): 3 (normal) expected for a recessive trait (Table 1).

Although several sequence differences were identified among the samples that were examined, none was likely to inactivate DXS (Table A1). The MITE insert that is present in w*-5200 albino, w*-5200 normal, and 616B normal samples is absent from the 616B albino sequence (Fig. 6), which suggests that the excision may be the cause of the albino mutation in 616B. However, the MITE element was absent from both 612A samples, both 612N samples, and from 612M normal, but present in the 612M albino sequence. The presence or absence of the MITE element is independent of the albino phenotype and not its cause.

The w*-5200 Dxs1 sequence included neither a large-scale disruption, such as the hypothesized Robertson's Mutator transposable element insertion, nor any smaller variations that would obviously result in failure to produce a functional gene product.

The unexpected absence of obvious molecular evidence for the cause of the mutation in either w*-5200 or 616B may be due to one of two possible explanations. The first is that the albino mutation that affects the w14 gene product is not present within the transcribed sequence of the locus. Gene expression may be affected by 5′ or 3′ sequence features outside the coding region that was examined. Although the MITE element that was detected in this study was present in the transcribed region of the locus, it raises the question of whether another MITE element may be inserted outside the transcribed region in a location able to affect expression. Reduced expression of ZmRAP2.7, a flowering time repressor gene, is associated with increased methylation in a regulatory element (Vgt1) that bears a highly methylated MITE element although a causative relationship between the two phenomena has not been demonstrated (Castelletti et al., 2014; Salvi et al., 2007). An ancient transposable element insertion ~60 kb upstream of tb1 locus contributes to the enhanced expression of the gene and the resulting apical dominance seen in modern maize (Studer et al., 2011). A MITE insertion in the 3'UTR of TaHSP16.9 (wheat) affects gene expression by stabilizing transcripts after exposure to heat stress (Li et al., 2014).

Amplification of the 5′ and 3′ UTRs identified no large-scale insertions or deletions that might prevent expression of Dsx1 (Figs. 7 and 8). Although no large-scale changes were detected within 2,000 bp of the gene, smaller scale changes may be present that would have to be detected by more focused sequence analysis as was done for the transcription unit in this study. Modifications to more distant elements that may affect transcription of the Dxs1 would be detected only by more elaborate genomic analyses. While disruption of a regulatory region is a plausible explanation for altered gene expression in individual cases, the likelihood that both mutation events (w*-5200 and

616B), each generated in a mutagenesis project of known mechanism, would result from such disruptions seems small.

The second possible explanation for the lack of evidence of mutations in this locus is that Dxs1 is not the locus affected in w14 mutations. Although the circumstantial evidence on which the hypothesis of w14 involvement was based seems solid, to date it has not been confirmed by molecular means.

While it has been impossible to identify a sequence change that would obviously affect the expression of the w14 gene, it may be that a change eluded detection by sequencing that can be detected by other means. In future studies isolation and comparison of mRNA from mutant and normal sibling individuals should be performed. Northern analysis would provide information about the presence or absence of the transcripts from w14 and their sizes. In the case that mRNA is detected by Northern analysis, Real Time PCR should be employed to more precisely determine the relative levels of transcript accumulation between mutant and normal siblings.

While it seems unlikely that two independent mutations affecting the same locus would leave no molecular evidence in the transcribed sequence, it is possible. Fortunately, due to the long and active history of mutational analysis of maize, several other independent mutations affecting the w14 locus exist. Three of these will be propagated during the 2017 crop season to prepare DNA for sequence analysis. While this study did not yield a satisfying answer regarding the identity of the w14 locus or the nature of the mutations affecting it, data from additional w14 mutations and results from further experiments described above may shed light on the question.

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## Appendix

*Table A1:* List of all sequence differences between B73 and 616B albino, w*-5200 albino, w*-5200 normal, 616B normal and effects on protein synthesis

| Difference Relative to B73 | Effect on Amino Acid Sequence | *616B* Albino | *w*-5200* Albino | *w*-5200* Normal | *616B* Normal |
|---|---|---|---|---|---|
| G15T | 5'UTR | √ | √ | | |
| C50T | 5'UTR | √ | √ | | |
| A236G | silent | √ | √ | | |
| C293T | silent | √ | √ | | |
| A294C | N31H | √ | √ | √ | |
| A1396G | intron | | √ | √ | |
| C2674A | silent | | √ | _ | |
| G2769A | S436N | √ | √ | √ | √ |
| T2904C | intron | √ | √ | √ | |
| A2951C | intron | √ | √ | √ | |
| C2952G | intron | √ | √ | √ | |
| T2953A | intron | √ | √ | √ | |
| C2954T | intron | √ | √ | √ | |
| G2955C | intron | √ | √ | √ | |
| A2956G | intron | √ | √ | √ | |
| ins 2956TTT | intron | √ | √ | √ | |
| G3063A | D484N | √ | _ | √ | |
| G3083A | silent | √ | √ | √ | |
| C3084A | silent | √ | √ | √ | |
| T3140G | silent | √ | √ | √ | |
| C3239A | silent | √ | √ | √ | |
| G3314A | G568D | √ | √ | √ | |
| C3333T | intron | √ | √ | √ | |
| C3335A | intron | √ | √ | √ | |
| C3452T | A586V | √ | _ | √ | |
| T3492C | silent | √ | √ | √ | |
| C3633A | silent | √ | √ | √ | |
| C3680T | silent | √ | √ | √ | |
| C3707A | intron | √ | √ | √ | |
| del 3753GA | intron | √ | √ | √ | |
| A3761C | intron | √ | √ | √ | |
| del 3787AA | intron | √ | √ | √ | |
| A3975T | silent | √ | √ | √ | |
| A4015G | 3'UTR | √ | √ | √ | |

```
w5200          gcacactctctcccctgccacttcccaaatccgcccgccattcatgcactcttctgtgca
616B           gcacactctctcccctgccacttcccaaatccgcccgccattcatgcactcttctgtgca
w5200norma     gcacactctctccccggccacttcccaaatccgcccgccattcatgcacccttctgtgca
Dxs1           gcacactctctccccggccacttcccaaatccgcccgccattcatgcacccttctgtgca
616Bnormal
```

```
w5200          ctgtcagcgccaccattagctcgcagctcaagctcgccactaccattttggtcggttctt
616B           ctgtcagcgccaccattagctcgcagctcaagctcgccactaccattttggtcggttctt
w5200normal    ctgtcagcgccaccattagctcgcagctcaagctcgccactaccattttggtcggttctt
Dxs1           ctgtcagcgccaccattagctcgcagctcaagctcgccactaccattttggtcggttctt
616Bnormal
```

```
w5200          gaggaaatcgatcgaaccgttggagtgccaccactggcagaggctgttgcattcttgagt
616B           gaggaaatcgatcgaaccgttggagtgccaccactggcagaggctgttgcattcttgagt
w5200normal    gaggaaatcgatcgaaccgttggagtgccaccactggcagaggctgttgcattcttgagt
Dxs1           gaggaaatcgatcgaaccgttggagtgccaccactggcagaggctgttgcattcttgagt
616Bnormal
```

```
w5200          tgagcaggaagaggagaggaagcaATGGCTCTGTCGACGTTCTCTGTCCCAAGGGGTTC
616B           tgagcaggaagaggagaggaagcaATGGCTCTGTCGACGTTCTCTGTCCCAAGGGGTTC
w5200normal    tgagcaggaagaggagaggaagcaATGGCTCTGTCGACGTTCTCTGTCCCAAGGGGATTC
Dxs1           tgagcaggaagaggagaggaagcaATGGCTCTGTCGACGTTCTCTGTCCCAAGGGGATTC
616Bnormal
```

```
w5200          CTCGGCGTGCCGGCTCAGGACTCCCATTTCGCTTCGGCGGTCGAGCTCCATGTTCACAAG
616B           CTCGGCGTGCCGGCTCAGGACTCCCATTTCGCTTCGGCGGTCGAGCTCCATGTTCACAAG
w5200normal    CTCGGTGTGCCGGCTCAGGACTCCCATTTCGCTTCGGCGGTCGAGCTCCATGTCAACAAG
Dxs1           CTCGGTGTGCCGGCTCAGGACTCCCATTTCGCTTCGGCGGTCGAGCTCCATGTCAACAAG
616Bnormal
```

```
w5200          CTGCTCCAGGCCAGGCCTATCAATCTCAAGgtaagcttcctctgccagttgtacgcaagc
616B           CTGCTCCAGGCCAGGCCTATCAATCTCAAGgtaagcttcctctgccagttgtacgcaagc
w5200normal     CTGCTCCAGGCCAGGCCTATCAATCTCAAGgtaagcttcctctgccagttgtacgcaagc
Dxs1           CTGCTCCAGGCCAGGCCTATCAATCTCAAGgtaagcttcctctgccagttgtacgcaagc
616Bnormal
```

```
w5200          taaattttctcagttccgttccggttagtttgatggccaatgctgcgtgcagCCTCGGCG
616B           taaattttctcagttccgttccggttagtttgatggccaatgctgcgtgcagCCTCGGCG
w5200normal    taaattttctcagttccgttccggttagtttgatggccaatgctgcgtgcagCCTCGGCG
Dxs1           taaattttctcagttccgttccggttagtttgatggccaatgctgcgtgcagCCTCGGCG
616Bnormal
```

```
w5200          GAGGCCGGCATGCGTGTCGGCGTCGCTGTCGTCGGAGCGCGAGGCGGAGTACTACTCGCA
616B           GAGGCCGGCATGCGTGTCGGCGTCGCTGTCGTCGGAGCGCGAGGCGGAGTACTACTCGCA
w5200normal    GAGGCCGGCATGCGTGTCGGCGTCGCTGTCGTCGGAGCGCGAGGCGGAGTACTACTCGCA
Dxs1           GAGGCCGGCATGCGTGTCGGCGTCGCTGTCGTCGGAGCGCGAGGCGGAGTACTACTCGCA
616Bnormal     ------------------------------------------------------------
```

```
w5200          GAGGCCGCCCACGCCGCTGCTGGACACCATCAACTACCCCGTCCACATGAAGAACCTGTC
616B           GAGGCCGCCCACGCCGCTGCTGGACACCATCAACTACCCCGTCCACATGAAGAACCTGTC
w5200normal    GAGGCCGCCCACGCCGCTGCTGGACACCATCAACTACCCCGTCCACATGAAGAACCTGTC
 Dxs1          GAGGCCGCCCACGCCGCTGCTGGACACCATCAACTACCCCGTCCACATGAAGAACCTGTC
616Bnormal     ------------------------------------------------------------
```

```
w5200          TGTGAAGGAGCTGCGGCAGCTGGCCGACGAGCTCCGGTCCGACGTCATCTTCCACGTCTC
616B           TGTGAAGGAGCTGCGGCAGCTGGCCGACGAGCTCCGGTCCGACGTCATCTTCCACGTCTC
w5200normal    TGTGAAGGAGCTGCGGCAGCTGGCCGACGAGCTCCGGTCCGACGTCATCTTCCACGTCTC
 Dxs1          TGTGAAGGAGCTGCGGCAGCTGGCCGACGAGCTCCGGTCCGACGTCATCTTCCACGTCTC
616Bnormal     ------------------------------------------------------------
```

```
w5200                CAAGACCGGCGGCCACCTCGGGTCCAGCCTCGGCGTGGTGGAGCTCACCGTCGCGCTGCA
616B                 CAAGACCGGCGGCCACCTCGGGTCCAGCCTCGGCGTGGTGGAGCTCACCGTCGCGCTGCA
w5200normal          CAAGACCGGCGGCCACCTCGGGTCCAGCCTCGGCGTGGTGGAGCTCACCGTCGCGCTGCA
Dxs1                 CAAGACCGGCGGCCACCTCGGGTCCAGCCTCGGCGTGGTGGAGCTCACCGTCGCGCTGCA
616Bnormal           ------------------------------------------------------------

w5200                CTACGTCTTCAACGCGCCGCAGGACCGCATCCTCTGGGACGTCGGCCACCAGgtacgctg
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w5200normal          CTACGTCTTCAACGCGCCGCAGGACCGCATCCTCTGGGACGTCGGCCACCAGgtacgctg
 Dxs1                CTACGTCTTCAACGCGCCGCAGGACCGCATCCTCTGGGACGTCGGCCACCAGgtacgctg
616Bnormal           ------------------------------------------------------------


w5200                atgcggcatgggccgcgcgcgcggcatggctctgggtaacgtgcgctccatgtgagcgtg
616B                 atgcggcatgggccgcgcgcgcggcatggctctgggtaacgtgcgctccatgtgagcgtg
w5200normal          atgcggcatgggccgcgcgcgcggcatggctctgggtaacgtgcgctccatgtgagcgtg
Dxs1                 atgcggcatgggccgcgcgcgcggcatggctctgggtaacgtgcgctccatgtgagcgtg
616Bnormal           ------------------------------------------------------------


w5200                ccgggcaggtcgcggacaggctagctaattaaccaccccggacccgggttttgtttgtct
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W5200normal          ccgggcaggtcgcggacaggctagctaattaaccaccccggacccgggttttgtttgtct
Dxs1                 ccgggcaggtcgcggacaggctagctaattaaccaccccggacccgggttttgtttgtct
616Bnormal           ------------------------------------------------------------


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616B                 gattcgcgcgcatgcagTCGTACCCGCACAAGATCCTGACGGGGCGGCGCGACAAGATGC
w5200normal          gattcgcgcgcatgcagTCGTACCCGCACAAGATCCTGACGGGGCGGCGCGACAAGATGC
Dxs1                 gattcgcgcgcatgcagTCGTACCCGCACAAGATCCTGACGGGGCGGCGCGACAAGATGC
616Bnormal           ------------------------------------------------------------


w5200                CGACGATGCGGCAGACCAACGGCCTGGCGGGCTTCACCAAGCGCGCCGAGAGCGAGTACG
616B                 CGACGATGCGGCAGACCAACGGCCTGGCGGGCTTCACCAAGCGCGCCGAGAGCGAGTACG
w5200normal          CGACGATGCGGCAGACCAACGGCCTGGCGGGCTTCACCAAGCGCGCCGAGAGCGAGTACG
Dxs1                 CGACGATGCGGCAGACCAACGGCCTGGCGGGCTTCACCAAGCGCGCCGAGAGCGAGTACG
616Bnormal


w5200                ACAGCTTCGGCACGGGCCACAGCTCCACCACCATCTCCGCGGCGCTCGGGATGGCGGTGG
616B                 ACAGCTTCGGCACGGGCCACAGCTCCACCACCATCTCCGCGGCGCTCGGGATGGCGGTGG
w5200normal          ACAGCTTCGGCACGGGCCACAGCTCCACCACCATCTCCGCGGCGCTCGGGATGGCGGTGG
Dxs1                 ACAGCTTCGGCACGGGCCACAGCTCCACCACCATCTCCGCGGCGCTCGGGATGGCGGTGG
616Bnormal


w5200                GCCGGGACCTCAAGGGCGGCAAGAACAACGTGGTCGCGGTGATCGGCGACGGCGCCGTGA
616B                 GCCGGGACCTCAAGGGCGGCAAGAACAACGTGGTCGCGGTGATCGGCGACGGCGCCATGA
w5200normal          GCCGGGACCTCAAGGGCGGCAAGAACAACGTGGTCGCGGTGATCGGCGACGGCGCCATGA
Dxs1                 GCCGGGACCTCAAGGGCGGCAAGAACAACGTGGTCGCGGTGATCGGCGACGGCGCCATGA
616Bnormal


w5200                   CGCCCGGGCAGGCGTACGAGGCCATGAACAACGCCGGGTACCTGGACTCCGACATGATCG
616B                    CGGCCGGGCAGGCGTACGAGGCCATGAACAACGCCGGGTACCTGGACTCCGACATGATCG
w5200normal             CGGCCGGGCAGGCGTACGAGGCCATGAACAACGCCGGGTACCTGGACTCCGACATGATCG
Dxs1                    CGGCCGGGCAGGCGTACGAGGCCATGAACAACGCCGGGTACCTGGACTCCGACATGATCG
616Bnormal
```

```
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616B                 TCATCCTCAACGACAACAAGCAGGTGTCCTTGCCCACGGCGACGCTCGACGGGCCGGTGC
w5200normal          TCATCCTCAACGACAACAAGCAGGTGTCCTTGCCCACGGCGACGCTCGACGGGCCGGTGC
Dxs1                 TCATCCTCAACGACAACAAGCAGGTGTCCTTGCCCACGGCGACGCTCGACGGGCCGGTGC
616Bnormal           ACGACAACAAGCAGGTGTCCTTGCCCACGGCGACGCTCGACGGGCCGGTGC

w5200                CGCCCGTGGGCGCGCTCAGCAGCGCCCTCAGCAAGCTGCAGTCAAGCAGGCCGCTCAGGG
616B                 CGCCCGTGGGCGCGCTCAGCAGCGCCCTCAGCAAGCTGCAGTCAAGCAGGCCGCTCAGGG
w5200normal          CGCCCGTGGGCGCGCTCAGCAGCGCCCTCAGCAAGCTGCAGTCAAGCAGGCCGCTCAGGG
Dxs1                 CGCCCGTGGGCGCGCTCAGCAGCGCCCTCAGCAAGCTGCAGTCAAGCAGGCCGCTCAGGG
616Bnormal          CGCCCGTGGGCGCGCTCAGCAGCGCCCTCAGCAAGCTGCAGTCAAGCAGGCCGCTCAGGG

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w5200normal          AGCTCAGGGAGGTGGCCAAGGtacgtccgcacaaaccttggatgggacgctacatcaccc
Dxs1                 AGCTCAGGGAGGTGGCCAAGGtacgtccgcacaaaccttggatgggacgctacatcaccc
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W5200normal          tcttgacagcccggccggttaggcaagcgccacgtaagggcttgttcggttattcccaat
Dxs1                 tcttgacagcccggccggttaggcaagcgccacgtaagggcttgttcggttattcccaat
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Dxs1                 acacatggattggatgagattggaaaaaattatgaagaagtttgagctgtttgggattca
616Bnormal           acacatggattggatgagattggaaaaaattatgaagaagtttgagctgtttgggattca

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Dxs1                 aacccatccaatcccgctcaatccacatggattgagagctaaccgaacaagccctaacac
616Bnormal           aacccatccaatcccgctcaatccacatggattgagagctaaccgaacaagccctaacac

w5200                gtcaaatttccagactgtcctcgttctcacggaagcgcgtagattttctggaatcttgat
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W5200normal          gtcaaatttccagactgtcctcgttctcacggaagcgcgtagattttctggaatcttgat
Dxs1                 gtcaaatttccagactgtcctcgttctcacggaagcgcgtagattttctggaatcttgat
616Bnormal           gtcaaatttccagactgtcctcgttctcacggaagcgcgtagattttctggaatcttgat

w5200                tagtagtaattcctatagttgcacatatttttttatcaaatcattaagataaataaataa
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W5200normal          tagtagtaattcctatagttgcacatatttttttatcaaatcattaagataaataaataa
Dxs1                 tagtagtaattcctatagttgcacatatttttttatcaaatcattaagataaataaataa
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w5200normal          ccttgcctttggaattaaatggaaaactgtcaacagtcatcgggcagcagcagacgtaca
Dxs1                 ccttgcctttggaattaaatggaaaactgtcaacagtcatcgggcagcagcagacgtaca
616Bnormal           ccttgcctttggaattaaatggaaaactgtcaacagtcatcgggcagcagcagacgtaca

w5200                tgacgcgagctatggagcttcttgaatctactgcacgaaagcgtctgaatgaaacttgtt
616B                 tgacgcgagctatggagcttcttgaatctactgcacgaaagcgtctgaatgaaacttgtt
W5200normal          tgacgcgagctatggagcttcttgaatctactgcacgaaagcgtctgaatgaaacttgtt
Dxs1                 tgacgcgagctatggagcttcttgaatctactgcacgaaagcgtctgaatgaaacttgtt
616Bnormal           tgacgcgagctatggagcttcttgaatctactgcacgaaagcgtctgaatgaaacttgtt
```

```
w5200              tagtcttagcgaacatgccaaaataggctgcgtcagatgcagagtgacgtgcactaatct
616B               tagtcttagcgaacatgccaaaataggctgcgtcagatgcagagtgacgtgcactaatct
w5200normal        tagtcttagcgaacatgccaaaataggctgcgtcagatgcagagtgacgtgcactaatct
Dxs1               tagtcttagcgaacatgccaaaataggctgcgtcagatgcagagtgacgtgcactaatct
616Bnormal         tagtcttagcgaacatgccaaaataggctgcgtcagatgcagagtgacgtgcactaatct

w5200              gcttctaggggcatgcatggacacatgttcaccagaaaggccgatgacgaagcgtatac
616B               gcttctaggggcatgcatggacacatgttcaccagaaaggccgatgacgaagcgtatac
w5200normal        gcttctaggggcatgcatggacacatgttcaccagaaaggccgatgacgaagcgtatac
Dxs1               gcttctaggggcatgcatggacacatgttcaccagaaaggccgatgacgaagcgtatac
616Bnormal         gcttctaggggcatgcatggacacatgttcaccagaaaggccgatgacgaagcgtata

w5200              gagctagcattctagcaacagtccgtagattcgagtaatgcccactactaggcaaacttt
616B               gagctagcattctagcaacagtccgtagattcgagtaatgcccactactaggcaaacttt
W5200normal        gagctagcattctagcaacagtccgtagattcgagtaatgcccactactaggcaaacttt
Dxs1               gagctagcattctagcaacagtccgtagattcgagtaatgcccactactaggcaaacttt
616Bnormal         gagctagcattctagcaacagtccgtagattcgagtaatgcccactactaggcaaacttt

w5200              gtataaacaaagctactcaaagcatggatgatggatctcaggtcgcagcaagttgactct
616B               gtataaacaaagctactcaaagcatggatgatggatctcaggtcgcagcaagttgactct
W5200normal        gtataaacaaagctactcaaagcatggatgatggatctcaggtcgcagcaagttgactct
Dxs1               gtataaacaaagctactcaaagcatggatgatggatctcaggtcgcagcaagttgactct
616Bnormal         gtataaacaaagctactcaaagcatggatgatggatct----------------------

w5200              agaaatagtttatcatgctactcgagctgtatcca-gtttgactgacattggttcatctt
616B               agaaatagtttatcatgctactcgagctgtatccaagtttgactgacattggttcatctt
W5200normal        agaaatagtttatcatgctactcgagctgtatccaagtttgactgacattggttcatctt
Dxs1               agaaatagtttatcatgctactcgagctgtatccaagtttgactgacattggttcatctt
616Bnormal         ------------------------------------------------------------

w5200              ctactggactggtcataatattacctgggctaggttttgaccggcccattcttgttgggc
616B               ctactggactggtcataatattacctgggctaggttttgaccggcccattcttgttgggc
W5200normal        ctactggactggtcataatattacctgggctaggttttgaccggcccattcttgttgggc
Dxs1               ctactggactggtcataatattacctgggctaggttttgaccggcccattcttgttgggc
616Bnormal         ------------------------------------------------------------

w5200              cgacaaggattgtggaaggaatgggcggcccaggtttgcagctctgagtctctgaccgat
616B               cgacaaggattgtggaaggaatgggcggcccaggtttgcagctctgagtctctgaccgat
W5200normal        cgacaaggattgtggaaggaatgggcggcccaggtttgcagctctgagtctctgaccgat
Dxs1               cgacaaggattgtggaaggaatgggcggcccaggtttgcagctctgagtctctgaccgat
616Bnormal         ------------------------------------------------------------

w5200              cgctggcgtgcgtgcttgtgcttgtgcaggGAGTGACGAAGCAGATCGGTGGCTCAGTGC
616B               cgctggcgtgcgtgcttgtgcttgtgcaggGAGTGACGAAGCAGATCGGTGGCTCAGTGC
w5200normal        cgctggcgtgcgtgcttgtgcttgtgcaggGAGTGACGAAGCAGATCGGTGGCTCAGTGC
Dxs1               cgctggcgtgcgtgcttgtgcttgtgcaggGAGTGACGAAGCAGATCGGTGGCTCAGTGC
616Bnormal         ------------------------------------------------------------

w5200              ACGAGCTGGCGGCGAAGGTGGACGAGTACGCCCGCGGCATGATCAGCGGGCCCGGCTCCT
616B               ACGAGCTGGCGGCGAAGGTGGACGAGTACGCCCGCGGCATGATCAGCGGGCCCGGCTCCT
w5200normal        ACGAGCTGGCGGCGAAGGTGGACGAGTACGCCCGCGGCATGATCAGCGGGCCCGGCTCCT
Dxs1               ACGAGCTGGCGGCGAAGGTGGACGAGTACGCCCGCGGCATGATCAGCGGGCCCGGCTCCT
616Bnormal         ------------------------------------------------------------
```

```
w5200              CGCTCTTCGAGGAGCTCGGTCTCTACTACATCGGCCCCGTCGACGGCCACAACATCGACG
616B               CGCTCTTCGAGGAGCTCGGTCTCTACTACATCGGCCCCGTCGACGGCCACAACATCGACG
w5200normal        CGCTCTTCGAGGAGCTCGGTCTCTACTACATCGGCCCCGTCGACGGCCACAACATCGACG
Dxs1               CGCTCTTCGAGGAGCTCGGTCTCTACTACATCGGCCCCGTCGACGGCCACAACATCGACG
616Bnormal         ------------------------------------------------------------

w5200              ACCTCATCACCATCCTCAACGACGTCAAGAGCACCAAGACCACCGGCCCCGTCCTCATCC
616B               ACCTCATCACCATCCTCAACGACGTCAAGAGCACCAAGACCACCGGCCCCGTCCTCATCC
w5200normal        ACCTCATCACCATCCTCAACGACGTCAAGAGCACCAAGACCACCGGCCCCGTCCTCATCC
Dxs1               ACCTCATCACCATCCTCAACGACGTCAAGAGCACCAAGACCACCGGCCCCGTCCTCATCC
616Bnormal         ............................................................

w5200              ACGTCGTCACCGAGAAGGGCCGCGGCTACCCCTACGCCGAGCGAGCCGCCGACAAGTACC
616B               ACGTCGTCACCGAGAAGGGCCGCGGCTACCCCTACGCCGAGCGAGCCGCCGACAAGTACC
w5200normal        ACGTCGTCACCGAGAAGGGCCGCGGCTACCCCTACGCCGAGCGAGCCGCCGACAAGTACC
Dxs1               ACGTCGTCACCGAGAAGGGCCGCGGCTACCCCTACGCCGAGCGAGCCGCCGACAAGTACC
616Bnormal         ............................................................

w5200              ACGGTacaacgcaccacatgagctagcatatgtgccactgtttgcctgttgcacagcaca
616B               ACGGTacaacgcaccacatgagctagcatatgtgccactgtttgcctgttgcacagcaca
w5200normal        ACGGTacaacgcaccacatgagctagcatatgtgccactgtttgcctgttgcacagcaca
Dxs1               ACGGTacaacgcaccacatgagctagcatatgtgccactgtttgcctgttgcacagcaca
616Bnormal         ............................................................

w5200              gatcgtaccccgaccggaatctgtgcgtcatcttggctctgttgtttgatgcgtgcgtgc
616B               gatcgtaccccgaccggaatctgtgcgtcatcttggctctgttgtttgatgcgtgcgtgc
W5200normal         gatcgtaccccgaccggaatctgtgcgtcatcttggctctgttgtttgatgcgtgcgtgc
Dxs1               gatcgtaccccgaccggaatctgtgcgtcatcttggctctgttgtttgatgcgtgcgtgc
616Bnormal         ............................................................

w5200              gtgcaggtGTCGCCAAGTTTGATCCGGCGACCGGGAAGCAGTTCAAGTCCCCCGCCAAGA
616B               gtgcaggtGTCGCCAAGTTTGATCCGGCGACCGGGAAGCAGTTCAAGTCCCCCGCCAAGA
w5200normal         gtgcaggtGTCGCCAAGTTTGATCCGGCGACCGGGAAGCAGTTCAAGTCCCCCGCCAAGA
Dxs1               gtgcaggtGTCGCCAAGTTTGATCCGGCGACCGGGAAGCAGTTCAAGTCCCCCGCCAAGA
616Bnormal         ............................................................

                   2641

w5200              CGCTGTCCTACACCAACTACTTCGCCGAGGCGCTAATCGCCGAGGCGGAGCAGGACAGCA
616B               CGCTGTCCTACACCAACTACTTCGCCGAGGCGCTCATCGCCGAGGCGGAGCAGGACAGCA
w5200normal        CGCTGTCCTACACCAACTACTTCGCCGAGGCGCTCATCGCCGAGGCGGAGCAGGACAGCA
Dxs1               CGCTGTCCTACACCAACTACTTCGCCGAGGCGCTCATCGCCGAGGCGGAGCAGGACAGCA
616Bnormal         ............................................................

w5200              AGATCGTGGCCATCCACGCGGCCATGGGCGGCGGCACGGGGCTCAACTACTTCCTCCGCC
616B               AGATCGTGGCCATCCACGCGGCCATGGGCGGCGGCACGGGGCTCAACTACTTCCTCCGCC
w5200normal        AGATCGTGGCCATCCACGCGGCCATGGGCGGCGGCACGGGGCTCAACTACTTCCTCCGCC
Dxs1               AGATCGTGGCCATCCACGCGGCCATGGGCGGCGGCACGGGGCTCAACTACTTCCTCCGCC
616Bnormal         ............................................................

w5200              GCTTCCCGAACCGGTGCTTCGACGTCGGGATCGCGGAGCAGCACGCCGTCACGTTCGCGG
616B               GCTTCCCGAACCGGTGCTTCGACGTCGGGATCGCGGAGCAGCACGCCGTCACGTTCGCGG
w5200normal        GCTTCCCGAACCGGTGCTTCGACGTCGGGATCGCGGAGCAGCACGCCGTCACGTTCGCGG
Dxs1               GCTTCCCGAGCCGGTGCTTCGACGTCGGGATCGCGGAGCAGCACGCCGTCACGTTCGCGG
616Bnormal         ............................................................
```

```
w5200              CCGGCCTGGCCTGCGAGGGCCTCAAGCCCTTCTGCGCCATCTACTCGTCTTTCCTGCAGC
616B               CCGGCCTGGCCTGCGAGGGCCTCAAGCCCTTCTGCGCCATCTACTCGTCTTTCCTGCAGC
w5200normal      CCGGCCTGGCCTGCGAGGGCCTCAAGCCCTTCTGCGCCATCTACTCGTCTTTCCTGCAGC
Dxs1               CCGGCCTGGCCTGCGAGGGCCTCAAGCCCTTCTGCGCCATCTACTCGTCTTTCCTGCAGC
616Bnormal

w5200                GCGGCTACGACCAGGTgcgcacgccgccgtgtgcccgccggccgggccgttcttcgcatt
616B                 GCGGCTACGACCAGGTgcgcacgccgccgtgtgcccgccggccgggccgttcttcgcatt
w5200normal          GCGGCTACGACCAGGTgcgcacgccgccgtgtgcccgccggccgggccgttcttcgcatt
Dxs1                 GCGGCTACGACCAGGTgcgcacgctgccgtgtgcccgccggccgggccgttcttcgcatt
616Bnormal

w5200                tgcttgctgctcgatcgtttcgttttcttcttttgtgcgggcgcggtcctcgactgacgc
616B                 tgcttgctgctcgatcgtttcgttttcttcttttgtgcgggcgcggtcctcgactaacgc
W5200normal          tgcttgctgctcgatcgtttcgttttcttcttttgtgcgggcgcggtcctcgactgacgc
Dxs1                 tgcttgctgctactcgcgttttcttcttttgtgcgggcgcggtcctcgactgacgc---
616Bnormal

W5200                cgtacgcacgtcgccgatgggccggtgtgggtggtggcgcaggtcgTGCACG
616B                 cgtacgcacgtcgccgatgggccggtgtgggtggtggcgcaggtcgTGCACG
W5200normal          cgtacgcacgtcgccgatgggccggtgtgggtggtggcgcaggtcgTGCACG
Dxs1                 cgtacgcacgtcgccgatgggccggtgtggccgtgtgggtggtggcgcaggtcgTGCACG
616Bnormal

w5200                 ACGTCAATCTGCAGAAGCTACCGGTAAGGTTCGCCATGGACAGGGCCGGGCTGGTCGGCG
616B                  ACGTCAATCTGCAGAAGCTACCGGTAAGGTTCGCCATGGACAGGGCCGGGCTGGTCGGCG
w5200normal           ACGTCAATCTGCAGAAGCTACCGGTAAGGTTCGCCATGGACAGGGCCGGGCTGGTCGGCG
Dxs1                  ACGTCGATCTGCAGAAGCTACCGGTGCGGTTCGCCATGGACAGGGCCGGGCTGGTCGGCG
616Bnormal

w5200                CGGACGGGCCGACCCACTGCGGGGCGTTCGACGTCGCGTACATGGCCTGCCTGCCCAACA
616B                 CGGACGGGCCGACCCACTGCGGTGCGTTCGACGTCGCGTACATGGCCTGCCTGCCCAACA
w5200normal          CGGACGGGCCGACCCACTGCGGGGCGTTCGACGTCGCGTACATGGCCTGCCTGCCCAACA
Dxs1                 CGGACGGGCCGACCCACTGCGGGGCGTTCGACGTCGCGTACATGGCCTGCCTGCCCAACA
616Bnormal

w5200                TGGTCGTCATGGCCCCGTCCGACGAGGCCGAGCTCTGCCACATGGTCGCCACAGCCGCGG
616B                 TGGTCGTCATGGCCCCGTCCGACGAGGCCGAGCTCTGCCACATGGTCGCCACAGCCGCGG
w5200normal TGGTCGTCATGGCCCCGTCCGACGAGGCCGAGCTCTGCCACATGGTCGCCACAGCCGCGG
Dxs1                 TGGTCGTCATGGCCCCGTCCGACGAGGCCGAGCTCTGCCACATGGTCGCCACCGCCGCGG
616Bnormal

w5200                CAATCGACGACCGCCCGTCCTGCTTCCGCTACCCGAGAGGCAACGGCGTTGGCGTCCCGT
616B                 CAATCGACGACCGCCCGTCCTGCTTCCGCTACCCGAGAGGCAACGGCGTTGGCGTCCCGT
w5200normal          CAATCGACGACCGCCCGTCCTGCTTCCGCTACCCGAGAGGCAACGGCGTTGGCGTCCCGT
Dxs1                 CCATCGACGACCGCCCGTCCTGCTTCCGCTACCCGAGAGGCAACGGCGTTGGCGTCCCGT
616Bnormal

   w5200             TGCCGCCCAACTACAAAGACACTCCCCTCGAGGTATGTATACATCGATCGATGGTGTATT
   616B              TGCCGCCCAACTACAAAGACACTCCCCTCGAGGTATGTATACATCGATCGATGGTGTATT
   w5200normal       TGCCGCCCAACTACAAAGACACTCCCCTCGAGGTATGTATACATCGATCGATGGTGTATT
   Dxs1              TGCCGCCCAACTACAAAGGCACTCCCCTCGAGGTACGTATACATCGATCGATGGTGTCTT
   616Bnormal
```

```
w5200          TTACTCATTTTTTATCAATCGGGGATCTTCAGTTTTGGTAATCGGCCTATATAGGTCGGC
616B           TTACTCATTTTTTATCAATCGGGGATCTTCAGTTTTGGTAATCGGCCTATATAGGTCGGC
w5200normal    TTACTCATTTTTTATCAATCGGGGATCTTCAGTTTTGGTAATCGGCCTATATAGGTCGGC
Dxs1           TTACTCATTTTTTATCAATCGGGGATCTTCAGTTTTGGTAATCGGCCTATATAGGTCGGC
616Bnormal     ..............................................................

w5200          AAAGGCAGGATCCTGCTGGAGGGCGACCGGGTGGCGCTGCTGGGGTACGGGTCGGCAGTG
616B           AAAGGCAGGATCCTGCTGGAGGGCGACCGGGTGGTGCTGCTGGGGTACGGGTCGGCAGTG
w5200normal    AAAGGCAGGATCCTGCTGGAGGGCGACCGGGTGGTGCTGCTGGGGTACGGGTCGGCAGTG
Dxs1           AAAGGCAGGATCCTGCTGGAGGGCGACCGGGTGGCGCTGCTGGGGTACGGGTCGGCAGTG
616Bnormal     ..............................................................

w5200          CAGTACTGCCTGACCGCCGCGTCCCTGGTGCAGCGCCACGGCCTCAAGGTCACCGTCGCC
616B           CAGTACTGCCTGACCGCCGCGTCCCTGGTGCAGCGCCACGGCCTCAAGGTCACCGTCGCC
w5200normal    CAGTACTGCCTGACCGCCGCGTCCCTGGTGCAGCGCCACGGCCTCAAGGTCACCGTCGCC
Dxs1           CAGTACTGCCTGACTGCCGCGTCCCTGGTGCAGCGCCACGGCCTCAAGGTCACCGTCGCC
616Bnormal     ..............................................................

w5200          GACGCGAGGTTCTGCAAGCCGCTGGACCACGCCCTGATCAGGAGCCTGGCCAAGTCCCAC
616B           GACGCGAGGTTCTGCAAGCCGCTGGACCACGCCCTGATCAGGAGCCTGGCCAAGTCCCAC
w5200normal    GACGCGAGGTTCTGCAAGCCGCTGGACCACGCCCTGATCAGGAGCCTGGCCAAGTCCCAC
Dxs1           GACGCGAGGTTCTGCAAGCCGCTGGACCACGCCCTGATCAGGAGCCTGGCCAAGTCCCAC
616Bnormal     ..............................................................

w5200          GAGGTGCTCATCACCGTGGAGGAAGGCTCCATCGGAGGGTTCGGCTCGCACGTCGCCCAG
616B           GAGGTGCTCATCACCGTGGAGGAAGGCTCCATCGGAGGGTTCGGCTCGCACGTCGCCCAG
w5200normal    GAGGTGCTCATCACCGTGGAGGAAGGCTCCATCGGAGGGTTCGGCTCGCACGTCGCCCAG
Dxs1           GAGGTGCTCATCACCGTGGAGGAAGGCTCCATCGGCGGGTTCGGCTCGCACGTCGCCCAG
616Bnormal     ..............................................................

w5200          TTCATGGCCCTGGACGGCCTTCTTGACGGCAAACTCAAGgcaagtctcacactagctagc
616B           TTCATGGCCCTGGACGGCCTTCTTGACGGCAAACTCAAGgcaagtctcacactagctagc
w5200normal    TTCATGGCCCTGGACGGCCTTCTTGACGGCAAACTCAAGgcaagtctcacactagctagc
Dxs1           TTCATGGCCCTGGACGGCCTTCTCGACGGCAAACTCAAGgcaagtctcaccctagctagc
616Bnormal     ..............................................................

w5200          tgctcggtcgccctaatgataacgagagagagagaaaaaaactccgaactccatcttt
616B           tgctcggtcgccctaatgataacgagagagagagaaaaaaactccgaactccatcttt
w5200normal    tgctcggtcgccctaatgataacgagagagagagaaaaaaactccgaactccatcttt
Dxs1           tgctcggtcgccctaatgataacgagagagagagaaaaaaaatccgaactccatcttt
616Bnormal     ..............................................................

w5200              agctgacaagtgatgaactcgacttttatttgggtgggtgcagTGGCGACCGCTGGTG
616B               agctgacaagtgatgaactcgacttttatttgggtgggtgcagTGGCGACCGCTGGTG
w5200normal        agctgacaagtgatgaactcgacttttatttgggtgggtgcagTGGCGACCGCTGG
Dxs1                agctgacaaaagtgatgaactcgacttttatttgggtgggtgcagTGGCGACCGCTGGTG
616Bnormal          ------------------------------------------------------------

w5200                CTTCCTGACAGGTACATCGACCATGGATCGCCGGCCGATCAGCTGGCCGAGGCTGGGCTG
616B                 CTTCCTGACAGGTACATCGACCATGGATCGCCGGCCGATCAGCTGGCCGAGGCTGGGCTG
w5200normal          CTTCCTGACAGGTACATCGACCATGGATCGCCGGCCGATCAGCTGGCCGAGGCTGGGCTG
Dxs1                 CTTCCTGACAGGTACATCGACCATGGATCGCCGGCCGATCAGCTGGCCGAGGCTGGGCTG
616Bnormal           ..............................................................

w5200          ACGCCGTCACACATCGCCGCGTCGGTGTTCAACATCCTGGGGCAGAACAGGGAGGCTCTT
616B           ACGCCGTCACACATCGCCGCGTCGGTGTTCAACATCCTGGGGCAGA----   GGAGGCTCTT
w5200normal    ACGCCGTCACACATCGCCGCGTCGGTGTTCAACATCCTGGGGCAGAACAGGGAGGCTCTT
Dxs1           ACGCCGTCACACATCGCCGCGTCGGTGTTCAACATCCTGGGGCAGAACAGGGAGGCTCTT
616Bnormal       ------------------------------------------------------------
```

```
w5200                GCCATCATGGCAGTGCCTAACGCGTAGaacttgtgctgatcttggcctatagagatggtt
616B                 GCCATCATGGCAGTGCCTAACGCGTAGaacttgtgctgatctgggcctatagagatggtt
w5200normal         GCCATCATGGCAGTGCCTAACGCGTAGaacttgtgctgatcttggcctatagagatggtt
Dxs1                 GCCATCATGGCAGTGCCAAACGCGTAGaacttgtgctgatctgggcctatagagatgatt
616Bnormal

w5200                gtacattttgtcgttaactagagtgtctgaacttgggagattagtcttctttggatgaaa
616B                 gtacattttgtcgttaactagagtgtctgaacttgggagattagtcttctttggatgaaa
W5200normal         gtacattttgtcgttaactagagtgtctgaacttgggagattagtcttctttggatgaaa
Dxs1                 gtacattttgtcgttaactagagtgtctgaacttgggagattagtcttctttggatgaaa
616Bnormal

W5200                gtgtcgccggaacaacagttaccg
616B                 gtgtcgccggaacaacagttaccg
W5200normal         gtgtcgccggaacaacagttaccg
Dxs1                 gtgtcgccggaacaacagttaccg
616Bnormal
```

*Figure A1:* Multiple sequence alignment of Dxs1 alleles from normal and mutant maize seedlings. Dxs1: B73 sequence. Intron sequences are in small cap and exon sequences are in all caps. Grey shading highlights the differences between sequences. Dashes (-) indicate gaps inserted to optimized the alignment

```
w5200                tattgacagcccggccggataggcaagcgccacgtaagggcttgttcggttattcccaat
616B                 tcttgacagcccggccggttaggcaagcgccacgtaa----------------------
W5200normal          tcttgacagcccggccggttaggcaagcgccacgtaagggcttgttcggttattcccaat
Dxs1                 tcttgacagcccggccggttaggcaagcgccacgtaagggcttgttcggttattcccaat
616Bnormal           tcttgacagcccggccggttaggcaagcgccacgtaagggcttgttcggttattcccaat
612A                 tcttgacagcccggccggttaggcaagcgccacgtaa----------------------
612Anormal           tcttgacagcccggccggttaggcaagcgccacgtaa----------------------
612M                 tcttgacagcccggccggttaggcaagcgccacgtaa----------------------
612Mnormal           tcttgacagcccggccggttaggcaagcgccacgtaagggcttgttcggttattcccaat
612N                 tcttgacagcccggccggttaggcaagcgccacgtaa----------------------
612Nnormal           tcttgacagcccggccggttaggcaagcgccacgtaa----------------------

w5200                acacatggattggatgggattggaaaaaattatgaagaagtttgagctgtttgggattca
616B                 ------------------------------------------------------------
w5200normal          acacatggattggatgggattggaaaaaattatgaagaagtttgagctgtttgggattca
Dxs1                 acacatggattggatgagattggaaaaaattatgaagaagtttgagctgtttgggattca
616Bnormal           acacatggattggatgagattggaaaaaattatgaagaagtttgagctgtttgggattca
612A                 ------------------------------------------------------------
612Anormal           ------------------------------------------------------------
612M                 ------------------------------------------------------------
612Mnormal           acacatggattggatgggattggaaaaaattatgaagaagtttgagctgtttgggattca
612N                 ------------------------------------------------------------
612Nnormal           ------------------------------------------------------------

w5200                aacccatccaatcccgctcaatccacatggattgagagctaaccgaacaagccctaacac
616B                 ------------------------------------------------------------cac
w5200normal          aacccatccaatcccgctcaatccacatggattgagagctaaccgaacaagccctaacac
Dxs1                 aacccatccaatcccgctcaatccacatggattgagagctaaccgaacaagccctaacac
616Bnormal           aacccatccaatcccgctcaatccacatggattgagagctaaccgaacaagccctaacac
612A                 ------------------------------------------------------------cac
612Anormal           ------------------------------------------------------------cac
612M                 ------------------------------------------------------------cac
612Mnormal           aacccatccaatcccgctcaatccacatggattgagagctaaccgaacaagccctaacac
612N                 ------------------------------------------------------------cac
612Nnormal           ------------------------------------------------------------cac
```

```
w5200              gtcaaatttccagactgtcctcgttctcacggaagcgcgtagattttctggaatcttgat
616B               gtcaaatttccagactgtcctcgttctcacggaagcgcgtagattttctggaatcttgat
W5200normal        gtcaaatttccagactgtcctcgttctcacggaagcgcgtagattttctggaatcttgat
Dxs1               gtcaaatttccagactgtcctcgttctcacggaagcgcgtagattttctggaatcttgat
616Bnormal         gtcaaatttccagactgtcctcgttctcacggaagcgcgtagattttctggaatcttgat
612A               gtcaaatttccagactgtcctcgttctcacggaagcgcgtagattttctggaatcttgat
612Anormal         gtcaaatttccagactgtcctcgttctcacggaagcgcgtagattttctggaatcttgat
612M               gtcaaatttccagactgtcctcgttctcacggaagcgcgtagattttctggaatcttgat
612Mnormal         gtcaaatttccagactgtcctcgttctcacggaagcgcgtagattttctggaatcttgat
612N               gtcaaatttccagactgtcctcgttctcacggaagcgcgtagattttctggaatcttgat
612Nnoraml         gtcaaatttccagactgtcctcgttctcacggaagcgcgtagattttctggaatcttgat
```

*Figure A2:* Multiple sequence alignment of MITE region in Dxs1 alleles from normal and mutant maize seedlings. Dxs1: B73 sequence. Intron sequences are lower case and exon sequences are upper case. Dashes (-) indicate the deletion of MITE transposable element sequences leaving a TAA footprint.

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# Amphimermis thezamica Sp. N. (Nematoda: Mermithidae) a New Species of Nematode from Georgia

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*Abstract-* Female and male individuals of *Amphimermis thezamica* sp. n. (Nematoda: Mermithidae) have been described. The host organism is unknown. The material was collected from the soil of private gardening plot, located in village Tezami of Mtskheta-Mtianeti Region (East Georgia). A new species is characterized by the combination of the following features: amphids cup-shaped, less oval and with average size, vagina prolonged, S-shaped, round eggs with smooth surface and thick envelope, 56 papillae, arranged in three rows in genital part, long double spicule, intertwined in some sections. By morphological and morphometric data a new species is close to the group of *A. bogongae*, especially to *A. litoralis*. New species resembles *A. litoralis* by the shape of amphids, S-shaped vagina, ending of a tail and twisted spicule. It differs from *A. Litoralis* by the length of vagina, structure of spicule and twisted parts, presented in its different sections, by the length of twisted and untwisted parts; by the shape of stoma.

*Keywords:* *amphimermis thezamica sp.n., nematoda, mermithidae, parasitic, Georgia.*

*GJSFR-C Classification:* *FOR Code: 060899*


*Strictly as per the compliance and regulations of :*

# Amphimermis thezamica Sp. N. (Nematoda: Mermithidae) a New Species of Nematode from Georgia

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*Abstract-* Female and male individuals of *Amphimermis thezamica* sp. n. (Nematoda: Mermithidae) have been described. The host organism is unknown. The material was collected from the soil of private gardening plot, located in village Tezami of Mtskheta-Mtianeti Region (East Georgia). A new species is characterized by the combination of the following features: amphids cup-shaped, less oval and with average size, vagina prolonged, S-shaped, round eggs with smooth surface and thick envelope, 56 papillae, arranged in three rows in genital part, long double spicule, intertwined in some sections. By morphological and morphometric data a new species is close to the group of *A. bogongae*, especially to *A. litoralis*. New species resembles *A. litoralis* by the shape of amphids, S-shaped vagina, ending of a tail and twisted spicule. It differs from *A. Litoralis* by the length of vagina, structure of spicule and twisted parts, presented in its different sections, by the length of twisted and untwisted parts; by the shape of stoma. We present the list of species of the genus Amphimermis, distributed in Holarct ic, with brief information on morphological characters, hosts and places of distribution.

*Keywords: amphimermis thezamica sp.n., nematoda, mermithidae, parasitic, Georgia.*

## I. Introduction

Nematodes, united in the genus Amphimermis are polyphages;they are of economic importance as agents of biological control of harmful insects (Poinar& Welch, 1981; Chen &Yang, 1985). They parasitize on both, terrestrial and soil insects belonging to orders Orthopthera, Lepidopthera, Coleopthera, Hymenoptera and cause their death (Poinar, 1975; Rubtsov, 1977, 1978; Ipatieva & Pimenova, 1985; Poinar et al., 2006). Development of nematodes of this group takes place mainly in the soil. They differ from mermatids of other genera by a very long, twisted spicule and bent S-shaped long vagina (Rubtsov, 1978). More than 20 species are described from this genus worldwide. In Georgia, in particular in West Georgia, only one species of the genus Amphmermis - *Amphimermis lagidzae* Rubzov (Rubtsov, 1975) was registered so far. Morphological, anatomic and morphometric analysis of nematodes, isolated from soil has shown that the described nematode is a new form and it belongs to the genus Amphimermis.

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## II. Material and Methods

Mermitid nematodes have been collected using the shovel from the soil of private gardening plot, located in village Tezami[41°53'2"C,44°57'28" B] of Mtskheta-Mtianeti Region (East Georgia) in April of 2015.The nematodes were found at 20-25 cm depth from the soil surface. Adult female and male nematodes were observed alive and then killed by immersion in 60°C distilled water for 2 min, fixed in TAF (7 ml formalin, 2 ml triethanolamine, and 91 ml distilled water) (Poinar, 1975) and processed to glycerol by Seinhorst's method for taxonomic studies (Polozhentsev & Artyukhovski, 1963; Curran & Hominick, 1980). Drawings and pictures have been done using both alive and dead individuals of nematodes. Measurements were made using the light microscope (Motic–DMB1) on 4x, 10x, 40x and 100x magnification. Some small-size details of nematodes, significant for the diagnosis (stoma, amphids, etc.) were examined using the immersion objective of 100x magnification. Drawings and photos of nematodes mounted on slides were performed using a digital video camera Genius (G-Shot) DV 1110. Host of the nematode is unknown. The range of the characters is given following the mean value. The range of the characters is given following by the mean value. Type material deposited in the collection of the museum 16 of the Institute of Zoology of Ilia State University, Georgia

## III. Results

The description of the new species is based on the forms of nematodes that were taken in the East Georgia region in the village of Tezami. This is the second record of this kind in Georgia. New species of mermithid are morphologically different from *Amphimermis lagidzae* which was recorded in Georgia, as well as from all species united in this genus.

a) *Diagnisis of genus Amphimermis Kaburaki & Imamura, 1932*

=*Complexomermis* Filipjev, 1934, Diagnosis (Kaiser, 1991 *emend.*)

Mermetids are of medium and big size – from 13 to 260 mm. Body is thin and long, frontal part is significantly narrowed, while the posterior part is less narrowed. The six head papillae are arranged in the

same circle. Stoma opening is terminal; sometimes slightly displaced to the abdominal side. Stoma tube is long; it makes 60% of the body length. The distal part of adult, sexually mature forms does not extend beyond the inner head capsule. Amphids are big in size, located at the level of papillae or are bent backwards. Cavity of amphids is whether sharply prolonged or barrel-shaped. Cuticle is thick, with well-defined fibrous crossed filaments. Hypodermal chordae six in number. Vagina is S-shaped by shape, with three nodes and very long; very often the frontal part is widened. Vulva has a shape of an oblique fissure. Eggs numerous, of medium size and round, with thick envelope, without villi. Spicules long (from 1.0 mm to 3.6 mm), partly twisted. Mermithids have three orders of sexual papillae: middle papillae doubles in the region of sexual sphere. At the end of rounded tail under the cuticle they have uneven margins. Terminus of a tail of parasite and postparasite larvae is equipped with mucro.

*b) Taxonomy*

i. *Amphimermis thezamica sp. n. (Figures 1- 4).*

*Type Host and locality*: Host of the described nematode is unknown. It was collected from the soil of private gardening plot, located in village Tezami of Mtskheta-Mtianeti Region (East Georgia) [41°53'2"C, 44°57'28" B]. The species *Amphimermis thezamica* sp.n. 4 was named according to the place of collection.

*Type material:* slide of the holotype (adult female and male**):** Mtskheta-Mtianeti Region (East Georgia), village Tezami, April 2015 (the code villi. Tezami -2015 0-01); paratypes (18 females -16 slides; 7 males- 5 slides and 20 invasive larvae - 6 slides) have the same characters as the holotypes. Holotypes of a new species are preserved in the collection of the museum of the Institute of Zoology of the Ilia State University.

ii. Measurements

*Allotype (female):* length=205 mm; head diameter at the level of cephalic papillae: 68 (μm; body diameter at the level of the nerve ring: 144 (μm; maximum body diameter at vulva: 372 (μm; body diameter at the posterior end of trophosome: 253 (μm; body diameter at vulva: 405 (μm; distance from the anterior end to the nerve ring: 322 (μm; V%=47.7; length of vagina: 2.1

mm; width of vagina: 114 μm.

Female (paratypes; n=18): length=242±77 (71-282) mm; diameter of head at the level of cephalic papillae: 68±5 (64-79) (μm; body diameter at the level of nerve ring: 148±7 (133-152) μm; maximum body diameter at vulva: 390±24 (349-420) μm; body diameter at the posterior end of trophosome: 247±22 (228-260) μm; distance from the anterior end to the nerve ring: 360±24 (292-360) μm; distance from the end of trophosome to the end of tail 480±96 (315-580) μm;V%=47.5±3 (41.8-47.7); length of vagina: 2.2 ±0,8 (1.8-2.7) mm; width of vagina: 120±11 (100-140) *μ*m.

*Holotype (male):* Length=35 mm; head diameter at the level of cephalic papillae: 60 μm; body diameter at the level of the nerve ring: 114 μm; maximum body diameter:178 μm; body diameter at the level of anus: 159 μm; distance from the anterior end to the nerve ring: 228 μm; length of the spicule: 1.773 mm; width of the spicule: 16μm.

Male (paratypes; n=7):Length=40±8 (29-54) mm; head diameter at the level of cephalic papillae: 62±3 (56-68) μm; body diameter at the level of the nerve ring: 112±4 (107-118) μm; maximum body diameter: 182±12 (151-188) μm; body diameter at the level of anus: 169±23 (132-195) μm; distance from the anterior end to the nerve ring: 232±3 (224-232) μm; length of the spicule: 1.770±0.4(1.668-1.776) mm; width of the spicule: 15±0.8 (14-16) μm.

*Invasive larva*: (n=20). Body length 1.1±0.1 (1-1.3) mm; maximum width 22±2 (19-22) μm; larva has a style, which length fluctuates from 25 to 26 μm. Body diameter at the level of the head papillae 10±1 (9-11) μm; at the nerve ring - 19±3 (15-19) μm; distance from the apical end of the head to the nerve ring makes 53±4 (53-57) μm, while from the rectum from 83±6 (79-86) μm length till the end of tail larva is spindle-shaped. It is characterized by the movement of plectoidnematodes. Anterior part of the body is wide, but is very narrow at the terminal part and ends with micro bulb-like tail. Tail terminus is used by the larva to attach to the substrate.

iii. *Description*

a. *Morphology*

*Female*. Nematode is of white colour. Body is thin and long. Body diameter increases at the 45.5 mm distance from the apical end of anterior part and then it is of nearly even width till the end of tail. Head is rounded and slightly flattened (Figures 1A, 2A,B). Diameter of the head capsule 9.2 μm. Cuticle is thick, it contains crossed fibrous threads; stoma is symmetrically situated at the end of the head. The 6 head papillae are well defined. Each papilla has 2-3 sensils. Amphids have shape of cup, are less oval and middle size (length of amphids 15.2 μm, width 9.6 μm). Hollows of amphids open behind the papillae at 15 and 19 μm distance. Stoma is narrow (1.5 μm wide); length of stoma 19.4 μm. Stoma is without collar. Oesophagus 5.4mm long, diameter 5.5 μm. Channel of vagina starts obliquely. It is long1.5 mm. (Figures 1C, 2C) cylindrical and bent S-shaped, nearly of even width along the whole length. Eggs round (length fluctuates from 83 to 87 μm, width 79-87 μm); with smooth surface; envelope thick - 4.2 μm wide. Size of cells of trophosome fluctuates from 27 to 140μm. Lateral chordae narrow, size of chordae, located at dorsal and ventral sides fluctuates from 15 to 19μm. Tail is slightly conical (Figures 1B, 2D,E); its terminal part is rounded and unevenly immersed under the cuticle.

*Male.* In comparison with female, male is smaller in size. Oesophagus is 1.5 mm long. Long, twisted, double spicule is characteristic of the male (Figure 3D). It contains twisted and non-twisted parts (Figures 3B, C, D, E, 4C,E,F). Length of distal untwisted part of spicule is 117.5 μm (see Figure 3B), length of the next, twisted part is 428.7 μm, length of the central, untwisted part is 252.1 μm; the next, twisted part is 663.2 μm long, and length of the proximal, untwisted part makes 312.4 μm. Terminal part of spicule is conical and its end is rounded. In the terminal part of spicule 2 notches are visible (Figure 3E), which is characteristic to this species. Cloaca diameter 159.6 μm. In postanal part 2 well defined nucleoli are visible (38 μm long and 30 μm wide), which are not found in other Amphimermitids. Sexual papillae are arranged in three orders; in preanal part 10 quite big papillae are situated medially, though on each of lateral parts 7-7 small papillae are presented. In postanal part 20 papillae are situated medially (among them the first, second, nineteenth and twentieth papillae are small in size, though the rest papillae are big in size) and on each of its lateral part 6-6 papillae are situated. Total 56 papillae are situated in the genital part. Size of spermatocytes fluctuates from 7 to 11 μm. Tail is 266.5 μm long. It is slightly conical(Figures 3C, 4D), rounded at the end.

c) *Diagnosis and Relationships*

By its morphological and morphometric characters a newly described species - *A. thezamica* sp. n. belongs to the genus Amphimermis. By the haracter of twisting of spicule and shape of amphids the nematodes, united in the genus Amphimermis are divided into four groups (Bacer & Poinar, 1994):1. *volubilis* (A.volubilis); 2. *avoluta* ( *A. avoluta, A. acridiorum, A. buraki*); 3. *bogongae (A. bogongae, A. maritime, A. litoralis, A. tinui, A. bonaerensis, A. mirabinda);* 4. *elegans A. elegans, A. zuimushi, A. tongaensis, A. artyukhovskii, A. australoelegans).*

A new species, which is characterized by a twisted spicule, was compared with nematodes of all four groups. It turned out that, by morphological and morphometric characters it is the most close to the group of *A.bogongae*, especially to the species *A. litoralis*.

*A. thezamica* sp. n. Resembles *A. litoralis* by the shape of amphids (both species have cup-shaped amphids), S-shaped vagina and end of tail; (both *A. thezamica* sp. n. and *A. litoralis* are characterised by the uneven deepening under the cuticle at the end of the tail, twisted spicule and diametric dimensions of the body.

*A. thezamica* sp. n. differs from *A. Litoralis* by the following characters: size of the body (in a new species male L=40 (29-54) mm, in *A. litoralis* – 57 (34-76) mm; in female – correspondingly 242 mm and 136 mm; by the diameter of the body - (in a new species diameter of the body is 182 (151-188) μm, though that of *A. litoralis*is 267 (232-471) μm; by the length of spicule - in a new species length of spicule is 1.773 (1.668-1.776) mm – in A. *litoralis* - correspondingly 3.200 (2.900-3.600) mm; by the length of twisted parts of spicule and their positioning: length of the first untwisted part of spicule in a new species is 117 μm; in *A. litoralis* it is equal to 194 (41-336) μm. Length of the first twisted part of spicule in a new species is 428 μm, in *A. litoralis* – 647 μm; length of a central untwisted part in a new species is 253 μm; in *A. litoralis* – 302 μm; length of the next twisted part in a new species is 663 μm, though in *A. litoralis* it is 646 μm long. Terminal part of spicule in a new species is untwisted (length 312.4 μm), though in *A. litoralis* it is twisted till the end. Vagina of the female of a new species is longer 1.5 mm, than that of *A. litoralis*(960 μm). Part of stoma, which from the apical part of head connects to the esophagus, is slightly widened in *A. thezamica* sp.n., though this is not a case in *A. litoralis*.Terminal part of a head is rounded in *A. litoralis*, though in a new species it is flattened. These two species differ from each other by the positioning of vulva towards the body: V% for *A. litoralis* is 50, though vulva of a new species is located slightly ahead (V%=47). *A. thezamica sp.n.,*and *A. litoralis*differ also by the thickness of the cuticle: thickness of cuticle at the head capsule - 13 μm; at vulva - 12 μm; at the end of tail – 51 μm; in a new species these indexes are correspondingly 30, 38 and 41 μm. Comparison has shown that despite the fact that by morphological and morphometric characters new species, described by us, is quite close to *A. bogongae*, by the key diagnostic characters it differs from the latter: by the length of vagina, structure of spicule and presence of twisted segments at different places; by the length of twisted and untwisted parts; by the shape of stoma and thickness of the cuticle. Based on the above we consider that the mermithid, described by us can be regarded as a new species.

## IV. Discussion

*Female* individual of *A. thezamica sp. n.* is of white colour. Body is thin and long. At the 45.3 mm distance from the apical end of the body its diameter increases, and then body is nearly of the same width till the tail. Head is rounded and slightly flattened. Cuticle is thick and it contains crossed fibrous threads. Stoma is situated symmetrically at the end of the head. Number of head papillae – 6. Amphids are cup-shaped. Stoma is narrow, without collar. Channel of vagina starts obliquely. It is long, cylindrical, bent S-shaped, and has the same width along the whole length. Eggs are round and have smooth surface. Tail is slightly conical. Its terminal part is rounded and unevenly deepened under the cuticle.

*Male* individual is smaller in size than the female one. Male is characterized by the long, double spicule. It is composed of twisted and untwisted parts. The 2 notches are well defined at the end of the spicule, which is characteristic to this species. Total 56 papillae present in the genital part. Tail is slightly conically, rounded at the end.

Despite the resemblance of morphological and morphometric characters of A. Thezamica sp.n. with those of species, belonging to the genus Amphimermis, by the structures of spicule and presence of twisted segment at its different parts, length of twisted and untwisted parts, shape of stoma, a new species differs from Holarctic species, united in this genus, such as: *A. zuimushi* Kaburaki & Imamura, 1932; *A. acridiorum* Baker & Poinar,1994; *A. artyukhovskii* Artyukhovski & Karchenko, 1965; *A. australoelegans* Baker & Poinar,1994; *A. avoluta* Rubtsov & Koval, 1975; *A. bogongae* Welch, 1963; *A. bonaerensis* Miralles & Camino, 1982; *A. buraki*Baker & Poinar,1994; *A. elegans* (Hagmeier, 1912) Welch, 1963; *A. lagidzae* Rubstsov, 1975; *A. litoralis* Artyllkhovski & Karchenko, 1971; *A. longiscapus* Rubtsov, 1976;*A. maritime* Rubstov, 1971; *A. mirabinda* Baker & Poinar,1994; *A. mongolica* Rubtsov, 1976; *A. polaris*Spiridonov& Lantsov, 1996;*A. tinyi* Nickle, 1972; *A. tongaensis* Spiridonov, 1987; *A. Volubilis* Rubtsov & Koval, 1975;

*A. zuimushi* - parasitizes on Lepidoptera: Noctuidae- *Agrotis infusa* (Boisd.), Pyralidae- *Chilo simplex* (Butler). Cavity of amphidin a lateral position is extended along the longitudinal axis of the body. It differs from all genera by a very long and stretched spicule and long, S-shaped bent vagina, Spicules 1.02-1.45 mm long. Distribution: Japan.

*A. acridiorum*- parasitizes on Orthoptera: Acrididae- *Phaulacridium vittatum* (Sjostedt); *Oedaleus australis* (Saussure); *Chortoicetes terminifera* (Walker)., Amphid opening posterior to lateral cephalic papilla. Vagina short and broad (in lateral view), vagina long and narrow (in lateral view). Distribution: Australia.

*A. artyukhovskii* - parasitizes on Lepidoptera: Lymantriidae - *Lymantriadispar* (L), Geometridae – *Operopthera brumata* (L)., Spicule 1.6-1.8 mm. Body length 26-53 mm. Tail canoid, rounded. Female body length 36-190 mm. Distribution: Voronezh region, (Russia).

*A. australoelegans*- parasitizes on Orthoptera: Acrididae- *Phaulacridium vittatum* (Sjostedt), *Chortoicetesterminifera* (Walker); Coleoptera : Scarabaedae - *Sericesthis sp*., Tail conoid, pointed terminus. Vagina long (1.5 mm), thin walled. Distribution: Australoia.

*A. avoluta* - parasitizes on Coleoptera: Chrysomelidae- *Leptinotarsa decemlineata* Say. Proximal half of spicule untwisted (=avoluta group), vagina medium (0.5 mm). Distribution: Ukraine.

*A. bogongae* - parasitizes on Lepidoptera: Noctuidae- *Agrotisinfusa* (Boisd.). Tail bluntly rounded, distance of proximal papillae from cloaca=20% of spicule length; medium sized amphids in relation to head diameter, thick-walled. Distribution: Australia.

*A. bonaerensis* -parasitizes on Orthopera: Acrididae- *Laplatacris dispar* Rhen., Tail pointed, distance proximal papillae to cloaca≤spicule length. Vulva with flanges. Distribution: Australia.

*A. buraki* - parasitizes on Orthopera:Tettigoniidae- *Conocephalus sp.* ; Body short ( 30 mm ), spicule length 750-975 μm, terrestrial, amphids thick - walled, situated anterior to neck region with opening immediately posterior to lateral head papillae. Distribution: Australia.

*A. elegans* - parasitizes on Orthoptera: Acrididae: Stenobothrus sp. Tail bruntly rounded. Body length 195-260 mm. Distribution: Germany.

*A. lagidzae* - the host organism and male individuals are not known. Amphids situated in neck region with opening well posterior to lateral head papillae. Body 74 mm. Distribution: Geogia.

*A. litoralis* - unknown. Spicule long (2.9-3.6 mm) in relation to the body length (45-70 mm). Body length 136 mm, terrestrial. Distribution: Voronezh Region (Russia).

*A. longiscapus* - parasitizes on Lepidoptera: Lymantriidae: *Lymantria dispar* (L). Male individuals are not known. Amphids situated anterior to the neck region wit opening, immediately posterior to the lateral head papillae. Body 43 mm. Distribution: Kirgizstan.

*A. maritima* - parasitizes, unknown. Spicule 2.2 mm. Bodi 53 mm. Junction of vagina and uterus at an obtuse anterior-ventral angle. Distribution: Primorsk Region (Russia).

*A. mirabinda* - parasitizes on Orthoptera: Acrididae: *Phaulacridium vittatum* (Sjostedt ). Spicule 1.2-1.5 mm. Body 35-42 mm. large amphids in relation to the head diameter, thin-walled. Distribution: Australia;

*A. mongolica* - parasitizes, unknown. Amphids thin-walled, situated in neck region with opening well posterior to lateral head papillae. Distribution: Mongolia;

*A. polaris*- parasitizes on Diptera: Tipulidae -*Tipula (Savtshenkia) glaucocinerea* Lundtsr. Body length of male - 6.065 mm, length of spicule – 1.648 mm, length of females – 10.740 mm; usually on the tail terminus is developed 12 μm long cuticular appendix. Distribution: Taimyr, Dolgano-Nenetskyi Autonomous region.

*A. tinyi* - parasitizes on Odanata: Coenagrionidae: Ischuraposita (Hagen), *Annomalagrion bastatum* (Say). Male body short (11-17 mm), spicule 700-860 μm, aquatic. Female body length 30 mm, aquatic. Distribution: USA.

*A. tongaensis* - parasitizes, unknown. Diameter of amphid is greater than two thirds of head width. Female unknown. Distribution: Tonga.

*A. volubilis* - parasitizes on Coleoptera: Chryso-melidae: *Leptinotarsa decemlineata* Say. Spicule twisted entire length (= volubilis group), vagina long (0.7 mm).

## V. Acknowledgenments

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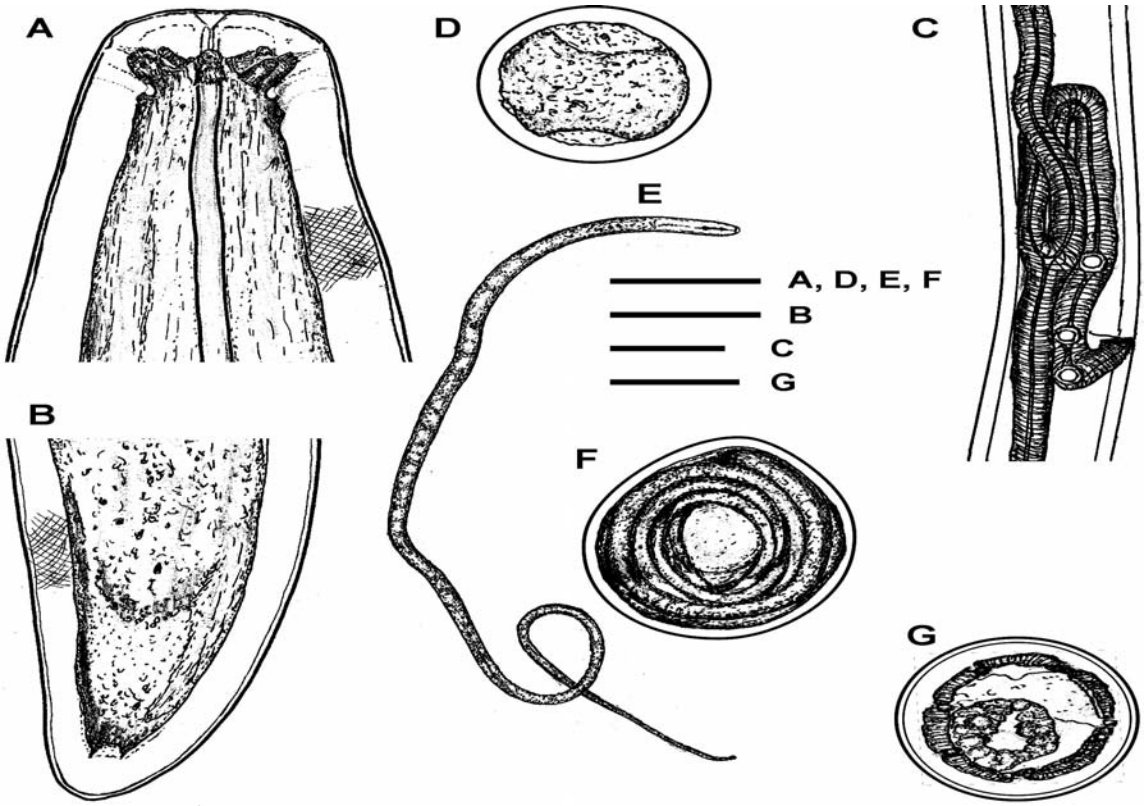


*Figure 1:* *Amphimermis thezamica* sp. n., female, holotype. A: Anterior region of the body, lateral view; B: Posterior end, lateral view; C: Vagina, lateral view; D: Uterine egg; E: stage -2(infective), whole body; F: Last stage of larva in egg; G: Mid-body, cross section. Scale for A,D,E,F = 50 µm; B = 100 µm; C=400 µm; G=200 µm.

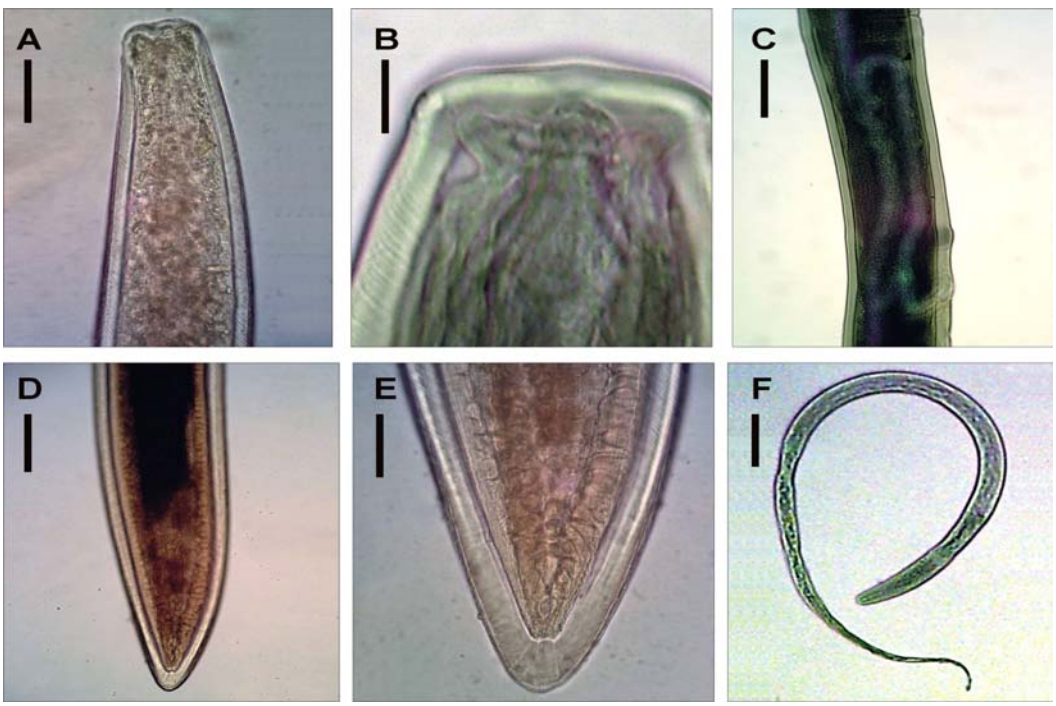


*Figure 2:* *Amphimermis thezamica* sp. n., female, holotype. A: Anterior region of the body, nerve ring, and pharyngeal tube, lateral view; B: Head, lateral view; C: Vagina, lateral view; D: Posterior region of the body, lateral view; E: Tail end; F: stage -2 (infective juveniles) whole body. Scale for A,E=25 µm; for B=30 µm; for C,D=100 µm; for F=100 µm.

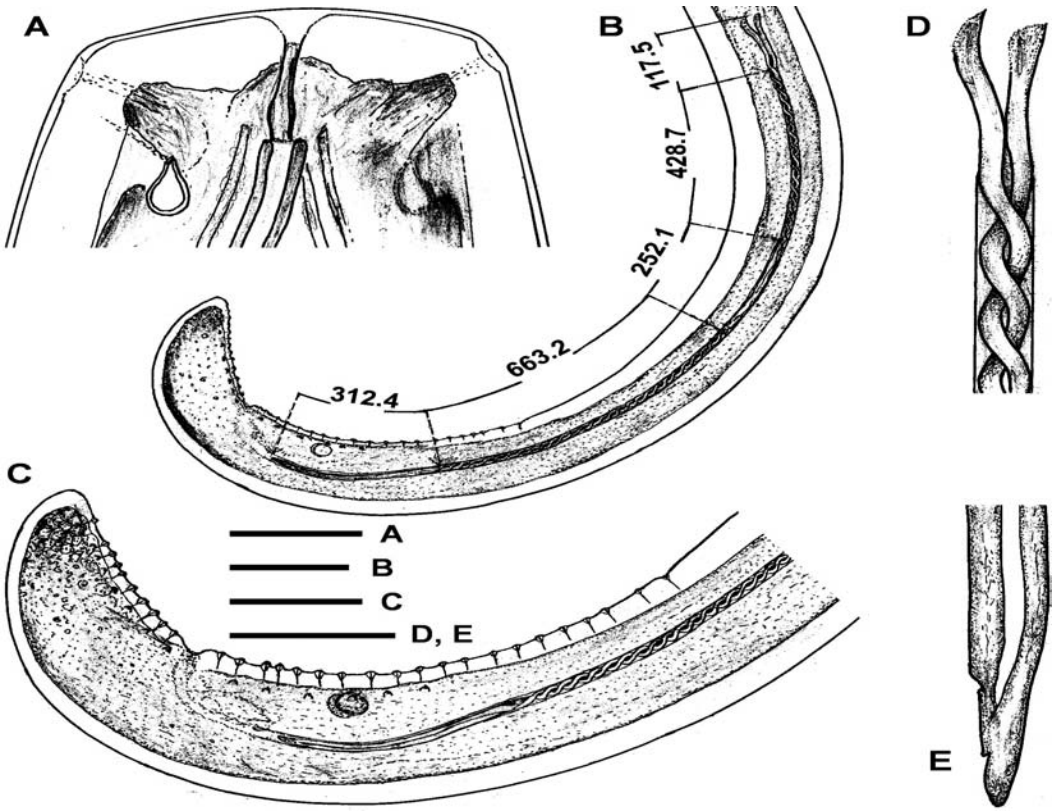


*Figure 3:* *Amphimermis thezamica* sp. n., male, holotype. A: Head, wide of the cuticle, lateral view; B: Tail, with whole spicula, lateral view; C: An enlarged tail with a half spicule; D: Non twisted and twisted head of spicule; E: Last past of non-twisted spicule. Scales for A=20 μm; B=150 μm; C=100 μm; D,E=25 μm.

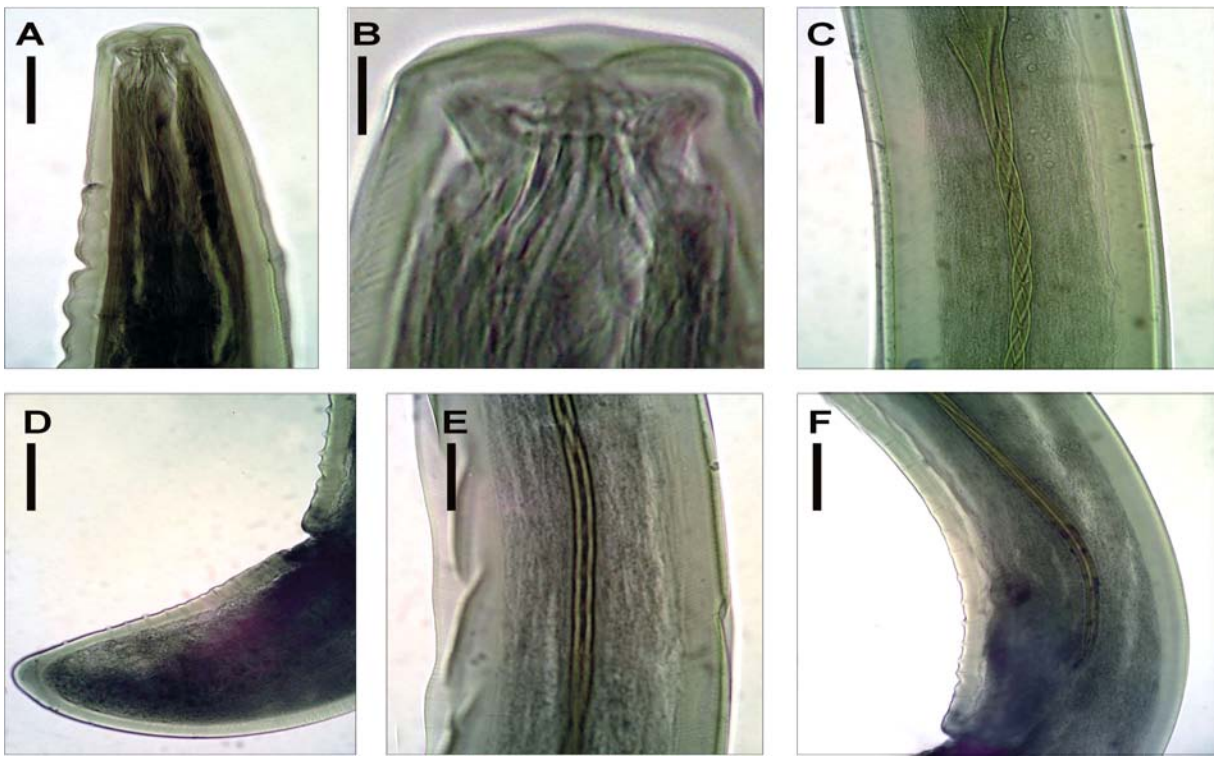


*Figure 4:* *Amphimermis thezamica* sp. n., male, holotype. A: Anterior region of body, lateral view; B: Head, lateral view; C: Non - twisted and twisted part of spicule head; D: Tail, lateral view; E: Middle part of non-twisted spicule; F: End of non-twisted spicule, lateral view; Scales for A,C,D,E,F=25 μm; for B=30 μm.

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# Effects of Common Bean Varieties and Densities Intercropped with Rice on the Performance of Associated Components in Kaffa and Benchi Maji Zones, Southwestern Ethiopia

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*Keywords:* *economic feasibility, land equivalent ratio, gross monetary value and productivity.*

*GJSFR-C Classification:* *FOR Code: 060799*


*Strictly as per the compliance and regulations of :*

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Mitiku Woldesenbet [α] & Getachew Mekonnen [σ]

*Abstract-* Intercropping of cereals and legumes is important for sustainable food production in the tropics aimed at minimizing risks associated with monoculture. A field experiment was conducted at kuja site in Guraferda Woreda of Bechi Maji zone and Gojeb site in Ghimbo woreda of Kaffa zone, southwestern Ethiopia, during 2016 cropping season to determine the effects of the density and varieties of common bean intercropped with rice on growth, yield components and yield of the associated crops and productivity of the system. Rice variety 'NARICA-4' was intercropped with three varieties of common bean (Red Wolaita, Awash Melka and Nasir) in a factorial combination of *three* population densities of 25% (62,500 plant $ha^{-1}$), 50% (125,000 $ha^{-1}$) and 75% (187,500 plants $ha^{-1}$) of the recommended population density along with sole crops of the respective varieties of common bean and rice in randomized complete block design with three replications. The results of the study showed that days to 50% heading, days to 90% maturity, were significantly affected by common bean density. The shortest days to 50% heading (95.58 days) and the shortest days to 90% maturity (120.33 days) of the associated rice were recorded at 75% planting density of common bean and significantly increased with the decrease in density of common bean to 25%. The plant height of rice was significantly affected by the main effect of variety, density and their interaction. Accordingly, the highest plat height (78 cm) was observed when Nasir variety was intercropped with rice at 75% planting density. It was observed that either the main effect or the interaction of common bean varieties and planting densities were not significant on the grain yield and harvest index of rice. Though it was not significant, the highest grain yields (3042 and 2855 kg ha-1) were obtained from intercropping Awash Melka variety at 50% planting population, respectively. It was observed that the highest dry biomass (12556 kg /ha) of the rice was recorded when intercropped with common bean at 25% planting density and both parameters decreased significantly with increase in planting density of common bean to 75%. The main effect of common bean varieties had significant ($<0.05$) effect on dry bio mass and highly significant ($p<0.01$) effect on days to 90% maturity, leaf area index, plant height, number of seed per pods hundred seed weight and harvest index of common bean. The shortest days to 90% maturity (83.6 days) and the highest plant height (53.00 cm) were recorded for the Nasir variety. Conversely, the highest plant height (57.33cm) and the highest number seeds per pod (4.7) were recorded in responses to 25% planting density and significantly increased as the bean planting density increased to 75%. The highest grain yield (1842 kg /ha) was recorded for variety Awash Melka at 75% planting density. The highest total LER of 2.38 and GMV of 30,883 ETB/ha were recorded when rice was intercropped with bean variety Awash Melka at planning density of 75%. Therefore, based on the above agronomic and economic evaluations, rice (100%) intercropped with common bean variety Awash Melka at planting density of 75% of the common bean can be recommended for intercropping of rice with common bean in the study area. However, the experiment has to be repeated across over seasons with consideration of farmers preference of the common bean varieties to reach at conclusive recommendation.

*Keywords: economic feasibility, land equivalent ratio, gross monetary value and productivity.*

## I. Introduction

Poor soil fertility management, poor crop husbandry and effects of climate change are the major challenges and contribute for low crop productivity. Agro-ecological intensification of land use is a prerequisite for increased agricultural productivity, natural resource conservation and sustainable development (CCRP, 2009). The limited land areas are facing pressure to meet basic demands, such as food, since most growers own very small plots of land, especially in Africa (Rezaei-Chianeh *et al.*, 2011). In view of this, there is need for increased production in small areas through intercropping, which utilize common limiting resources better than the species grown separately, as an efficient resource use method (Ghosh *et al*., 2006; Sobkwicz, 2006).

The bulk of agricultural output in Ethiopia comes from 13.3 million small scale subsistence households, each owning, on average about 0.93 ha of land and produces a number of different food and cash crops (CSA, 2008). Tef, maize, wheat, rice and sorghum are among the cereal crops used to be the staple food crops and target of most of the food security programs in Ethiopia (CSA, 2014). Rice was introduced and evaluated initially at different areas of Ethiopia such as Gambella, Pawe, and Fogera in the late 1960s.

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However, attention was not given to rice research prior mid 1990s. Since 1990, seven upland rice varieties including two NERICAs (New Rice for Africa) have been released. The average productivity of these varieties ranges from 2.5 to 4 t/ha on farmers fields (Wolelaw, 2005). Ethiopia has different rice agro-ecologies that can grow rain fed upland rice, rain fed lowland rice, and irrigated rice with a total potential land mass of 1 million hectare (Sewagegne, 2011). However, this yield is very low as compared to the world production due to different constraints among which soil fertility problem is the first.

The current trend in global agriculture is to search for highly productive, sustainable and environmentally friendly cropping systems (Crew and Peoples, 2004). With increasing economic and environmental costs associated with fertilizer use, the need for low-input agro-ecological systems is rising (Meighen and Marney, 2012). Traditional agriculture, as practiced through the centuries, has always included different forms of intercropping (Lithourgidis *et al.*, 2011). As in most tropical countries, in Ethiopia, traditional cropping systems are based on resource poor farmers' subsistence requirements, and are not necessarily the most efficient ones (Tesfa *et al.*, 2002). In southwestern Ethiopia, small scale farmer uses a combination of crops grown on the same land in such a way that cereals, pulses, and oil seeds are represented. However, this cereal-legume intercropping study was not scientifically justified for rice in southwestern Ethiopia, though the area is potential area for rice production. Therefore, this study was initiated with the objective of determining the effects of the varieties and densities of common bean intercropped with rice on performance of the components.

## II. Materials and Methods

*a) Description of the Study Site*

The experiment was conducted in two locations namely, Ghimbo district Gojeb site in Kaffa Zone and Guraferda district Kuja site in Benchi Maji Zone, Southwestern Ethiopia, during 2016 main cropping season. These sites were selected because of that they are the most potential areas of southwestern Ethiopia. The study sites at Gojeb and Kuja are located at 8° 06´ N, 36°29´ E, 1490 m.a.s.l. and 9° 07´ N, 37° 35´ E, 1238 m.a.s.l., respectively. The rainfall pattern of these areas is characterized by bimodal distribution with small rainy season *belg* (March-June) and main rainy season *meher* (July-November).

*b) Description of Experimental Materials*

An improved variety of rice named as *NERICA-4* (WAB-450-IB-P-9/1), which is currently grown extensively by the model farmers in the study areas, was used as a test crop. The variety was released in 2006 by the Pawe Agricultural Research Center for its high yield and promising agronomic performances (MoARD, 2007).

*c) Treatments and Experimental Design*

The treatments consisted of planting of rice at the density of 100% of sole population with three common bean varieties (Red Wolaita, Awash Melka and Nasir) at the density of 25% (62500 plants $ha^{-1}$ ), 50% (12500 plants $ha^{-1}$), and 75% (187500 plants $ha^{-1}$). The sole rice was planted at spacing of 70 cm between rows by drilling. The sole common beans were planted at spacing of 40 x 10 cm (250000 plants $ha^{-1}$), respectively. For intercropping, common bean was planted inside rice rows at intra-row spacing of 7 cm, 11 cm and 21 cm representing 75%, 50% and 25% of sole population density of common bean, respectively. The experiment was laid out in a Randomized Complete Block Design (RCBD) in factorial arrangement in three replications. There were sole crop rice and the three common bean varieties.

The plot size and spacing of the experiment for sole common bean was 4.2 m length, 40 cm inter-row spacing, 10 cm intra- row spacing with a gross plot size of 4.2 m x 0.4 m x 6 = 10.08 $m^2$ and the central four rows of three meters length (4 rows x 0.4 m x 3 m = 4.8 $m^2$) were harvested while for the intercrops four rows inside the rice with a plot size of (4 rows x 0.70 m x 3 m = 8.4 $m^2$) were harvested. Sole rice was planted in 4.2 m length, 70 cm inter rows spacing by drilling and with 6 rows and had a gross plot size of 4.2 m x 0.70 m x 6 rows = 17.64 $m^2$ and three central rows of three meters (3 rows x 0.70 m x 4.2 m = 8.82 $m^2$) were harvested both from sole and intercropped rice.

*d) Experimental Procedure*

Land preparation was done in mid June 2016 using daily labour and the rice seeds were sown in rows spaced 20 cm apart by hand drilling at the seed rate of 100 kg $ha^{-1}$. The sources of N and P were urea (46% N) and triple super phosphate (TSP, 46% $P_2O_5$), respectively. All P and half of the N fertilizer sources for the respective inorganic N and $P_2O_5$ treatments were applied at planting. The remaining half of the inorganic N fertilizer was applied at tillering stage by side drilling. Weeds were removed manually three times *i.e.* at early tillering, maximum tillering and booting stages. No insecticide or fungicide was applied as there was no serious incidence of insect pests or diseases. Harvesting was done manually using hand sickles. The harvested product was sun-dried to a constant weight and threshing and winnowing were done subsequently.

*e) Soil Analysis*

Prior to planting, surface (0 - 30 cm) soil samples, from five spots across the experimental fields, were collected in a zigzag pattern, composited, and analyzed for soil physico-chemical properties and the

results are depicted in Table 1. The soil physico chemical analysis of the study areas revealed that the soils of the experimental field were clay and clay loam in texture both at Gojeb and Kuja, respectively. The results also indicated that the soil of Gojeb and Kuja are moderately and slightly acidic with pH of 6.31 and 5.66, respectively. The soils have medium organic carbon (1.46) and total N (0.09%) at Kuja and low organic carbon (0.99) and total N (0.06%) at Gjebo. Available P is low both at Kuja (6.30 ppm) and Gojeb (5.90 ppm) (Table 1).

*Table 1:* Physico-chemical characteristics of soil of the experimental sites

| Soil parameters | Districts | |
|---|---|---|
| | Gojeb | Kuja |
| Textural composition (%) | | |
| Sand | 22.00 | 16.30 |
| Silt | 25.00 | 24.80 |
| Clay | 53.00 | 58.90 |
| Textural class | Clay | Clay loam |
| pH | 5.66 | 6.31 |
| Organic Carbon (%) | 0.99 | 1.46 |
| Total N (%) | 0.06 | 0.09 |
| Available P (mg kg) | 5.90 | 6.30 |
| CEC (cmol/kg) | 16.22 | 23.41 |

*f) Data Collection*

i. *Rice Component*

Days to heading were recorded when the ears or panicles were fully visible on 50% of the plants from each plot by visual observation and days to physiological maturity were recorded when 90% of the plants reached maturity in each plot, *i.e*. when grains were difficult to break with thumb nail. Number of productive tillers $m^{-2}$ was counted from two random 1m X 1m areas (5 rows of 1m length) within the net plot area at physiological maturity and the average was recorded as number of productive tiller $m^{-2}$. Plant height (cm) was determined from measurements of 10 randomly pre-tagged mother shoots from ground level to the top of the spike excluding the awns at physiological maturity. Likewise, the spikes in the pre-tagged 10 plants were collected and the total grains were counted to record the number of grains per spike. Thousand grains were counted in each plot using electronic seed counter from a bulk of threshed grain and their weight was measured using a sensitive balance at harvest and the weight was adjusted to 12.5% moisture content.

The total aboveground dry biomass yield including straw and spikes of plants in a net plot area was measured using spring balance after sun drying to a constant weight. Then threshed and the grain yield per net plot was weighed and adjusted to 12.5% moisture content. Harvest index was calculated as the ratio of grain yield to total aboveground dry biomass and expressed in percentage.

*g) Common Bean Component*

i. *Phenological and Growth Parameters*

Days to 50% flowering, 90% physiological maturity, Leaf area, Number of primary branches, Dry biomass, Stand count, Number of pods per plant, Number of seeds per pod, Hundred seed weight (g), Grain yield (kg/ha) and Harvest index.

ii. *Productivity and Economic Evaluation of the Intercropping System*

Productivity of the intercropping system was determined by calculating the Land Equivalent Ratio (LER) (Willey, 1979). Land Equivalent Ratio (LER) was a relative land area required as sole crop to produce the same yield as an intercrop system and calculated as

$$\text{LER} = \frac{Y_{ab}}{Y_{aa}} + \frac{Y_{ba}}{Y_{bb}}$$

Where $Y_{ab}$ is yield per ha of rice in intercrop with common bean; $Y_{aa}$ yield per ha of sole rice; $Y_{ba}$ were grain yield per ha of common bean in intercrop with rice; $Y_{bb}$ was grain yield per ha of sole common bean. LER values >1.00 indicate an agronomic advantage of intercropping over sole cropping.

The economic evaluation was done using Gross Monetary Values (GMV) as described by Willey (1979). To calculate the GMV of component crops, the prevailing prices at local market (18.00 Birr/kg at Gojeb

and 19.50 Birr/kg at Kuja for rice and 8.0 Birr per kg for common bean at Gojeb and 18.00 Birr/kg) were used.

*h) Statistical Data Analysis*

The agronomic data were subjected to analysis of variance (GLM procedure) using SAS software program version 9.2 (SAS Institute, 2003). Homogeneity of variances was evaluated using the F-test as described by Gomez and Gomez (1984) and since the F-test has showed homogeneity of the variances of the two locations for most of the agronomic parameters, the average data analysis was used for the two locations. The Fisher's protected least significant difference (LSD) test at 0.05 probability level was employed to separate treatment means where significant treatment differences existed.

## III. Results and Discussion

*a) Rice Component*

i. *Rice Crop Phenology*

The result of this study showed that the main effect of planting density of common bean had a highly significant ($P<0.01$) effect on days to 50% heading and days to 90% maturity of rice crop.

ii. *Rice Growth Parameters*

The result of this study indicated that the main effects of varieties and planting density of common bean as well as the interactions had no significant effect on leaf area and leaf area index of rice crop. This result is in agreement negatively with the finding of Wogayehu (2005) who reported that the leaf area index of maize was significantly ($P<0.05$) affected by intercrops of the associated bean varieties. Though it was not significant, the highest leaf area (1593 $cm^2$) of rice was obtained when Nasir variety was intercropped with rice compared to Awash Melka variety (1585 $cm^2$) and Red Wolaita variety (1424 $cm^2$) (Table 3). This might be due to less competition between rice and the associated Nasir variety for growth factors as it was early in maturity as compared to the two varieties, and may have required less nutrients during growth than the other variety.

The result of this study also indicated that Common bean variety and planting density had a highly significant ($P<0.01$) effect and the interaction had a significant ($P<0.05$) effect on plant height of rice. As a result, the highest plant height (78 cm) was obtained when rice was intercropped with common bean variety Nasir at planting density of 75% while the shortest plant height (64.7 cm) was obtained when common bean variety Red Wolaita was intercropped with rice at planting density of 25% (Table 2). In general as the planting density of common bean increased, the height of the associated rice increased indicating increased competition from the associated common bean for the limited resources. This result is in agreement with previous studies conducted by Sarma (1994) on *Sesamum indicum* which indicated that in narrow spacing plants compete more for available resources especially for light and result in more height than widely spaced plants. As sesame plants compete for light, high populations grow taller and faster than low populations (Langham, 2007). Unlike to the results of the present study, Demesew (2002) on maize/bean intercropping, Sisay (2004) on sorghum/green gram intercropping and Wogayehu (2005) on maize/common bean intercropping reported that the plant height of the cereal component was not significantly affected by the associated legume components.

*Table 2:* The average results of the interaction effect of varieties and planting density of commonbean on plant height (cm) of rice intercropped with common bean

| | Planting Density of Common bean (PD) | | | |
|---|---|---|---|---|
| Common bean Varieties (V) | 25% | 50% | 75% | |
| Red Wolaita | $64.7^{d}$ | $74.6^{ab}$ | $74.7^{ab}$ | |
| Awash Melka | $74.7^{ab}$ | $71^{bc}$ | $65^{d}.1$ | |
| Nasir | $72.2^{cd}$ | $69.7^{c}$ | $78^{a}$ | |
| Intercrop mean | | | | 74.3 |
| Sole rice mean | | | | 261.3 |
| | V × PD | | Sole vs Intercrop | |
| $LSD_{(0.05)}$ | 4.88 | | NS | |
| CV (%) | 4.7 | | 6.6 | |

*NS= Non-significantly different at 5% probability level; Means followed by the same letters are not significantly different at 5% level of significance according to LSD test.*

*b) Yield component and Yield of Rice*

Form this study it was observed that the number of grains per spike and 100 seed weights (g) were not significantly affected by either the main effects of the varieties and planting density of common bean or their interaction. In agreement to the result of this study, Tilahun (2002) reported non-significant effect of planting densities and planting patterns on maize 1000-kernel weight. Similarly, Wogayehu (2005) reported non-significant effect of the associated bean varieties on thousand-kernel weight of maize.

It was observed that, the number of grains per spike and 100 seed weights (g) decreased as the planting density of common bean increased from 25% to 75% (Table 1). This might be due to increased competition for growth resources from the associated common bean as its density increased.

The hundred seed weight recorded from intercropped rice was lower than that from sole crop although the difference was not significant (Table 3). In line with this result, Tamado and Eshetu (2002) from intercropping of maize and haricot bean reported that 1000-kernel weight of maize was not significantly affected by the cropping system. On the other hand, Bandyopadhyay and De (1986) attributed the highest sorghum grain yield in intercrops to greater panicle and 1000-grain weight.

*Table 3:* The average result of main effects of varieties and planting density of common bean on number of grains per spike and 100 seed of rice intercropped with common bean and grown under sole crop

| Treatment | Number of grains per spike | 100 seed weight(g) |
|---|---|---|
| Common bean Varieties | | |
| R + Red Wolaita | 16.06 | 37.1 |
| R + Awash Melka | 13.00 | 38.2 |
| R + Nasir | 17.04 | 39.7 |
| LSD (0.05) | NS | NS |
| Common bean Planting Density | | |
| R + 25 % B | 1.13 | 42.2 |
| R + 50 %B | 1.05 | 40.5 |
| R + 75 %B | 1.05 | 38.0 |
| LSD (0.05) | NS | NS |
| CV (%) | 17.6 | 6 |
| Cropping system | | |
| Intercrop | 1.23 | 172.7 |
| Sole crop | 1.13 | 193.3 |
| LSD(0.05) | NS | NS |
| CV (%) | 6 | 5.8 |

*NS= Non-significantly different at 5% probability level; Means followed by the same letters are not significantly different at 5% level of significance according to LSD test.*

It was also observed that the main effect as well as interaction effect of planting density and variety of common bean did not significantly affect the grain yield and harvesting index of rice. This could be due to stronger competitiveness of the rice component as it was planted early at full population as compared to common bean. Amare (1992) also found that intercropping different haricot bean varieties did not significantly affect maize grain yield.

*Table 4:* The average result of the main effects of varieties and planting density of common bean on grain yield (kg ha$^{-1}$), dry bio mass (kg ha$^{-1}$) and harvest index (%) of rice intercropped with common bean and grown under sole crop

| Treatment | Grain yield (kg ha$^{-1}$) | Dry biomass (kg ha$^{-1}$) | Harvest index (%) |
|---|---|---|---|
| Common bean Varieties | | | |
| R + Red Wolaita | 2811 | 12586 | 25.41 |
| R + Awash Melka | 3042 | 11968 | 26.51 |
| R + Nasir | 3011 | 11354 | 25.32 |
| LSD(0.05) | NS | NS | NS |
| Common bean Planting Density | | | |
| R+ 25 % B | 2732 | 12556$^{a}$ | 21.75 |
| R + 50 % B | 2855 | 11224$^{b}$ | 25.43 |
| R + 75 % B | 2733 | 11223$^{b}$ | 24.35 |
| LSD (0.05) | NS | 366 | NS |
| CV (%) | 7.6 | 3.8 | 7.7 |

| | | | |
|---|---|---|---|
| Cropping system | | | |
| Intercrop mean | 2622 | 11586 | 22.63 |
| Sole crop mean | 3125 | 11568 | 27.01 |
| LSD(0.05) | NS | NS | NS |
| CV (%) | 4.8 | 6.2 | 9.8 |

*NS= Non-significantly different at 5% probability level; Means followed by the same letters are not significantly different at 5% level of significance according to LSD test.*

However, among the intercropped common bean varieties, rice intercropped with variety Awash Melka gave the highest grain yield (3042 kg $ha^{-1}$) (Table 4). In agreement with this results of this study, Davis and Garcia (1987), Harwood *et al.* (2000), Tolessa *et al.* (2002) and Tolera *et al*. (2003) reported that planting beans in association had no appreciable effect on the grain yield of maize.

Dry biomass was highly significantly affected by the planting density of the associated common bean and non significantly affected by variety and the interaction of variety with planting density. The highest dry bio mass yield (12556 kg $ha^{-1}$) was obtained when rice was intercropped with common bean at plant population of 25% and the dry bio mass decreased as the planting density increase to 75% (Table 4). The reduction in dry biomass production in intercropped rice could be due to shading effect of the common bean during the early growth stage and inter-specific competition. Biscoe and Gallagher (1972) reported that the rate of dry bio mass production in crops depend up on the efficiency of the interception of photosyntheticically active radiation (PAR).

The harvest index of rice was non significantly affected by the main effect of common bean varieties, population and their interaction. In this study, though the difference was statistically non-significant, relatively higher harvest index was recorded from sole crop (27.01%) than the intercropped rice (22.63%) (Table 4). In conformity to this Karikari *et al.* (1999) in Bambara groundnut + maize and Bambara groundnut + sorghum intercropping, reported significantly higher harvest indices for sole maize (0.599) and sole sorghum (0.386) than those in intercrops.

*c) Common Bean Component*

i. *Crop Phenology*

The main effect of common bean varieties and planting density had significantly affected days to 50% emergence, days to 50% flowering and days to maturity.

ii. *Growth Parameters*

The main effect of varieties of common bean was highly significant ($P<0.01$) on leaf area, leaf area index, and plant height. Similarly the effect of planting density on leaf area index and plant height was highly significant and the interaction effect of varieties and planting density was significant on leaf area.

Common bean variety Nasir has the highest LAI (2.9) while the lowest was for variety Awash Melka (LAI=1.4). The variation in leaf area and leaf index observed due to varieties might be due to the difference in inherent characters of the varieties. Similarly, Wogayehu (2005) found significant difference in the leaf area index of common bean among the intercropped common bean varieties.

With regards to the common bean planting density, the highest leaf area index (2.9) was recorded when common bean was intercropped with rice at planting density of 75% and then the leaf area index decreased as planting density was decreased (Table 5). In line with this result, Sisay (2004) reported the highest LAI (2.321) when green gram was sown at its full rate (100%) while the lowest LAI (0.266) from the treatment containing the lowest rate (20%) of green gram. Although the difference is not statistically significant, higher leaf area index (2.53) of common bean was recorded from sole crop than the intercrop with LAI of 2.20 (Table 5).

*Table 5:* The average result of the main effects of varieties and planting density of common bean on days to maturity, leaf area index and plant height of common bean intercropped with rice and grown as sole crop

| Treatment | Days to 90 % maturity | LAI | Plant height (cm) |
|---|---|---|---|
| Common bean varieties | | | |
| Red Wolaita | 113.4b | 2.5[ab] | 52.56[b] |
| Awash Melka | 117.3a | 1.4[c] | 46.44[c] |
| Nasir | 83.6[c] | 2.9[a] | 53.00[b] |
| LSD (0.05) | 0.92 | 0.87 | 0.72 |
| Common bean Planting Density | | | |
| R + 25% | 98.1c | 1.5[b] | 53.83[c] |
| R + 50% | 99.9b | 2.2[ab] | 55.75[b] |
| R + 75% | 101.6a | 2.9[a] | 57.33[a] |
| LSD (0.05) | 0.79 | 0.75 | 0.62 |
| CV (%) | 0.9 | 35.8 | 1.3 |
| Intercrop mean | 116.3 | 2.03 | 55.64 |
| Sole crop mean | 100.0 | 2.53 | 55.83 |
| LSD (0.05) | 9.05 | NS | NS |
| CV% | 1.5 2.3 | 9.9 | 5.4 |

*NS= Non-significantly different at 5% probability level; Means followed by the same letters are not significantly different at 5% level of significance according to LSD test.*

With regards to the effect of planting density the highest plant height (57.33 cm) was recorded at common bean planting density of 75% and the height was significantly increased as planting density of common bean was increased (Table 5). The reduction in plant height of common bean with increase in planting density of common bean might be due to increased competition for growth resources such as radiation, soil moisture and nutrients with increased population of the intercropping system.

iii. *Yield Components and Yield*

In the present study, the main effect of common bean varieties had a highly significant ($P<0.01$) effect on number pods per plant, number seeds per pod, hundred seed weight, grain yield and harvest index. Moreover, the effect of planting density was highly significant on the above parameters except on hundred seed weight and harvest index which was non-significant. The interaction effect of varieties and planting density of common bean was significant ($P<0.05$) on number pods per plant and highly significant ($P<0.01$) on grain yield of common bean.

The highest number of pods per plant (16.33) was obtained from Nasir common bean variety at planting density of 25% and the lowest number of pods per plant (3.0) was obtained from Awash Melka variety at planting density of 75% (Table 6). In general, the number of pods per plant decreased with the increase in planting density for all the varieties. This decrease in number of pods per plant at higher density could be attributed to increased competition among plants for growth factors. In line with this, in sorghum + mung bean and sorghum + pigeon pea intercropping, Subramanian and Rao (1988) reported that decrease in grain number per unit area was responsible for lower grain yields in intercrops than in sole crops.

Above ground dry biomass (kg $ha^{-1}$) was significantly ($P<0.05$) affected by the common bean varieties and highly significantly ($P<0.05$) by the planting density. The highest above ground dry biomass (4466 kg $ha^{-1}$) was recorded for variety Awash Melka while the lowest (3121 kg $ha^{-1}$) was recorded for variety Red Wolaita (Table 6). With regards to the planting density, the highest above ground dry biomass (5292 kg $ha^{-1}$) was recorded at common planting density of 75% and it was decreased significantly with the decrease in planting density of common bean to 25% (Table 6). This decrease might be decrease in population of common bean in the intercropping system. In agreement with this result, Sisay (2004) recorded the highest above ground dry biomass from 100% green gram broadcast with sorghum followed by 80% green gram broadcast with sorghum. Similarly, intercropping of full density of barley with five planting densities of faba bean (100:12.5%, 100:25%, 100:37.5%, 100:50% and 100:62.5%) showed significant increment on dry biomass yield of intercropped faba bean from 653 kg/ha to 2494 kg/ha as plant density of faba bean increased from 12.5% to 62.5% (Getachew *et al.,* 2006). Though the difference was not statistically significant sole crop bean gave higher above ground dry biomass than the intercrop.

*Table 6:* Main effects of varieties and planting density of common bean on growth parameters and yield components of common bean intercropped with rice and grown as sole crop

| Treatment | Number of branches | Dry bio mass (kg/ha) | No. of seed per pod | 100 seed wt (g) | Harvest index (%) |
|---|---|---|---|---|---|
| Common bean varieties | | | | | |
| Red Wolaita | 2.93 | 3121[b] | 5.0[a] | 22.6[c] | 17.7[bc] |
| Awash Melka | 3.36 | 4416[a] | 3.5[c] | 22.3[a] | 27.7[a] |
| Nasir | 2.78 | 3166[b] | 4.8[a] | 20.3[c] | 18.7[b] |
| LSD (0.05) | NS | 839.2 | 0.35 | 8.3 | 3.1 |
| Common bean Planting Density | | | | | |
| R + 25% | 2.98 | 2323[c] | 4.7[a] | 31.7 | 18.7 |
| R + 50% | 2.92 | 3292[b] | 4.6[a] | 30.8 | 20.4 |
| R + 75% | 2.93 | 5292[a] | 3.9[b] | 30.6 | 20.2 |
| LSD (0.05) | NS | 726.8 | 0.3 | NS | NS |
| CV (%) | 25 | 23 | 7.7 | 25.6 | 16.9 |

| | | | | | |
|---|---|---|---|---|---|
| Intercrop mean | 2.9 | 3491 | 4.47 | 23.67 | 23.3 |
| Sole crop mean | 3.2 | 4147 | 4.47 | 32.84 | 23.7 |
| LSD (0.05) | NS | NS | NS | 8.1 | NS |
| CV (%) | 4.1 | 19 | 6.6 | 7.7 | 5.2 |

*NS= Non-significantly different at 5% probability level; Means followed by the same letters are not significantly different at 5% level of significance according to LSD test.*

The highest number of seeds per pod (5.0) was obtained from common bean variety Red Wolaita while the lowest number of seed per pod (3.5) was for variety Awash Melka (Table 6). This difference in number of seeds per pod might be due to the inherent behavior of the varieties where Awash melka with large seed size had the smallest number of seeds per pod.

The main effects of common bean varieties, planting density and the interaction had a highly significant ($P<0.01$) effect on grain yield of the intercropped common bean (Table 7). The highest grain yield (1842 kg $ha^{-1}$) was recorded for common bean variety Awash Melka at planting density of 75% while the lowest grain yield (180 kg $ha^{-1}$) was recorded for bean variety Red Wolaita at 25% planting density (Table 7). In general variety Awash Melka gave higher grain yield than the other varieties possibly due to its large seed size. Moreover, the grain yield of common bean was increased as the planting density increased which might be due to the increased population. In agreement with this result, Sisay (2004) reported the highest seed yield of green gram when it was intercropped with sorghum with full rate and the lowest seed yields of green gram from intercrop combinations containing 20% and 40% populations of green gram.

*Table 7:* The interaction effect of varieties and planting density of common bean on grain yield (kg $ha^{-1}$) of common bean intercropped with rice

| | Planting Density of Common bean (PD) | | | |
|---|---|---|---|---|
| Common bean Varieties (V) | 25% | 50% | 75% | |
| Red Wolaita | 180[g] | 407[e] | 712[c] | |
| Awash Melka | 546[d] | 1142[b] | 1842[a] | |
| Nasir | 182[g] | 421[d] | 691[g] | |
| Intercrop mean | | | | 708 |
| Sole common bean mean | | | | 845 |
| | V × PD | | Sole vs Intercrop | |
| $LSD_{(0.05)}$ | 133 | | NS | |
| CV (%) | 10.8 | | 13.7 | |

*NS= Non-significantly different at 5% probability level; Means followed by the same letters are not significantly different at 5% level of significance according to LSD test.*

In this study, there was no significant difference in grain yield of the intercropped common bean sole cropped bean. However, this is in contrast to results by Huxley and Maigu (1978) who reported that in cereals and legumes intercropping system, the grain yield of the legume component declined, on average, by about 50% of the sole crop yield, where as the cereals yield was reduced by only 11 %. In agreement with this finding, Pal *et al.* (1993) reported that grain yield of sole cropped maize; sorghum and bean were significantly higher than the intercropped yield of these crop

*d) Productivity and Economic Evaluation of Rice and Common Bean Intercropping*

i. *Land Equivalent Ratio*

The agronomic productivity of this experiment was evaluated by calculating total land equivalent ratio (LER) by summing up the partial land equivalent ratio (PLER) of common bean and rice as described by Willey (1979). The main effect of variety and planting density of common bean and their interactions had a highly significant ($P<0.01$) effect on total Land Equivalent Ratio (Appendix Table 7).

The highest total LER of 2.38 was recorded when rice was intercropped with bean variety Awash Melka at planning density of 75% while the lowest total LER of 1.01 was obtained when bean variety Red Wolaita was intercropped at planting density of 25% (Table 17). In general, as the planting density of common bean increased, the total LER was increased indicating the importance of increased bean population in the intercropping system in improving the agronomic efficiency of the intercropping system. The value of LER above 1 indicates that the intercropping system utilizes the available growth resources more efficiently than sole cropped. In this study all of the total LER values of the intercropping system were greater than one, indicating that intercropping of rice and common bean was productive and had yield advantage over growing either rice or common bean in sole. This could be due to the efficient utilization of resources by the intercropped crops. In line with the results of this study, Ofori and Stern (1987) pointed out that the value of LER follow the density of legume component. Similarly, Eyob (2007) reported the highest LER of 1.94 from intercropping of faba bean with 75% plant density of sorghum. In contrast to this, Yesuf (2003) reported that the LER decreased with the increase planting density. Intercropping had higher mean LER (1.80) than sole crop (1.0) .

*Table 8:* Interaction effect of varieties and planting density of common bean on total land equivalent ratio of the intercropped rice and common bean

| | Planting Density of Common bean (PD) | | | |
|---|---|---|---|---|
| Common bean Varieties (V) | 25% | 50% | 75% | |
| Omo | 1.01$^{f}$ | 1.23$^{de}$ | 1.47$^{b}$ | |
| Ibbando | 1.40$^{bc}$ | 1.92$^{a}$ | 2.38$^{a}$ | |
| Hawassa Bume | 1.10$^{ef}$ | 1.26$^{cde}$ | 1.49$^{b}$ | |
| Intercrop mean | | | | 1.7 |
| Sole mean | | | | 1.0 |
| | V × PD | | Sole vs Intercrop | |
| $LSD_{(0.05)}$ | 0.16 | | NS | |
| CV (%) | 6.3 | | 10.5 | |

*NS= Non-significantly different at 5% probability level; Means followed by the same letters are not significantly different at 5% level of significance according to LSD test.*

ii. *Gross Monetary Value*

The main effect of variety and planting density of common bean and their interactions had significant ($P<0.05$) effect on Gross Monetary Value (Appendix Table 7). The highest Gross Monetary Value of 30,883 ETB/ha was obtained from common bean variety Awash Melka intercropped with rice at planting density of 75% while the lowest Goss Monetary Value of 17,356 ETB/ha was obtained when bean variety Red wolaita was intercropped with rice at planting density of 25% (Table 18).

*Table 9:* Interaction effect of varieties and planting density of common bean on Gross Monetary Value of the inter cropped rice and common bean

| Common bean Varieties (V) | Planting Density of Common bean (PD) | | |
|---|---|---|---|
| | 25% | 50% | 75% |
| Red Wolaita | 17356 | 19584 | 21821 |
| Awash Melka | 21623$^{b}$ | 26787$^{a}$ | 30883$^{a}$ |
| Nasir | 18884$^{de}$ | 20130$^{bcd}$ | 22454$^{b}$ |

| | |
|---|---|
| Intercrop mean | 28984 |
| Sole rice mean | 17169 |
| Sole common bean | 7493 |

*NS= Non-significantly different at 5% probability level; Means followed by the same letters are not significantly different at 5% level of significance according to LSD test.*

As it was in total LER, as the planting density of common bean increased, the Gross Monetary Value increased indicating the importance of increased bean population in the intercropping system in increasing the economic efficiency of the intercropping *system as rice had high price (18 Birr/kg) than bean (5.6 Birr/kg) in the local market. In this study* all the intercrops gave higher gross monetary value than either of sole rice or sole bean (Table 18). In agreement with the result of this study, Tesfay (2012) reported the highest GMV (46375.2 ETB/ha) from additive mixture of faba bean and wheat variety HAR 2501 at the seed rate of 75% while the lowest GMV (32222.1 ETB/ha) was obtained from intercropping of faba bean and variety HAR 2501 with seed rate of 50%.

## IV. Conclusion

Intercropping is an important option for efficient utilization of resource especially under gradually decreasing cultivated land. Even though there is practice of intercropping cereals and legumes in the study area, the practice of intercropping rice with different densities of common bean is not common. Therefore, this study was initiated with the objective of determining the effect of density and varieties of common bean intercropped with rice on performance of the associated crops at Kuja and Gojeb, sothwestern Ethiopia. The treatment consisted of three improved common bean varieties (Red Wolaita, Awash Melka and Nasir) and three planting densities (25%, 50% and 75%) of the recommended seed rate of sole common bean laid out in randomized complete block design (RCBD) in factorial arrangement and replicated three times.

The results of the study showed that days to 50% heading, days to 90% maturity, leaf area index, number of seeds per spike, 100 seed weight, grain yield, dry bio mass and harvest index were not significantly affected by the varieties of the associated common bean. However, days to 50% heading, days to 90% maturity and dry bio mass were significantly affected by common bean density. The shortest days to 50% heading (95.58 days) and the shortest days to 90% maturity (120.33 days) of the associated rice were recorded at 25% planting density of common bean and significantly increased with the increase in density of common bean to 75%. The highest dry biomass (12556 kg/ha) of the rice crops were recorded when intercropped with common bean at 25% planting density and both parameters decreased significantly with increase in planting density of common bean to 75%.

The interaction effect of varieties and planting density was highly significant ($P<0.01$) on plant height of the rice intercropped with common bean varieties. The highest plant height (78 cm) was recorded when rice was intercropped with common bean variety Nasir at planting density of 25%. In general as the planting density of common bean increased the height of the associated rice was increased.

The main effect of common bean varieties had significant ($<0.05$) effect on dry bio mass and highly significant ($p<0.01$) effect on days to 90% maturity, leaf area index, plant height, number of seed per pods, hundred seed weight and harvest index. The shortest days to 90% maturity (83.6 days) and the highest plant height (53 cm) were recorded for the Nasir variety. Common bean variety Awash Melka was found to have the highest days to maturity (117.3 days), dry bio mass (4466 kg/ha), hundred seed weight (52.3 g) and harvest index (27.7%).

The main effect of common bean density had significant effects on days to 90% maturity, leaf area index, plant height, dry bio mass and number of seeds per pod. The shortest days to 90% maturity (83.6 days) and the lowest leaf area index (1.4) were recorded at common bean planting density of 25% and significantly increased as the bean planting density increased to 75%. Conversely, the highest plant height (57.33cm) and the highest number seeds per pod (5.0) were recorded in responses to 25% planting density and significantly increased as the bean planting density increased to 75%.

Days to 50% emergence, stand count difference, leaf area and number of pods per plant of common bean were significantly affected by the interaction effect of rice intercropped with common bean. Grain yield of common bean was also significant affected by the interaction effect of rice intercropped with common bean. The shortest days to 50% emergence (10 days) of common bean was recorded at the Awash Melka and Nasir at planting density of 25% and Red Wolaita at 75% planting density intercropped with rice crop. The highest leaf area (2.9 $cm^2$) and the highest number of pods per plant (16.33) were recorded for varieties Awash Melka and Red Wolaita at 25% planting density intercropped with rice, respectively.

The main effect of variety and planting density of common bean and their interactions had a highly significant ($P<0.01$) effect on total Land Equivalent Ratio. The highest total LER of 2.38 was recorded when rice was intercropped with bean variety Awash Melka at

planning density of 75% while the lowest total LER of 1.01 was obtained when bean variety Red Wolaita was intercropped at planting density of 25%. In general, as the planting density of common bean increased, the total LER was increased. As it was for LER, the main effect of variety and planting density of common bean and their interactions had significant ($P<0.05$) effect on Gross Monetary Value. The highest Gross Monetary Value of 30,883 ETB/ha was obtained from common bean variety Awash Melka intercropped with rice at planting density of 75% while the lowest Goss Monetary Value of 17,356 ETB/ha was obtained when bean variety Red Wolaita was intercropped with rice at planting density of 25%). In this study all the intercrops gave higher gross monetary value than either of sole rice or sole bean. Therefore, based on the above agronomic and economic evaluations, rice (100%) intercropped with common bean variety Awash Melka at planting density of 75% of the common bean can be recommended for intercropping of rice with common bean in the study area. However, as this is one field experiment, the experiment has to be repeated over seasons with consideration of farmers preference of the common bean varieties to reach at conclusive recommendation.

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# Evaluation of Glyphosate Toxicity on Arabian killifish, Aphanius dispar Collected from Southwestern Saudi Arabia

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*Keywords: glyphosate, fish toxicity, aphanius dispar, behavioral changes, histology, acute toxicity, environmental health.*

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In conclusion, this current study results revealed that glyphosate is very toxic leading not only to abnormal behavioral responses and tissue alterations, but might cause mass extinction of fish species. Therefore, glyphosate should be used carefully in/or near aquatic systems to avoid extinctions of life forms, particularly *Aphanius dispar*. Thence, protecting species diversity, which is a key issue for stability and resiliency of aquatic ecosystems.

*Keywords: glyphosate, fish toxicity, aphanius dispar, behavioral changes, histology, acute toxicity, environmental health.*

## I. Introduction

Glyphosate herbicide (Tiller 480 SL), which is a broad spectrum non-selective herbicide is used excessively to control and inhibit a great variety of annual, biennial and perennial grasses, sedges, broad leaved weeds and woody shrubs in agricultural, industrial, urban, forestry, aquatic ecosystems, fish ponds, lakes, and canals (Cavas and Konen, 2007; Langiano and Martinez, 2008; Sani and Idris, 2016; Tsui and Chu, 2008). Glyphosate has been reported to be the most important herbicide ever developed (WHO, 1994) to particularly be applied in plant varieties that are genetically modified to be better able to tolerate glyphosate treatment during weed control (Langiano and Martinez, 2008), without affecting crops (Sani and Idris, 2016). Glyphosate is not only used directly to control noxious weeds in aquatic systems, but also reported to reach aquatic systems after application in agricultural fields, thus affect non-targeted organisms indirectly like invertebrate, fish and other life forms from the first level up higher the food chains (Jofre et al., 2013), thence reduce species diversity, community structure affecting the stability and resilience of aquatic ecosystems (Perez et al., 2011).

Glyphosate mode of action is through competitive inhibition of phosphoenolpyruvate (PEP) on the active site of 5-enolpyruvylshikimate-3-phosphatethensate, an enzyme involved in the biosynthesis of aromatic amino acids (phenylalanine, Tyrosine, and tryptophan), which are essential for protein synthesis (Mallory-Smith, 2013, Tu et al., 2001). Glyphosate and its formulations, especially those containing surfactants are considered hazardous to the aquatic environment, which showed higher toxicity to most aquatic organisms than the active ingredient itself, which has been classified into very slight to high toxicants to aquatic organisms including fish species (WHO, 1994; Perez et al., 2011) due to its higher solubility varying from 10,000 to 15,000 mg/l at 25oC (Nwani et al., 2013). Glyphosate and its formulations acute and chronic effects on aquatic organisms including fish species have been reported to involve behavioral, histopathological, biochemical, and physiological changes (Langiano and Martinez, 2016; Jiraungkoorskul et al., 2002; Thanomsit et al., 2016), reflecting slight to severe concentration-related alterations over a short period of time (Perez et al., 2011). Several studies on fish species have reported variations for the acute toxicity of glyphosate concentrations of which: 10 mg/l for Asian sea bass, *Lates Calcarifer*, 13.69 mg/l for the Neotropical fish, *Prochilodus lineatus* (Langiano and Martinez, 2008), and 8.3 mg/l for *Oncorhynchus mykiss*, (Waynon, 1980), 16.8 mg/l for nile tilapia, *Oreochromis niloticus* (Jiraungkoorskul et al., 2002), 97.47 mg/l for catchama blanca, *Piaractus brachypomus* (Ramirez-Duarte et al.,

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2008), and 211.80 mg/L for *Tilapia zilli* (Nwani et al., 2013). *Aphanius dispar* juveniles has been reported to respond differently to different pesticides upon 24h acute exposure to different concentrations of synthetic pyrethroid pesticides, fenpropathrin and fenvalerate (Shoaib et al., 2013) expressing differently low LC50 values, indicating high sensitivity of fish juveniles to different pesticides as many environmental factors influence the bioassay results (Shoaib et al., 2012).

Fish behavior has been indicated to be the most sensitive indicators upon exposure to environmental stressors particularly in fish species (Banace et al., 2011; Zarei et al., 2013). Fish were observed in previous studies to express various abnormal behavioral changes such as hyperactivity, loss of schooling, overcrowding, hypoactivity, breathing difficulties, jumping out of water, surfacing, jerky swimming, rapid opercula movements, loss of righting response convulsion, loss of balance, after exposure to a number of environmental toxicants, thus their living standards is affected overwhelmingly before extinctions occur (Banace et al., 2011; Ba-Omar and Al-Jardani, 2011; Kumar et al., 2015; Zarei et al., 2013; Sani and Idris, 2016; Nwani et al., 2013). *Aphanius dispar* exposed to temphos, which is an organophosaphate pesticide expressed common behavioral changes such as restlessness, erratic swimming, convulsion, and loss of balance (Ba-Omar and Al-Jardani, 2011). Furthermore, the reported effects of glyphosate exposure in inducing histopathological changes of gills and liver tissues of fish included epithelial uplifting, interlamellar hyperplasia, hypertrophy of epithelial cells, shortening and folding of lamellae, necrosis of lamellar epithelium, lamellar fusion, aneurism, hyperplasia of chloride cells and mucus cells in the interlamellar spaces, clubbing, edema, and degeneration of filaments of the gills (Akinsorotan and Olele, 2013; ayoola, 2008; Deivasigamani, 2015; Jiraungkoorskul et al., 2002). while, they observed histopathological changes of the liver to include cytoplasmic and nuclear degeneration, hyperplasia, vacuolization of the cytoplasm, mild to severe infilteration of leukocytes, pyknotic nuclei, hypertrophy of hepatocytes, necrosis and bile stagnation. Ba-Omar and Al-Jardani, (2011) found lamellar damages including degradation of chloride cells, desquamation, epithelial uplifting, sloughing of epithelial cells, hypertrophy of lamellae, fusion of secondary lamellae, curling, tearing and collapsing of the lamellae after exposure of *Aphanius dispar* to the organophate temphos.

*Aphanius dispar* (Ruppell 1829), which also is known by the common name Arabian killifish has a wide distribution throughout Africa, Asia, and coast line of the red sea including Saudi Arabia. However, Arabian killifish population represents a single species, but with many color variations and patterns depending on locality. In Saudi Arabia, *Aphanius dispar* can breed all year round with vivid coloration of the males that attract females and can tolerate in their habitats a wide range of temperature, salinity, and other factors making *Aphanius dispar* able to tolerate stressors, which might jeopardize their existence due to reduced food availability, habitat degradation, exotic species, chemical contamination, and exploitation.

Since *Aphanius dispar* existence is threatened not only by their habitat degradation and food availability, but also by various environmental stressors (Saeed et al., 2015), the focus of this current study was to determine the acute toxicity of the commercially formulated glyphosate and its effects on behavior and histology of the gills and liver tissues in light of the excessive use of glyphosate in agriculture.

## II. Materials and Methods

### a) Chemicals

A commercial formulation of glyphosate (480 g/l glyphoste-isopropylamine salt) with trade name (Tiller 480 SL) manufactured by Astra Chem., Tabuk, KSA, was used in this current study.

### b) Experimental Fish

Male and female juvenile Arabian killifish, *Aphanius dispar* were collected from southwestern Saudi Arabia, specifically from sadder Weila valley, through netting using a hand net. The juveniles were transported in a clean-aerated freshwater to the laboratory with care to lessen stress. *Aphanius dispar* juveniles mean weight was 1.5 ± 0.3 g and 4.5 ± 0.5 cm of length were allocated to aquaria randomly and left to be acclimatized under laboratory conditions for two weeks before running the static bioassay. Fish were fed commercial diet (flaked- food) once daily. The average values of water quality were (temperature 22 ± 1.0˚C, pH 7.2 ± 0.1, dissolved oxygen 7.03± .02 mg/L, and total hardness 220 ± 2 mg/l). The light and dark cycle of 12 h: 12 h was maintained throughout the whole study duration.

### c) Acute Toxicity Test

The bioassay test was conducted according to the US EPA guidelines 712-C-6-118 (1996) to determine the 96-h LC50 values of commercial formulation of glyphosate (Tiller 480 SL). Fish were starved for 24 hours prior to and during the bioassay. The test was conducted in plastic aquaria (30 + 30 + 15 cm) containing 8 L of static water (10 fish per aquarium). Seven different concentrations of glyphosate (60, 90, 120, 150, 180, 210 and 240 mg/L) with two replicates plus the control were used for running the acute toxicity test. Fish mortality in each aquarium was recorded and dead fish were removed immediately throughout the bioassay duration. The LC50 value of the fish was determined using the Probit analysis method (Finney,1971), and their behavior was monitored daily

for any abnormal behavioral changes throughout the bioassay.

*d) Sub-Acute Toxicity Test*

In order to investigate the histopathological effect of glyphosate on the gill and liver tissues, fish were exposed to 1/4 th of the 96-h LC50 glyphosate dose for 14 days. At the end of the exposure, fish were sacrificed and the gill and liver tissues of the control and treated fish were immediately excised and fixed in Bouin's solution for 48 h at room temperature. After fixation, the tissues were washed with tap water, dehydrated through a graded ethanol series, cleared in xylene and embedded in paraffin wax. Sections of 6 µm were cut using a microtome (American Optical Co., USA), and stained with hematoxylin and eosin (Bancroft J and Steven A 1996). The stained sections were then examined for histopathological changes and photographed using Olympus light microscope (Olympus, Tokyo, Japan) equipped with a digital camera.

*e) Statistical Analysis*

The 96-h LC50 value of the Aphanius dispar was calculated using the Probit analysis method (Finney, 1971). One way ANOVA was performed using SPSS software to detect significant differences among groups. *P* value $< 0.05$ was considered statistically significant.

## III. Results

*a) Acute Toxicity Test*

The 96-h LC50 value (Fig.1) of glyphosate upon exposure of *Aphanius dispar* to different glyphosate concentrations (60, 120, 150, 200, 180, 210 and 240 mg/L, was determined to be 115.25 mg/L using the probit analysis method.

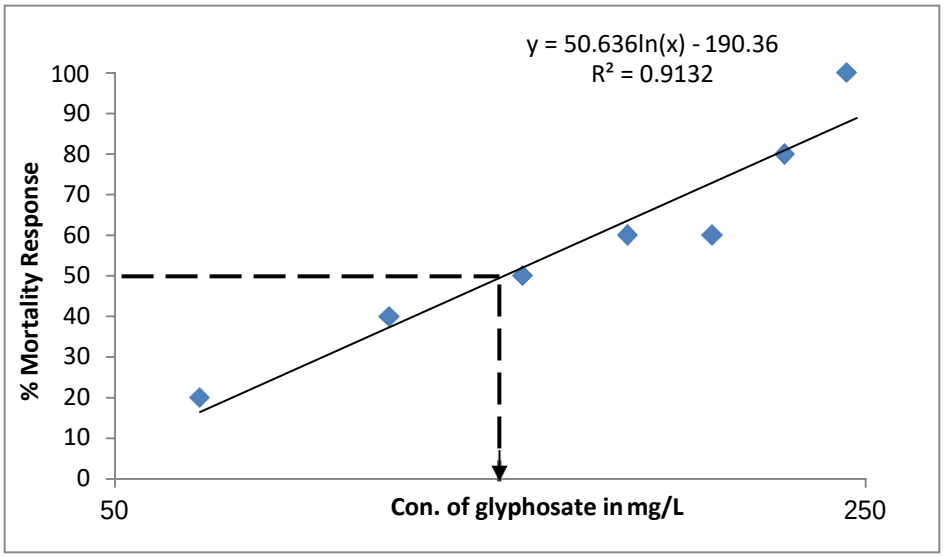


*Fig. 1:* The relationship between glyphosate concentrations and mortality percentages

*b) Fish behavior*

Unexposed fish group to glyphosate did not exhibit any adverse abnormal behavioral responses or any mortality throughout the duration of the bioassay as compared to the treated fish. However, exposed fish to glyphosate exhibited various abnormal behavioral responses, which were concentration-related as shown in Table1.

*Table 1:* Behavioral responses of the Arabian killifish, Aphanius dispar after acute exposure to different concentrations (mg/L) of glyphosate.

| Concentration mg/L | Behavioral responses of Aphanius dispar |
|---|---|
| 0 | Normal activity to mild hyperactivity on the first day, Normal feeding behavior |
| 60-89 | Mild hyperactivity associated with loss of schooling into schools Hyperactive |
| 90-119 | surfacing to the tank top as an avoidance response associated with rapid opercula movements |
| 120-149 | Erratic movements associated with opercula movements and rapid mouth movement rate gulping for air |
| 150-179 | Schooling on and off, frequent surfacing and jumping outside the aquaria, cannibalism, loss of balance associated with hanging vertically in the water column head-up tail-down, swirling with rapid speed |

| | |
|---|---|
| 180-209 | Exhaustion associated with hypoactivity, settling on the bottom of the tanks with less opercula and mouth movements |
| 210-240 | Exhaustion associated with hypoactivity and mortality |

*c) Histopathological Study*

Mild focal changes of the Arabian killifish, *Aphanius dispar* gill lamellae were observed in the control group (Fig. 2-a), while there were no observed histopathological changes in the liver tissues. However, exposed fish to the herbicide glyphosate (1/4 96 hLC50) concentration exhibited a wide range of mild to excessive histopathological alterations of the gills (Fig. 2, b-e) and Liver (Fig. 3, b-d) (Fig. 3-a).).

The gills of exposed fish exhibited histopathological changes of which epithelial uplifting, edema, epithelial hyperplasia, fusion of lamellae, clubbing of the tips of secondary lamellae, and thickening of lamellar epithelium (Figure 2, b-e). While, the liver organ exhibited histopathological changes of significance including deterioration and necrosis of the liver hepatocytes, hypertrophy of hepatocytes, eccentric nuclei and pyknosis, as well as mild to extensive vacuolization of hepatocytes with a foamy appearance (Figure 3, b-d).

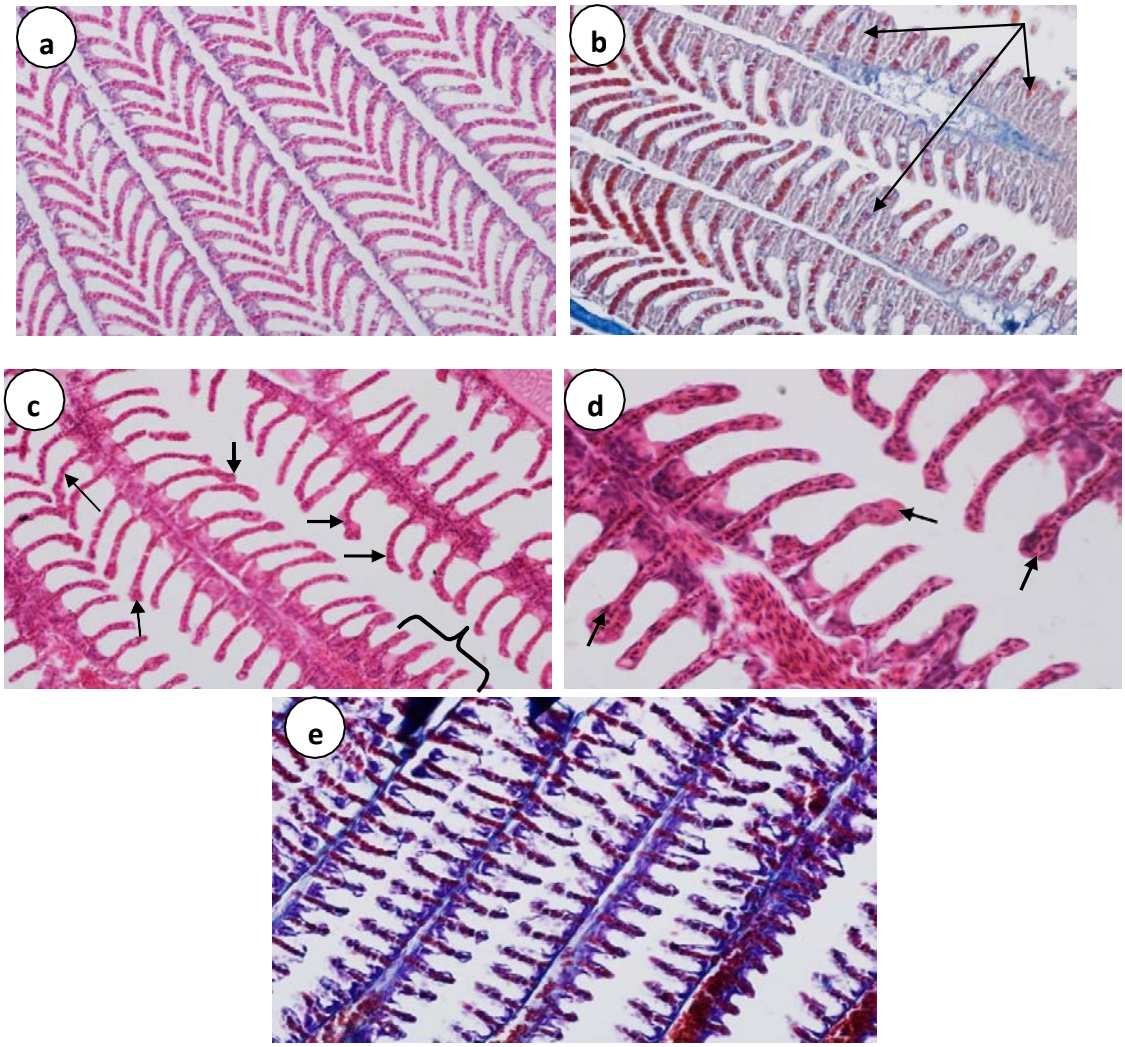


*Fig. 2 (a-e):* Histopathological changes of *Aphanius dispar* gills: Control group (a) and glyphosate treated (b-e). a: normal gill filaments and secondary lamellae. b: interlamellar hyperplasia of the filaments leading to fusion of secondary lamellae (arrows), c: shortening of secondary lamellae (bracket), elongation associated with curling, clubbing and elongation of secondary lamellae ((small arrows), d: clubbing of the secondary lamellae (arrows). e: gills showing generalized excessive epithelial uplifting from the basement membrane. (H & E) X 20.

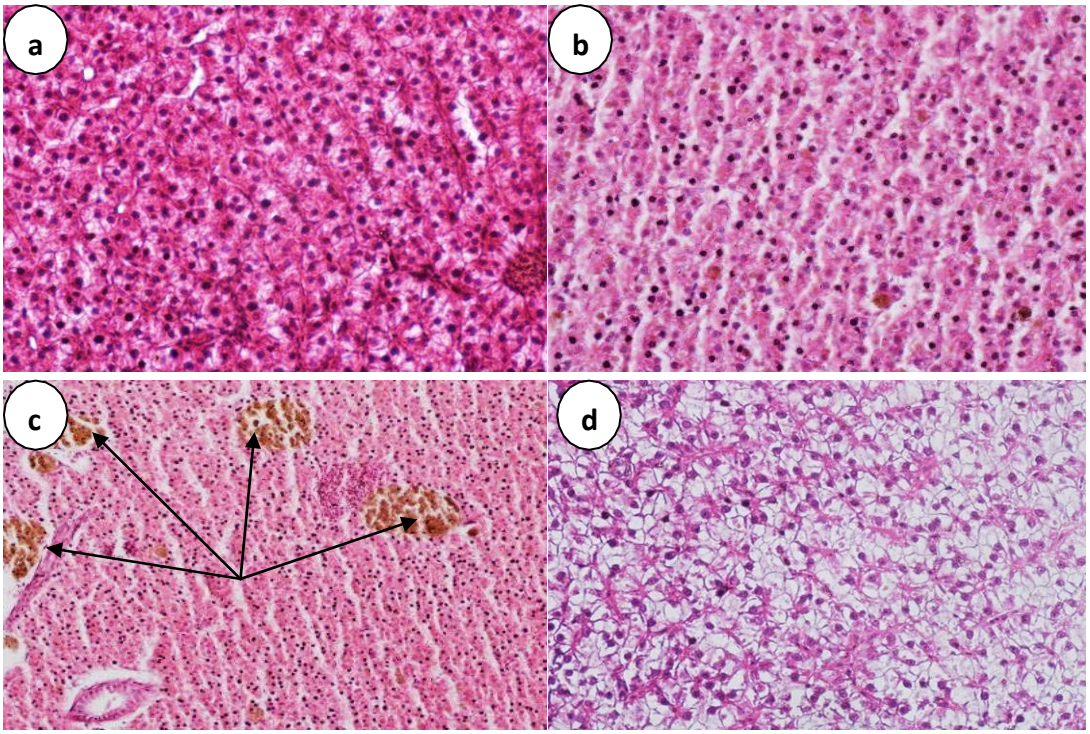


*Fig. 3:* Histopathological changes of Aphanius dispar liver: Control group (a) and glyphosate treated (b-d). a: normal liver of the control group with normal polygonal hepatocytes associated with very mild deterioration. b: deterioration of the hepatocytes and pyknosis of nucleolus. c: extensive deterioration and excessive bile stagnation (arrows). d: excessive cytoplasmic vacuolization with a foamy appearance . (H & E) X 20.

## IV. Discussion

*a) Acute Toxicity*

Mortality and extinction of many life forms is imposed by myriads of chemical pollutants including herbicides. The impacts of chemical pollutants reflect their concentrations, duration of exposure, environmental factors, and sensitivity of life forms. Thus determination of the lethal dose (LC50), the dose that might kill half of any population within a short period of time, is considered the first step prior to any physiological studies as with regard to chemical pollutants. In this current study, glyphosate formulation (Tiller 480 SL) 96 h LC50 of the Arabian killifish, *Aphanius dispar* juveniles valued at 115.25 mg/l (Figure. 1). The 96h LC50 values of glyphosate have been investigated on different fish species at different environmental conditions, indicating variation in concentrations (Neskovic et al., 1996; Jiraungkoorskul et al., 2002). Previously very limited studies have stated that *Aphanius dispar* juveniles responded differently to different pesticides (Shoaib et al., 2013), indicating variability in sensitivity to different pesticides being influenced by various environmental factors (Shoaib et al., 2012; Shoaib et al., 2013). Futhermore, Nwani et al. (2013) reported the 96 h LC50 for *Tilapia Zilli* at 211.80 mg/l upon exposure to glyphosate formulation (Forceup). The reported LC 50 values which are close to the results of this current study upon exposure to glyphosate for 96 h were found to be 97.47 mg/L for cachama blanca, *Piaractus brachypomus* (Ramirez-Duarte et al., 2008) and 86 mgl for the common carp, *Cyprinus carpio* (Deivasigamani, 2015). Additionally, lower 96 h LC50 values were recorded: 43.65 mg/L for the African catfish, *Clarias gariepinus* (Akinsorotan, 2013),13.69 mg/l for Neotropis fish, *Pochilodn lineatus* (Landgiano and Martinez, 2008), 10.0 mg/l for the Asian Bass, *Lates calcarifer* (Thunomsit et al. 2016), 1.05 mg/l for nile tilapia, *Oreochromis niloticus* (Ayoola 2008) and 0.05 mg/l for the African catfish, Clarias gariepinus (Ayanda et al., 2015).

Therefore, from the determined 96 h LC50 values (115.25 mg/l), we do believe that *Aphanius dispar* juveniles are very sensitive to glyphosate and its toxicity might be exacerbated further upon exposure under harsher environmental factors. Thus, as we compare this current study 96 h LC50 value to the previously reported studies, 96 h LC50 higher and lower values on different fish species and different environmental conditions, we believe that the determined 96 h LC50 value in our study on might be influenced by the ambient environmental conditions for this species.

*b) Fish behavior*

Behavioral responses have been indicated to be the most sensitive indicators upon exposure to potential toxic effects in fish species (Banace et al., 2011; Ba-Omar and Al-Jardani 2011). Our results

showed that the unexposed fish group did not reveal any adverse abnormal behavioral responses or mortality throughout the duration of the bioassay as compared to the treated fish groups other than mild hyperactivity at the onset of the experimental execution, which might be attributed to fish handling during allocation. On the contrary, exposed fish to glyphosate exhibited various abnormal behavioral responses (Table1) and were concentration-related similar to the previously reported observations after exposure of different fish species to the herbicide glyphosate (Akinsorotan et al., 2013; Ayoola 2008; Okayi et al., 2010). Abnormal behavioral changes such as mild to moderate erratic swimming, rapid rate of opercular and mouth movements, infrequent surfacing were observed at low to moderate concentrations, while at higher concentrations Aphanius dispar exhibited rapid swimming associated with frequent surfacing and jumping outside of the aquaria, hanging head-up tail-down position, and hypoactivity before the fish became weak, hypoactive, and settled at the bottom followed by exhaustion and death. These observations were consistent with the previously reported abnormal behavioral changes after exposure of various fish species to the herbicide glyphosate: Clarias gariepinus adult (Akinsorotan et al., 2013), Clarias gariepinus fingerlings (Okayi et al., 2010), juvenile African catfish, Clarias gariepinsus (Ayoola, 2008), the common carp, Cyprinus carpio (Deivasigamani, 2015), and Asian sea bass, Lates calcarifer (Thanomsit et al., 2016). Similarly, Apanius dispar juveniles exposed to the organophosphate temphos expressed abnormal behavioral changes (Ba-Omar and Al-Jardani 2011). These previously mentioned authors reported such abnormal behavioral changes and mortality to occur after acute and chronic toxicity indicating respiratory failure inflected by the effects of the glyphosate on the gills. Thus, fish mortalities observed in this study could be due to the destruction of gill tissues and impairment of gas-exchange capacity after fish became very lethargic and exhausted. Furthermore, fish respiratory failure might be an indication of physiological distress on juveniles resulting from potential progressive energy expenditure with time preceding mortality of fish.

Furthermore, according to Kumar et al. (2015), Zarei et al. (2013), Nwani et al., (2013), and Okayi et al., (2010) mucus secretion observed in this current study at the water surface at higher concentrations might suggest excessive impacts of glyphosate on fish gills forming a mucus film on the gills interrupting gaseous exchange and causing death of fish following exhaustion and lethargic responses. While, Pandey et al., (1990) attributed the secretion of mucus to dysfunction of the endocrine gland under toxic stress thus changes in the number and area of mucus glands and chromatophores. On the other hand, Sani and Idris, (2016) the previously reported behavioral changes have been indicated to occur as a result of not only metabolic dysfunction but also due to nervous disorder upon exposure to toxic glyphosate. Thus we do believe that fish exhibiting such abnormal behavioral changes and mucus secretion in Aphanius dispar in this current study might have been due to the toxic effects of glyphosate on gill tissues and respiration impairment.

*c) Histopathology*

Aphanius dispar upon exposure to glyphosate revealed gill and liver tissue alterations which were concentration-and-time related. Literatures on the impacts of noxious chemicals on fish histopathology of Aphanius dispar gills, liver, kidney and all other levels of biological organizations are scarce and very current. For example, the acute and chronic impacts of 3,4-dichloroaniline (DCA), sodium dodecyl sulfate, and zinc sulfate and chlorine on Aphanius dispar embryos development were studied (Saeed et al., 2015). While, the effects of the organophosphate temphos on the gills of Aphanius dispar revealed various concentration-related gill damages including hemorrhage of lamellae, epithelial uplifting, epithelial hypertrophay, swelling at the base and tips of lamellae, fusion of lamellae, tears in the filaments, sloughing of epithelial cells from the filaments and lamellae (Ba-Omar and Al-Jardani, 2011).

The gill organs of fish, which are very complex structure essential for gaseous exchange, acid-base balance, excretion, and osmoregulatory function are in contact with the outside environment. There was no recognizable alterations in the gills of the control fish. However fish exposed to 1/4th 96 h LC50 glyphosate exhibited various tissue alterations including fusion of secondary lamellae, epithelial uplifting, minor clubbing of the secondary lamella tips, hyperplasia of the primary filaments and secondary lamellae, and curling of the secondary lamellae. Similarly, previous studies have reported wide spectrum of gill histopathological changes after exposure of fish species to variety of noxious agents including glyphosate. Fish exposure to glyphosate for 96 h of Clarius gariepinus (Akinsorotan et al., 2013), Juveniles African catfish, Clarias gariepinus (Ayoola, 2008), common carp, Cyprinus carpio (Deivasigaman, 2015), Cyprinus carpio (Neskovic et al., 1996), Nile tilapia, Oreochromis niloticus (Jiraungkoorskul et al., 2002) and Aphanius dispar to temphos (Ba-Omar and Al-Jardani (2011) caused wide spectrum of gill histopathological changes of which epithelial hyperplasia, edema, lifting of epithelium, epithelial hyperplasia thickening of primary lamellar epithelium, clubbing, fusion of lamellae and secretion of mucus etc. The pronounced variety of insults of the gill organs which have been observed in this current study followed by exhaustion, lethargy and death of fish are clear indication of the impairment of gaseous exchange and reduced functional efficiency of the gills before suffocation of fish occurred. Therefore, the histopathological changes of fish gills can impair the

respiratory function by reducing the total surface area available for oxygen uptake and increase the diffusion distance between the external environment and the blood inside the lamellae preventing gaseous exchanges, which cause suffocation of fish and death (Ayoola 2008; Ba-Omar and Al-Jardani, 2011; Jiraungkoorskul et al., 2002;).

On the other hand, liver, which is an organ performing various functions associated with the metabolism of xenobiotics (Langiano et al., 2008) exhibited various histopathological alterations after exposure to glyphosate used in this current study including vacuolization of the cells cytoplasm, hypertrophy of hepatocytes, degeneration of hepatocytes, and bile stagnation. Similar findings on the impacts of glyphosate on fish species at varios concentrations were reported (Langiano et la., 2008; Ayola 2008; Akinsorotan et al., 2013; Jiraungkoorskul et al., 2002; Deivasigamani 2015; Neskovic et al., 1996). It is believed that the vacuolization of liver cells might indicate evidence of fatty degeneration (Deivasigamani 2015; Jiraungkoorskul et al., 2002; Ayoola 2008). The localized necrosis of hepatocytes suggest excessive work required by the fish to get rid of the toxicant from its body during the process of detoxification as well as failure of the liver cells to regenerate due to continuous exposure to noxious agents (Deivasigamani 2015; Ayoola 2008). Bile stagnation was observed in the control and treated Neotropical fish Prochilodus lineatus as manifestation of a physiopatholoical conditions caused by a lack of bile metabolism and secretion (Langiano et la., 2008; Ayoola 2008; Deivasigamani 2015), whereas we found that bile secretion by hepatocytes observed within the cells as yellowish droplets in the exposed fish, however, bile droplets were very intense and the degree of intensity was observed in the treated fish with higher glyphosate concentrations, which might indicate glyphosate effects as opposed to suggested nutritional deficiency by Langiano et la., (2008) since we were prompt on feeding the fish once daily and on time.

In conclusion, the results of this current study asserts the toxic impacts of glyphosate on fish behavior and histopathology of the gill and liver tissues. Thus, impairment of gills and liver functional efficiencies before exhaustion, suffocation, and death occurred. Therefore, we recommend regulating glyphosate usage in/or near aquatic environment and the importance of establishing environmental monitoring commission guidelines to regulate or discourage the use of glyphosate and other harmful chemicals. Thence, protecting not only life forms like fish from extinction, but also ensures biological diversity and stability for healthier ecosystems.

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# On the Investigation of Awareness Level of Family Planning among Rural Dwellers in Nigeria (Principal Component Analysis Approach)

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*Keywords: family planning, principal component, eigen values and vectors, marital status and contraceptives.*

*GJSFR-C Classification: FOR Code: 279999*


*Strictly as per the compliance and regulations of :*

# On the Investigation of Awareness Level of Family Planning among Rural Dwellers in Nigeria (Principal Component Analysis Approach)

Ayoola, F. J. [α], Adeboye, N. Olawale [σ] & Kayode Balogun [ρ]

*Abstract-* This study examined the awareness level and attitude of family planning among rural dwellers in the South western part of Nigeria and its consequences on population/economy of Nigeria. Data were gathered from 300 respondents who were randomly selected from Seriki rural communities of western Nigeria using proportional allocation of the stratified random sampling technique. The research considered to know the level of awareness between the traditional family planning methods and modern methods of contraception. The modern methodswere found to be more significantly embraced than traditional family planning methods based on the analysis of the result obtained which shows that 175% prefer modern methods while 125% preferred traditional methods. The factors considered in examining these are educational background, economic factors, marital status, social factor, ethnicity and illiteracy level; the data collected based on these factors was analyzed using principal component analysis technique in order to determine the most prevalent factor that causes attitudinal problems. The results give rise to the Eigen values and Eigen vectors of the components, whereby the variance proportion for each is given as 0.4844, 0.2391, 0.1480, 0.0597, 0.0461 and 0.0227 for economics, educational background, ethnicity, illiteracy, marital status and social status respectively; thereby qualified the factors as first PC, second PC, third PC, fourth PC, fifth PC and the sixth PC respectively. Thus, economic factor which is the first PC is the factor that is predominantly responsible for the discovered low level of awareness and poor attitudinal behavior towards family planning.

*Keywords: family planning, principal component, eigen values and vectors, marital status and contraceptives.*

## I. Introduction

Family planning implies the ability of individuals and couples to anticipate and attain their desired number of children by spacing and timing their births. It is achieved through the use of contraceptive methods and the treatment of involuntary fertility. The availability of family planning does more than enable women and men to limit family size. It safeguards individual health and right and improves the quality of life of couples and their children.

Family planning has attracted attentions all over the world due to its relevance in decision making, population growth and development. Family planning is defined as birth spacing, preventing unwanted pregnancies or secure wanted pregnancy (W.H.O, 1995). Beekle and McCabe (2006) defined family planning as the practice that helps individuals or couples to attain certain objectives such as avoiding unwanted pregnancies, bringing about wanted babies at the right time, regulating, the interval between pregnancies, controlling the time at which birth occurs in relation to the ages of parents and determining the number of children in the family. Family planning is a means of reproductive health. In-spite of the hue and cry in and outside Nigeria about family planning or birth control, many people are still confused about its meaning, the methods involved, the advantages and disadvantages and the factors hindering it's wide application in Nigeria, especially among the rural communities (Iffih and Ezeah,2004).Women's education enhances their capability and also their reproductive rights to decide freely and responsibly the number, spacing and timing of their children and to have other necessary information regarding reproductive rights. Studies have shown that education is a determinant of awareness of family planning practices in Nigeria, for instance; Anyanwuh et.al (2013) investigated the extent of family planning, the methods and contraceptive devices in use and the influence of education on family planning among couples in Nkanu Local Government Area of Enugu State. The findings revealed that educational background of the couples significantly influenced the choice of family planning in the community.

W.H.O (2013) found out that women's education is in line with lower fertility which constitutes management of reproductive resources. Maternal education has once been linked with reduction of child mortality among rural dwellers. Recent studies have also shown that religion is a good determinant of family planning practices. UNFPA (2013) identified some

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factors such as cultural background, and religions beliefs which place the women at disadvantaged position in reproductive health issues. Ezea and Iffih (2004) asserted that Catholic Church is rigid in their views of family planning. Catholics hold the view that the application of artificial method is wrong and should not be allowed. The Catholic Church is said to be comfortable with the use of Billings's ovulation method which is rather natural. Igbudu et. al (2011)conducted a study on the relationship between religious beliefs and family planning practices of married women in zone 5 barracks of the Nigeria police, comprising Edo, Delta, and Bayelsa state commands. The findings of the study revealed that attitudinal factor such as the strong religious desire for more children prevented women in these barracks from using family planning. Also noted were the insufficient knowledge on contraceptives methods, their fears and anxieties, rumor of others using contraceptives getting deleterious effect and poor delivery of health services (Hamau R.K et.al, 1996). Traditional methods such as coitus interrutus is described in Bible, periodic abstinence was used in ancient India and the precursor to the condom was used by the Egyptians back in 1350 BC (Odimegwu, 1999).Of recent, Ghulam Mustafa et al (2015) conducted a similar research in among rural dwellers in Pakistan and findings reveal that the majority knew about some modern contraceptive methods, but the overall contraceptive use was very low. Knowledge and use of any contraceptive method were particularly low. Reasons for not using family planning and modern contraception included incomplete family size, negative perceptions, in-laws' disapproval, religious concerns, side-effects, and lack of access to quality services.

The practices of modern contraceptives method offer many advantages compared to traditional approaches, in health and economy of the couples in the country. Family planning programs that stimulate modern approaches, policies and methods have become increasingly important in the last decades as a result of the social-economic problems influencing rapid population growth, as well as public health problems, especially control of sexually transmitted diseases (STDs) such as AIDS (UN, 1994).

The unwelcomed attitude of most rural dwellers to modern family methods has constitutes a continuous growth in African population size keeping it almost permanently underdeveloped, There is general believe that economic stagnation is often linked with over population and poor investment in health services and with Nigeria's population currently put at over 140 million people compared with Gross Domestic Product (GDP) that is not too encouraging and inflation still sky-rocketing, there is the fear that future living standards may substantially depreciate (Olatayo and Adeboye, 2013). In recent years, population growth has begun to be discussed in terms of its effect on global stability. Some of the supporters of this view have suggested that in post-cold war order, the growth of population has the potential to undermine the global stability. Thus, understanding of family planning scenario among different societies and communities, which by and large reside in urban slum and rural areas, might prove useful in increasing family planning acceptance by them and decreasing population growth (Sharma, V et.al, 2012).Within the context of above literatures, it has therefore becomes imperative to carry out this study in Seriki community; a rural community located in Ogun state, western region of Nigeria. The factors considered in examining this scenario are educational background, economic factors, marital status, social factor, ethnicity and illiteracy level; Principal component analysis technique shall be employed to fit model for each of the factor in order to determine the most prevalent among them that cause attitudinal problems to family planning among rural dwellers.

## II. Research Design and Methodology

The study area of this research is Seriki community in Ogun State and is accessible by land. The community comprises of different ethnic groups namely Yoruba's, Igbo, Hausa, Calabar, Isoko, Ijaws, just to mention but few. The Yoruba's are considered to be the more than any other tribes in the city, because it is located at the western part of Nigeria. According to the unpublished census figure of 2006, its population size was estimated at a total of 2,075. The community was divided into nine (9) strata and a sample size of 335 estimated through Taro Yanmeni's formula was randomly selected among focus group of age 15 – $30^{+}$comprises of single ladies, married couple and the divorced people using proportional allocation of Stratified random sampling technique.

Designed questionnaire was examined on the selected sample size under three (3) different categories. The instrument sought to solicit responses on area of residence, age at marriage, and highest educational qualification, duration or age of marriage, and number of children owned. The second category of the questionnaire covers the factors that encourage large families and their general perception on family planning. The major factors considered here are educational background, economic factors, marital status, social factor, ethnicity and illiteracy level. The third part purely seek information on family planning acceptance in both modern and traditional way. At the end of the survey, a cronbach's alpha validity rest was run on a sample of 10 respondents for reliability measures and the survey was found to be 81.2% reliable.

## III. Data Analysis

The analytical technique adopted for this research is a Multivariate analysis technique known as Principal Component Analysis.

It is a statistical procedure that uses an orthogonal transformation to convert a set of observations of possibly correlated variables into a set of values of linearly uncorrelated variables called principal components. This transformation is defined in such a way that the first principal component has the largest possible variance (that is, accounts for as much of the variability in the data as possible), and each succeeding component in turn has the highest variance possible under the constraint that it is orthogonal to the preceding components. The principal components are orthogonal because they are the Eigen vectors of the covariance matrix, which is symmetric.

If the variables $Y_1, Y_2, \ldots Y_p$ in Y are correlated, the ellipsoidal swarm of point is not oriented parallel to any of the axis. The axes can be rotated by multiplying each $y_i$ by an orthogonal matrix A and thus obtain

$$Z_i = A\,Y_i \tag{1}$$

Since A is an orthogonal, A'A=I and the distance to the origin is unchanged. Thus we want to sample covariance matrix of $Z\,S\,Z = A\,S\,A'$ to be diagonal.

$$Z\,S\,Z = A\,S\,A' = \begin{pmatrix} S^2z_1 & 0 \ldots\ldots 0 \\ 0 & S^2z_1 \ldots\ldots 0 \\ 0 & 0 \ldots\ldots S^2z_1 \end{pmatrix} \tag{2}$$

Thus the orthogonal matrix A that diagonalizes S is the transpose of the matrix C.

$$A = C^{I} = \begin{pmatrix} a_1 \\ a_2 \\ a_p \end{pmatrix}$$

*a) Eigenvalues and Eigenvectors*

For every square matrix A, a scalar $\lambda$ and a nonzero vector x can be found such that $Ax = \lambda x$. It should be known that$\lambda$ is called an eigenvalue of A, and x is an *eigenvector* of A corresponding to $\lambda$. To find $\lambda$ and x, we write the equation as as $(A - \lambda I)x = 0$.

If $|A - \lambda I| = 0$, then $(A - \lambda I)$ has an inverse and $x = 0$ is the only solution. Hence, in order to obtain nontrivial solutions, we set $|A - \lambda I| = 0$ to find values of $\lambda$ that can be used to find corresponding values of x.

Thus, in $(A - \lambda I)x = 0$, the matrix $A - \lambda I$ must be singular in order to find a solution vector x that is not 0.

Suppose we have a $3 \times 3$ matrix A with eigenvectors $X_1, X_2, X_3$, and eigenvalues $\lambda_1, \lambda_2, \lambda_3$ so that

$$Ax_1 = \lambda_1 x_1 \qquad Ax_2 = \lambda_2 x_2 \qquad Ax_3 = \lambda_3 x_3$$

thus,

$$A \begin{bmatrix} X_1 & X_2 & X_3 \end{bmatrix} = \begin{bmatrix} X_1 & X_2 & X_3 \end{bmatrix} \begin{bmatrix} \lambda 1 & 0 & 0 \\ 0 & \lambda_2 & 0 \\ 0 & 0 & \lambda_3 \end{bmatrix} \quad (4)$$

The eigenvalues will then be used to compute the variance of the principal components and we can speak of the proportion of variance explained by the first K component.

*b) Eigenvectors of Covariance Matrix*

We will derive our first algebraic solution to PCA using linear algebra. This solution is based on an important property of eigenvector decomposition. The sample correlation between the *j*th and *k*th variables is defined as Covariances$X^TX$ itself can be recognized as proportional to the empirical sample covariance matrix of the dataset X.

## IV. Result and Discussion

*a) Results*

*Table 1:* Ordinary correlations

| | E.F | E.B | ETHNICITY | ILLITERACY | MARRIAGE-STATUS | SOCIAL-FACTOR |
|---|---|---|---|---|---|---|
| ECONOMIC_FACTOR | 1.000000 | | | | | |
| E.B | 0.853992 | 1.000000 | | | | |
| ETHNICITY | -0.260151 | -0.174099 | 1.000000 | | | |
| ILLITERACY | -0.688533 | -0.683219 | 0.134808 | 1.000000 | | |
| MARRIAGE_STATUS | 0.353807 | 0.234607 | -0.719295 | -0.217871 | 1.000000 | |
| SOCIAL_FACTOR | -0.247535 | -0.224509 | 0.137374 | 0.281727 | -0.132131 | 1.000000 |

*Table 2:* Eigenvectors

| Variable | PC 1 | PC 2 | PC 3 | PC 4 | PC 5 | PC 6 |
|---|---|---|---|---|---|---|
| E.F | 0.525305 | 0.192413 | 0.174055 | 0.349704 | 0.099909 | -0.724197 |
| E.B | 0.498518 | 0.289330 | 0.192530 | 0.413398 | -0.079352 | 0.673428 |
| ETHNICITY | -0.295534 | 0.653014 | -0.005069 | -0.029402 | 0.695535 | 0.039670 |
| ILLITERACY | -0.463585 | -0.295967 | -0.043416 | 0.825845 | 0.116089 | -0.010534 |
| M.S | 0.339941 | -0.601188 | 0.065349 | -0.117261 | 0.696298 | 0.141992 |
| S.F | 0.240255 | -0.061762 | 0.962526 | -0.100871 | -0.040569 | -0.013651 |

*Table 3:* *Sum of the Variance Proportion*

Eigenvalues: (Sum = 6, Average = 1)

| Number | Value | Difference | Proportion | Cumulative Value | Cumulative Proportion |
|---|---|---|---|---|---|
| 1 | 2.906611 | 1.472286 | 0.4844 | 2.906611 | 0.4844 |
| 2 | 1.434325 | 0.546397 | 0.2391 | 4.340936 | 0.7235 |
| 3 | 0.887928 | 0.529765 | 0.1480 | 5.228865 | 0.8715 |
| 4 | 0.358163 | 0.081266 | 0.0597 | 5.587028 | 0.9312 |
| 5 | 0.276897 | 0.140822 | 0.0461 | 5.863925 | 0.9773 |
| 6 | 0.136075 | --- | 0.0227 | 6.000000 | 1.0000 |

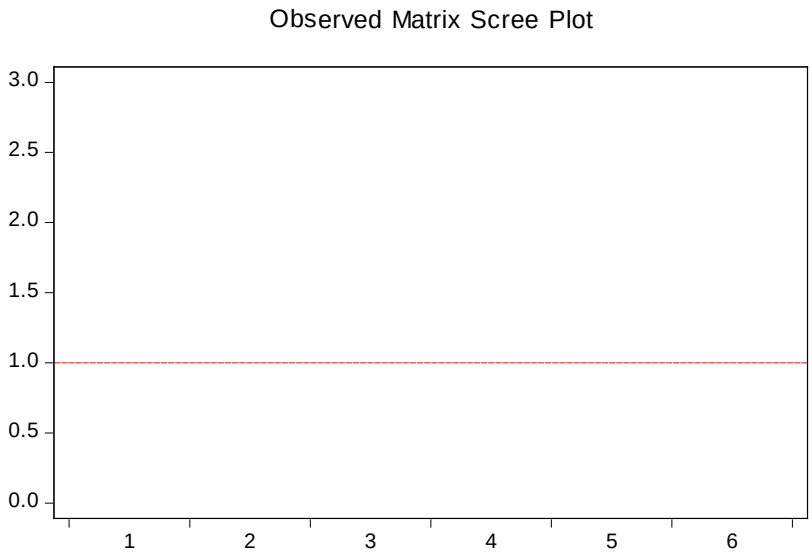


*Figure 1:* Observed matrix Scree plot

*Table 4:* Estimate of the Orthogonal

| | Unrotated Loadings F1 | F2 | Communality | Uniqueness |
|---|---|---|---|---|
| ECONOMIC_FACTOR | 0.888934 | 0.188602 | 0.825774 | 0.174226 |
| E.D | 0.840691 | 0.301768 | 0.797825 | 0.202175 |
| ETHNICITY | -0.436040 | 0.651089 | 0.614048 | 0.385952 |
| ILLITERACY | -0.711991 | -0.246761 | 0.567822 | 0.432178 |
| MARRIAGE_STATUS | 0.511711 | -0.620111 | 0.646385 | 0.353615 |
| SOCIAL_FACTOR | -0.300303 | -0.013913 | 0.090376 | 0.909624 |

| Factor | Variance | Cumulative | Difference | Proportion | Cumulative |
|---|---|---|---|---|---|
| F1 | 2.546057 | 2.546057 | 1.549884 | 0.718772 | 0.718772 |
| F2 | 0.996173 | 3.542231 | --- | 0.281228 | 1.000000 |
| Total | 3.542231 | 3.542231 | | 1.000000 | |

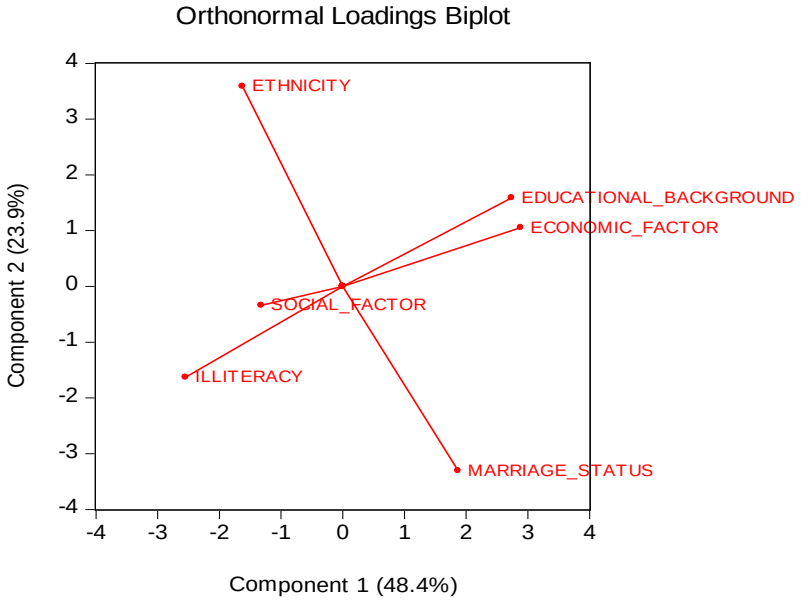


*Figure 2:* Orthogonal loadings

*b) Discussion*

In table 1, correlations among the causative factors were considered. The most prevalent dependence was exhibited between Educational background and Economic factor with a high positive correlation of 0.85 and that shows at what level the The model for the equation above are:

correlation value will be of importance. Some of the factors equally exhibited negative correlation with one another while others exhibited weak positive correlation. According to Table 2, the main component variables are defined as linear combinations of the original variables.

PC1 = 0.525305 (E.F) + 0.498518 (E.D) - 0.295534 (ethnicity) - 0.463585 (illiteracy) + 0.339941(M.S) - 0.240255 (S.F). (5)

PC2= 0.192413(E.F) + 0.289330 (E.B) + 0.653014 (ethnicity) - 0.295967(illiteracy) -0.601188 (M.S) -0.061762 (S.F). (6)

PC3 = 0.174055 (E.F) + 0.192530 (E.B) - 0.005069 (ethnicity) - 0.043416 (illiteracy) +0.065349 (M.S) +0.962526 (S.F). (7)

PC4 = 0.349704 (E.F) + 0.413398(E.B) -0.029402 (ethnicity) + 0.825845 (illiteracy) -0.117261 (M.S) -0.100871 (S.F). (8)

PC5 = 0.099909 (E.F) -0.079352 (E.B) + 0.695535(ethnicity) +0.116089 (illiteracy) + 0.696298(M.S) -0.040569 (S.F). (9)

PC6 = - 0.724197(E.F) + 0.673428 (E.B) + 0.039670 (ethnicity) -0.010534 (illetracy) + 0.141992 (M.S) - 0.013651 (S.F). (10)

In line with equations 5 to 10, the first principal component is positively correlated with three out of six of the original variables while others experienced negative correlation. Thus, the first principal component increases with the increase in economic factor, educational background and marriage status and this suggest that these three criteria vary together. If one increases, then the remaining two also increase. This component can be viewed as a measure of the quality of economic factor, educational background and marriage status. Furthermore, we see that the first principal component correlate moderately with the economic factor. In fact, we could state that based on the correlation of 0.525 that this principal component is a primary measure of the economic factor. It would follow that communities with high values would tend to have a lot of economic factor available around them in terms of economic status, type of family and this shows that economic factor will affect family planning in the community.

The second principal component also increases with ethnicity, educational background and economic factor. This component can be viewed as a measure of the degree of ethnicity in the community with a positive value of 0.65. Thus, this principal component is the primary measure of ethnicity which indicates that ethnicity also affect the attitudes to family planning in the community.

The third principal component is highly correlated with social factor with value of 0.962 follow by educational background, economic factor and marriage status. It implies that this component is the primary measure of social factor in the community. Thus, a degraded level of social factor within the community have had serious effect on their family planning attitudes.

The fourth principal component also strongly correlated with illiteracy level in the community with a value of 0.825, follow by educational background and economic factor. This component is the primary measure of illiteracy in the community. Which indicate that there is high level of illiteracy in the community which is also affecting the family planning attitudes in the area.

The fifth principal component is the primary measure of marital status with a value of 0.696. This implies that marriage status of the focus group have strongly impacted on the community's attitudes to family planning in the area.

The sixth principal component is the primary measure of educational background with value of 0.67 follow by marriage status. Which indicate that educational background contributes a lot to the attitudes of family planning in the area.

Table3 shows that Economic factor has the highest variance proportion of 0.484 follows by Educational background with a variance proportion of 0.239 and it thus appears that the poor attitudes of the community dwellers are mostly affected by the first two principal components, and together they explained 72.35% of the total variation in the acceptability of family planning attitudes under study. This leads us to the conclusion that a two factor solution will probably be sufficient with the eigenvalues of 2.906611, difference of 1.472286, variance of 0.4844 and 1.434, difference of 0.5463, variance of 0.2391. The remaining other four components jointly explained 27.65 % of the attitudes.

Figure 2 Scree plot is a principal component analysis chart to determine how many important components are present in the data, the scree graph plot the Eigen values against the component number. It can be easily observed that only the first two components crossed above the cut-off line, meaning that the remaining components account for smaller amounts of the total variance. Generally we are interested in keeping only those principal component with Eigen values greater than one. However, two factor were extracted based on significance of Kaiser Criterion (Kaiser, 1960).

*Table 4* shows a section displaying the estimates of the unrotated orthogonal loading, communalities and uniqueness estimates obtained from the estimates. we see that veliger MAP method has retained two factors, labeled F1 and F2 a brief examination of the unrotated loadings indicate that economic factor and educational background load on the first factor while ethnicity load on the second factor. To the right of the loadings are communality and uniqueness which apportion the diagonals of the correlation matrix into common (explained) and individual (unexplained) components therefore, the communalities explained what each variable can be accounted for with their weight(uniqueness). Thus, all the variables (factors) considered in this research are correlated since the ellipsoidal swarm of point is not oriented parallel to any of the axis as exhibited in figure 2.

## V. Conclusion/Recommendation

This research provides insights into the local contexts related to family planning knowledge, attitudes, perceptions, and practices and also highlights the need for contraceptives, both modern and traditional methods. In the wake of changing attitudes towards family planning and desired family size among rural dwellers, more women and couples will be seeking family planning services. Addressing obstacles such as access, affordability, and availability will help meet these needs and ensure that women and couples can meet their childbearing and reproductive health goals. In addition, a very low perceived need for contraception was found amongst the respondents wishing to bear more children. The result shows that the most important factors that affect people awareness are economic factor and educational background and they jointly have the highest variance proportion and the highest Eigenvalue which is greater than 1.

In view of the above, the following recommendations were however made:

1. That government should provide social economic factors that would increase the knowledge of education among the people which will improve their level of understanding of family planning.
2. In Nigeria, the bulk of the population lives in the rural areas. The government should therefore emphasize modern methods of family planning to complement the traditional methods, provide adequate fund for family planning officials to enable them procure the necessary devices and reach the rural dwellers.
3. Due to the fact that economic factors in the country is very poor, it is advisable for the people to reduce their child bearing in order to reduce the population of the country so that the provision of government amenities will be sufficient enough for community.
4. The study also recommendstrong need for involving men in healthcare programs designed to improve women's and newborns' health as they mostly influence decision-making at the household level and this will also result in active male participation and community ownership. Young, especially first time, fathers need support and empowerment. Encouraging communication between wife and husband about family planning and birth spacing should also be part of such campaigns to promote mutual decision-making between wife and husband and make husbands responsible partners in family planning/birth spacing decisions and ease the burden of decision-making on women.

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# Global Journals Inc. (US) Guidelines Handbook 2017

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# FELLOWS

## FELLOW OF ASSOCIATION OF RESEARCH SOCIETY IN SCIENCE (FARSS)

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*The following benefits can be availed by you only for next three years from the date of certification:*

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We shall provide you intimation regarding launching of e-version of journal of your stream time to time.This may be utilized in your library for the enrichment of knowledge of your students as well as it can also be helpful for the concerned faculty members.

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We shall provide you intimation regarding launching of e-version of journal of your stream time to time.This may be utilized in your library for the enrichment of knowledge of your students as well as it can also be helpful for the concerned faculty members.

The MARSS member can apply for approval, grading and certification of standards of their educational and Institutional Degrees to Open Association of Research, Society U.S.A.


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The IBOARS can initially review research papers of their institute and recommend them to publish with respective journal of Global Journals. It can also review the papers of other institutions after obtaining our consent. The second review will be done by peer reviewer of Global Journals Incorporation (USA) The Board is at liberty to appoint a peer reviewer with the approval of chairperson after consulting us.

The author fees of such paper may be waived off up to 40%.

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The IBOARS can organize symposium/seminar/conference in their country on behalf of Global Journals Incorporation (USA)-OARS (USA). The terms and conditions can be discussed separately.

The Board can also play vital role by exploring and giving valuable suggestions regarding the Standards of "Open Association of Research Society, U.S.A (OARS)" so that proper amendment can take place for the benefit of entire research community. We shall provide details of particular standard only on receipt of request from the Board.


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We shall provide you intimation regarding launching of e-version of journal of your stream time to time. This may be utilized in your library for the enrichment of knowledge of your students as well as it can also be helpful for the concerned faculty members.


After nomination of your institution as "Institutional Fellow" and constantly functioning successfully for one year, we can consider giving recognition to your institute to function as Regional/Zonal office on our behalf.
The board can also take up the additional allied activities for betterment after our consultation.

**The following entitlements are applicable to individual Fellows:**


Open Association of Research Society, U.S.A (OARS) By-laws states that an individual Fellow may use the designations as applicable, or the corresponding initials. The Credentials of individual Fellow and Associate designations signify that the individual has gained knowledge of the fundamental concepts. One is magnanimous and proficient in an expertise course covering the professional code of conduct, and follows recognized standards of practice.

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Disbursement of 40% Royalty earned through Global Journals : Researcher = 50%, Peer Reviewer = 37.50%, Institution = 12.50% E.g. Out of 40%, the 20% benefit should be passed on to researcher, 15 % benefit towards remuneration should be given to a reviewer and remaining 5% is to be retained by the institution.

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- Fellow can also join as paid peer reviewer and earn 15% remuneration of author charges and can also get an opportunity to join as member of the Editorial Board of Global Journals Incorporation (USA)
- • This individual has learned the basic methods of applying those concepts and techniques to common challenging situations. This individual has further demonstrated an in–depth understanding of the application of suitable techniques to a particular area of research practice.

**Note :**

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- In future, if the board feels the necessity to change any board member, the same can be done with the consent of the chairperson along with anyone board member without our approval.
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- In case of "Difference of Opinion [if any]" among the Board members, our decision will be final and binding to everyone.

"

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- Author Name in Font Size of 11 with one column as of Title.
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- First Character must be three lines Drop capped.
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**You can use your own standard format also.**
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1. General,

2. Ethical Guidelines,

3. Submission of Manuscripts,

4. Manuscript's Category,

5. Structure and Format of Manuscript,

6. After Acceptance.

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Manuscript submission is a systematic procedure and little preparation is required beyond having all parts of your manuscript in a given format and a computer with an Internet connection and a Web browser. Full help and instructions are provided on-screen. As an author, you will be prompted for login and manuscript details as Field of Paper and then to upload your manuscript file(s) according to the instructions.

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Complete support for both authors and co-author is provided.

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Based on potential and nature, the manuscript can be categorized under the following heads:

Original research paper: Such papers are reports of high-level significant original research work.

Review papers: These are concise, significant but helpful and decisive topics for young researchers.

Research articles: These are handled with small investigation and applications

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The recommended size of original research paper is less than seven thousand words, review papers fewer than seven thousands words also.Preparation of research paper or how to write research paper, are major hurdle, while writing manuscript. The research articles and research letters should be fewer than three thousand words, the structure original research paper; sometime review paper should be as follows:

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(b) A brief Summary, "Abstract" (less than 150 words) containing the major results and conclusions.

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(d) An Introduction, giving necessary background excluding subheadings; objectives must be clearly declared.

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(g) Discussion should cover the implications and consequences, not just recapitulating the results; conclusions should be summarizing.

(h) Brief Acknowledgements.

(i) References in the proper form.

Authors should very cautiously consider the preparation of papers to ensure that they communicate efficiently. Papers are much more likely to be accepted, if they are cautiously designed and laid out, contain few or no errors, are summarizing, and be conventional to the approach and instructions. They will in addition, be published with much less delays than those that require much technical and editorial correction.

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*Language: The language of publication is UK English. Authors, for whom English is a second language, must have their manuscript efficiently edited by an English-speaking person before submission to make sure that, the English is of high excellence. It is preferable, that manuscripts should be professionally edited.*

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Metric SI units are supposed to generally be used excluding where they conflict with current practice or are confusing. For illustration, 1.4 l rather than 1.4 × 10-3 m3, or 4 mm somewhat than 4 × 10-3 m. Chemical formula and solutions must identify the form used, e.g. anhydrous or hydrated, and the concentration must be in clearly defined units. Common species names should be followed by underlines at the first mention. For following use the generic name should be constricted to a single letter, if it is clear.

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Many researchers searching for information online will use search engines such as Google, Yahoo or similar. By optimizing your paper for search engines, you will amplify the chance of someone finding it. This in turn will make it more likely to be viewed and/or cited in a further work. Global Journals Inc. (US) have compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

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- It may take the discovery of only one relevant paper to let steer in the right keyword direction because in most databases, the keywords under which a research paper is abstracted are listed with the paper.
- One should avoid outdated words.

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Even though low quality images are sufficient for review purposes, print publication requires high quality images to prevent the final product being blurred or fuzzy. Submit (or e-mail) EPS (line art) or TIFF (halftone/photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Do not use pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings) in relation to the imitation size. 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).

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**6. AFTER ACCEPTANCE**

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**6.2 Early View of Global Journals Inc. (US) (Publication Prior to Print)**

The Global Journals Inc. (US) are enclosed by our publishing's Early View service. Early View articles are complete full-text articles sent in advance of their publication. Early View articles are absolute and final. They have been completely reviewed, revised and edited for publication, and the authors' final corrections have been incorporated. Because they are in final form, no changes can be made after sending them. The nature of Early View articles means that they do not yet have volume, issue or page numbers, so Early View articles cannot be cited in the conventional way.

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Online production tracking is available for your article through Author Services. Author Services enables authors to track their article - once it has been accepted - through the production process to publication online and in print. Authors can check the status of their articles online and choose to receive automated e-mails at key stages of production. The authors will receive an e-mail with a unique link that enables them to register and have their article automatically added to the system. Please ensure that a complete e-mail address is provided when submitting the manuscript.

**6.4 Author Material Archive Policy**

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Before start writing a good quality Computer Science Research Paper, let us first understand what is Computer Science Research Paper? So, Computer Science Research Paper is the paper which is written by professionals or scientists who are associated to Computer Science and Information Technology, or doing research study in these areas. If you are novel to this field then you can consult about this field from your supervisor or guide.

## TECHNIQUES FOR WRITING A GOOD QUALITY RESEARCH PAPER:

**1. Choosing the topic:** In most cases, the topic is searched by the interest of author but it can be also suggested by the guides. You can have several topics and then you can judge that in which topic or subject you are finding yourself most comfortable. This can be done by asking several questions to yourself, like Will I be able to carry our search in this area? Will I find all necessary recourses to accomplish the search? Will I be able to find all information in this field area? If the answer of these types of questions will be "Yes" then you can choose that topic. In most of the cases, you may have to conduct the surveys and have to visit several places because this field is related to Computer Science and Information Technology. Also, you may have to do a lot of work to find all rise and falls regarding the various data of that subject. Sometimes, detailed information plays a vital role, instead of short information.

**2. Evaluators are human:** First thing to remember that evaluators are also human being. They are not only meant for rejecting a paper. They are here to evaluate your paper. So, present your Best.

**3. Think Like Evaluators:** If you are in a confusion or getting demotivated that your paper will be accepted by evaluators or not, then think and try to evaluate your paper like an Evaluator. Try to understand that what an evaluator wants in your research paper and automatically you will have your answer.

**4. 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.

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

**6. Use of computer is recommended:** As you are doing research in the field of Computer Science, then this point is quite obvious.

**7. Use right software:** Always use good quality software packages. If you are not capable to judge good software then you can lose quality of your paper unknowingly. There are various software programs available to help you, which you can get through Internet.

**8. Use the Internet for help:** An excellent start for your paper can be by using the Google. It is an excellent search engine, where you can have your doubts resolved. You may also read some answers for the frequent question how to write my research paper or find model research paper. From the internet library you can download books. If you have all required books make important reading selecting and analyzing the specified information. Then put together research paper sketch out.

**9. Use and get big pictures:** Always use encyclopedias, Wikipedia to get pictures so that you can go into the depth.

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

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

**12. Make all efforts:** Make all efforts to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in introduction, that what is the need of a particular research paper. Polish your work by good skill of writing and always give an evaluator, what he wants.

**13. Have backups:** When you are going to do any important thing like making research paper, you should always have backup copies of it either in your computer or in paper. This will help you to not to lose any of your important.

**14. Produce good diagrams of your own:** Always try to include good charts or diagrams in your paper to improve quality. Using several and unnecessary diagrams will degrade the quality of your paper by creating "hotchpotch." So always, try to make and include those diagrams, which are made by your own to improve readability and understandability of your paper.

**15. Use of direct quotes:** When you do research relevant to literature, history or current affairs then use of quotes become essential but if study is relevant to science then use of quotes is not preferable.

**16. Use proper verb tense:** Use proper verb tenses in your paper. Use past tense, to present those events that happened. Use present tense to indicate events that are going on. Use future tense to indicate future happening events. Use of improper and wrong tenses will confuse the evaluator. Avoid the sentences that are incomplete.

**17. Never use online paper:** If you are getting any paper on Internet, then never use it as your research paper because it might be possible that evaluator has already seen it or maybe it is outdated version.

**18. Pick a good study spot:** To do your research studies always try to pick a spot, which is quiet. Every spot is not for studies. Spot that suits you choose it and proceed further.

**19. Know what you know:** Always try to know, what you know by making objectives. Else, you will be confused and cannot achieve your target.

**20. Use good quality grammar:** Always use a good quality grammar and use words that will throw positive impact on evaluator. Use of good quality grammar does not mean to use tough words, that for each word the evaluator has to go through dictionary. Do not start sentence with a conjunction. Do not fragment sentences. Eliminate one-word sentences. Ignore passive voice. Do not ever use a big word when a diminutive one would suffice. Verbs have to be in agreement with their subjects. Prepositions are not expressions to finish sentences with. It is incorrect to ever divide an infinitive. Avoid clichés like the disease. Also, always shun irritating alliteration. Use language that is simple and straight forward. put together a neat summary.

**21. 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 to your topic. You may also maintain your arguments with records.

**22. Never start in last minute:** Always start at right time and give enough time to research work. Leaving everything to the last minute will degrade your paper and spoil your work.

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

**24. Never copy others' work:** Never copy others' work and give it your name because if evaluator has seen it anywhere you will be in trouble.

**25. Take proper rest and food:** No matter how many hours you spend for your research activity, if you are not taking care of your health then all your efforts will be in vain. For a quality research, study is must, and this can be done by taking proper rest and food.

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

**27. Refresh your mind after intervals:** Try to give rest to your mind by listening to soft music or by sleeping in intervals. This will also improve your memory.

**28. Make colleagues:** Always try to make colleagues. No matter how sharper or intelligent you are, if you make colleagues you can have several ideas, which will be helpful for your research.

**29. Think technically:** Always think technically. If anything happens, then search its reasons, its benefits, and demerits.

**30. Think and then print:** When you will go to print your paper, notice that tables are not be split, headings are not detached from their descriptions, and page sequence is maintained.

**31. Adding unnecessary information:** Do not add unnecessary information, like, I have used MS Excel to draw graph. Do not add irrelevant and inappropriate material. These all will create superfluous. Foreign terminology and phrases are not apropos. One should NEVER take a broad view. Analogy in script is like feathers on a snake. Not at all use a large word when a very small one would be sufficient. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Amplification is a billion times of inferior quality than sarcasm.

**32. Never oversimplify everything:** To add material in your research paper, never go for oversimplification. This will definitely irritate the evaluator. Be more or less specific. Also too, by no means, ever use rhythmic redundancies. Contractions aren't essential and shouldn't be there used. Comparisons are as terrible as clichés. Give up ampersands and abbreviations, and so on. Remove commas, that are, not necessary. Parenthetical words however should be together with this in commas. Understatement is all the time the complete best way to put onward earth-shaking thoughts. Give a detailed literary review.

**33. Report concluded results:** Use concluded results. From raw data, filter the results and then conclude your studies based on measurements and observations taken. Significant figures and appropriate number of decimal places should be used. Parenthetical remarks are prohibitive. Proofread carefully at final stage. In the end give outline to your arguments. Spot out perspectives of further study of this subject. Justify your conclusion by at the bottom of them with sufficient justifications and examples.

**34. After 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 to 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 in 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 criterion for grading the final paper by peer-reviewers.

**Final Points:**

A purpose of organizing a research paper is to let people to interpret your effort selectively. The journal requires the following sections, submitted in the order listed, each section to start on a new page.

The introduction will be compiled from reference matter and will reflect the design processes or outline of basis that direct you to make study. As you will carry out the process of study, the method and process section will be constructed as like that. The result segment will show related statistics in nearly sequential order and will direct the reviewers next to the similar intellectual paths throughout the data that you took to carry out your study. The discussion section will provide understanding of the data and projections as to the implication of the results. The use of good quality references all through the paper will give the effort trustworthiness by representing an alertness of 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 the 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 evade

- Insertion a title at the foot of a page with the subsequent text on the next page
- Separating a table/chart or figure - impound each figure/table to a single page
- Submitting a manuscript with pages out of sequence

In every sections of your document

· Use standard writing style including articles ("a", "the," etc.)

· Keep on paying attention on the research topic of the paper

· Use paragraphs to split each significant point (excluding for the abstract)

· Align the primary line of each section

· Present your points in sound order

· Use present tense to report well accepted

· Use past tense to describe specific results

· Shun familiar wording, don't address the reviewer directly, and don't use slang, slang language, or superlatives

· Shun use of extra pictures - include only those figures essential to presenting results

**Title Page:**

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

**Abstract:**

The summary should be two hundred words or less. It should briefly and clearly explain the key findings reported in the manuscript--must have precise statistics. It should not have abnormal acronyms or abbreviations. It should be logical in itself. Shun citing 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 approach 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. Yet, use comprehensive sentences and do not let go readability for briefness. You can maintain it succinct by phrasing sentences so that they provide more than 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 maintain the initial two items to no more than one ruling each.

- Reason of the study - 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 quantitative data; results of any numerical analysis should be reported
- Significant conclusions or questions that track from the research(es)

Approach:

- Single section, and succinct
- As a outline of job done, it is always written in past tense
- A conceptual should situate on its own, and not submit to any other part of the paper such as a form or table
- Center on shortening results - bound background information to a verdict or two, if completely necessary
- What you account in an conceptual must be regular with what you reported in the manuscript
- Exact spelling, clearness 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 to comprehend and calculate the purpose of your study without having to submit to other works. The basis for the study should be offered. Give most important references but shun difficult to make a comprehensive appraisal of the topic. In the introduction, describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will have no attention in your result. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here. Following approach can create a valuable beginning:

- Explain the value (significance) of the study
- Shield the model - why did you employ this particular system or method? What is its compensation? You strength remark on its appropriateness from a abstract point of vision as well as point out sensible reasons for using it.
- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
- Very for a short time explain the tentative propose and how it skilled 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 with every section. If you make the four points listed above, you will need a least of four paragraphs.

- Present surroundings information only as desirable in order hold up a situation. The reviewer does not desire to read the whole thing you know about a topic.
- Shape the theory/purpose 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 sound written Procedures segment allows a capable scientist to replacement 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 for the least amount of information that would permit another capable scientist to spare 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 object, mention the specific item describing a way but draw the basic principle while stating the situation. The purpose is to text 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:

- Explain materials individually only if the study is so complex that it saves liberty this way.
- Embrace particular materials, and any tools or provisions that are not frequently found in laboratories.
- Do not take in frequently found.
- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

Methods:

- Report the method (not 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 - details how procedures were completed not how they were exclusively performed on a particular day.
- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

Approach:

- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
- Use standard style in this and in 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 a 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. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
- In manuscript, explain each of your consequences, point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise 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 in manuscript form.

What to stay away from

- Do not discuss or infer your outcome, report surroundings information, or try to explain anything.
- Not at all, take in raw data or intermediate calculations in a research manuscript.
- Do not present the similar data more than once.
- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

- As forever, use past tense when you submit to 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 part.

Figures and tables

- If you put figures and tables at the end of the details, make certain that they are visibly distinguished from any attach appendix materials, such as raw facts
- Despite of position, each figure must be numbered one after the other and complete with subtitle
- In spite of position, each table must be titled, numbered one after the other and complete with heading
- All figure and table must be adequately complete that it could situate on its own, divide from text

**Discussion:**

The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a 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 implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly 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 with prospect, and let it drop at that.

- Make a decision if each premise is supported, 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 the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One 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 available information
- Submit to work done by specific persons (including you) in past tense.
- Submit to generally acknowledged facts and main beliefs in present tense.

Please carefully note down following rules and regulation before submitting your Research Paper to Global Journals Inc. (US):

**Segment Draft and Final Research Paper:** You have to strictly follow the template of research paper. If it is not done your paper may get rejected.

- The **major constraint** is that you must independently make all content, tables, graphs, and facts that are offered in the paper. You must write each part of the paper wholly on your own. The Peer-reviewers need to identify your own perceptive of the concepts in your own terms. NEVER extract straight from any foundation, and never rephrase someone else's analysis.

- Do not give permission to anyone else to "PROOFREAD" your manuscript.

- Methods to avoid Plagiarism is applied by us on every paper, if found guilty, you will be blacklisted by all of our collaborated research groups, your institution will be informed for this and strict legal actions will be taken immediately.)
- To guard yourself and others from possible illegal use please do not permit anyone right to use to your paper and files.

## Criterion for Grading a Research Paper (Compilation)
## by Global Journals Inc. (US)

**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 Inc. (US).**

| 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<br><br>Above 200 words | No specific data with ambiguous information<br><br>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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