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Discovering Thoughts, Inventing Future

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Contents of the Issue

i. Copyright Notice
ii. Editorial Board Members
iii. Chief Author and Dean
iv. Contents of the Issue

1. Male Meiotic and Taxonomical Studies in *Withania Somnifera* (L.) Dunal. *1-5*
2. Engineering Microfluids, Biosensors and Chip Analysis. *7-18*
3. Floral Biology, Field Controlled Pollination and Hybrid Obtention of *Dioscorea Dumetorum(*Kunth) Pax) in Cameroon. *19-34*

v. Fellows
vi. Auxiliary Memberships
vii. Preferred Author Guidelines
viii. Index

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Male Meiotic and Taxonomical Studies in *Withania Somnifera* (L.) Dunal

By Vishnu Prasad Bhala & Rakesh Chandra Verma

Vikram University

Abstract- Detailed male meiosis has been made in one species of genus *Withania* collected from Ujjain region of Madhya Pradesh, India. The study revealed $2n=48$ for the species. In addition to this, some cells showed the occurrence of various meiotic irregularities for example, lagging chromosomes (1.76%), chromatin bridges (1.76%) and cytomixis (0.87%). The fertility of pollen grains was also determined which was observed 97.52%. The species are widely used for various medicinal purposes by local/tribal people of the state.

Keywords: male meiosis, meiotic abnormalities, pollen sterility, solanaceae.

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Male Meiotic and Taxonomical Studies in *Withania Somnifera* (L.) Dunal

Vishnu Prasad Bhala [α] & Rakesh Chandra Verma [σ]

Abstract- Detailed male meiosis has been made in one species of genus *Withania* collected from Ujjain region of Madhya Pradesh, India. The study revealed $2n=48$ for the species. In addition to this, some cells showed the occurrence of various meiotic irregularities for example, lagging chromosomes (1.76%), chromatin bridges (1.76%) and cytomixis (0.87%). The fertility of pollen grains was also determined which was observed 97.52%. The species are widely used for various medicinal purposes by local/tribal people of the state.

Keywords: male meiosis, meiotic abnormalities, pollen sterility, solanaceae.

I. Introduction

The genus *Withania* belongs to the family Solanaceae. The family comprises of 100 genera and about 2500 species (Hunziker 2001, Olmstead *et al.* 2008). The genus is distributed throughout the tropical and sub-tropical regions of the world with 26 species (Ahmad 2014). *W. somnifera* is well distributed in India with and is growing well in dry parts of tropical and subtropical regions extending to the elevation of 1500 m. The plant possesses antitumor, antimicrobial and anti-inflammatory properties. Since the plant is toxic in nature so it should be used with caution (Purohit and Yyas 2004). Flowers of its species are used to treat nervous exhaustion, insomnia and impotence. The meiotic course of the species reveals the presence of $2n=48$ from West Pakistan (Baquar 1967) as well as outside India. *W. somnifera* commonly known as "Ashwangandha" or "Indian ginseng" is extensively studied from India by Bir *et al*. (1978) Bir and Sidhu (1979 and 1980) from Punjab plains, Koul *et al.* (1976) from Jammu and Kashmir, Madhavadian (1968) from Tamil Nadu, Bhaduri (1933), Datta *et al.* (2005), Iqbal and Datta (2007) from West Bengal. It is also extensively worked out from Pakistan by Baquar (1967) and Khatton and Ali (1982) and from Saudi Arabia by Al-Turki *et al*. (2000). Earlier meiotic studies reveals the presence of intraspecific diploid cytotype ($2n=24$), tetraploid cytotype ($2n=48$) and hexaploid cytotypes ($2n=72$). The karyotype analysis of the species shows seven groups of the chromosomes with occurrence of metacentric and sub-metacentric types (Samaddar *et al.* 2012). The species also shows polysomatomy ($2n=12$, 18, 24, 36, 48, 72) with predominance of $2n=48$. Present research work is undertaken by keeping in view the existence of cytological diversity.

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II. Materials and Methods

a) Collection of plants materials

Species was collected from Ujjain city especially in the premises of Vikram University Ujjain. The photographs of the collected plants species were preferably taken from their natural habitats. The photography was done during the complete flowering seasons. For identification of plants species, different floras were consulted during the present study such as Flora of Madhya Pradesh Western Part by Singh (2012), various internet sources. The plant is an important medicinal plant of the Indian subcontinent. In additions to this, the plant specimens were taken to some taxonomists for further documentation. The identified plants specimens have been reposted in School of Studies in Botany, Vikram University, Ujjain (India).

b) Fixation and preservation of plant materials

Floral buds were fixed in freshly prepared Carnoy's fixative (6 parts of absolute alcohol: 3 parts of chloroform: 1 part of glacial acetic acid) for 24-48 hours. Afterwards, these were transferred to 70% ethyl alcohol and stored in refrigerator at 4°C until use. For chromosomal preparations, anthers were crushed and tapped to prepare a smear of pollen mother cells (PMCs) in 1% acetocarmine (Belling 1921). A number of PMCs were observed and chromosome counts were confirmed. In case of species with meiotic abnormalities, large numbers of PMCs are observed to confirm frequency of various abnormalities. Pollen fertility was observed by mounting the pollen grains in 50% glycerol-aceto carmine (1:1) solution (Marks 1954). Pollen grains with stained nuclei were taken as fertile and viable, whereas, unstained pollen grains marked as sterile ones.

c) Photomicrography

Photomicrographs were taken from freshly prepared temporary slides. The photographs were taken with the aid of the microscope Camera (Digital Eyepiece) on a field of 40% objective lens and 10X eyepiece of Olympus microscope.

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III. Results

a) Taxonomical and morphological studies

Kingdom: Plantae (Plants);
Sub-kingdom: Tracheobionta (Vascular plants);
Super division: Spermatophyta (Seeds plants);
Division: Angiosperma
Class: Dicotyledons
Order: Tubiflorae
Family: Solanaceae
Genus: *Withania*
Species: *Somnifera Dunal*

The plant is usually erect, green, branched/unbranched herb, up to 1.25 m in height. The aerial part, especially the stem, leaves and calyx are sparsely covered with fine hairy tomentum. Branches are round; leaves simple, petiolated, ovate, entire, shiny, smooth and opposite; flowers inconspicuous, greenish or yellow, in axillary umbellate cymes, bisexual; fruit is berry in a persistent calyx; seeds are small, flat, yellow, reniform, very light. In this species, the period of flowering and fruiting occurred during the months from August to October. The plant has tap root system having 15-25 cm in length and light yellow in colour. The branches are erect and are of about 60-120 cm in length. It requires dry weather conditions for development of better root quality and alkaloid content.

Male meiotic studies In this plant species, meiotic characterization of anthers revealed regular 24 bivalents ($2n = 48$) in cells at diakinesis as well as metaphase I. At anaphase I, the chromosomes successively demonstrated normal segregation of 24:24 chromosomes towards opposite poles (Figs. A-I). In addition to this, some cells showed the occurrence of various meiotic irregularities (Figs. A-I; Table-1-3), for example, lagging chromosomes (1.76%), chromatin bridges (1.76%) and cytomixis (0.87%). The incidence of chromosome association in the form of ring and rod bivalents per PMC ranged from 13 to 22 (mean = 17.2) and 2 to 11 (mean = 6.8), respectively. The frequency of chiasma per cells, on an average, was observed 45.8 (SD = ±3.25) in which 41.20 chiasma (SD = ±3.12) were found terminalized and 4.60 (SD = ±0.80) were identified as untereminalized. As a result, the terminalization coefficient was calculated as 0.90 (Table-8). The fertility of pollen grains was also determined which was observed 97.52%.

IV. Discussion and Conclusions

Meiosis is most sensitive stage in the life cycle for all sexual species and has direct relevance to natural selection; it leads to the formation of gametes, contributes to genome stability and generates genetic diversity. The process of meiosis depends upon interrelated events of homologous chromosome recognition, intimate association, synapsis and recombination (Hamant *et al.* 2006, de Muyt *et al.* 2009). In plants, it is affected by various genetic and environmental factors (Ahmad *et al.* 1984, Viccini and Carvalho, 2002, Sun *et al*. 2004, Bajpai and Singh 2006, Rezaei *et al*. 2010). There are various meiotic abnormalities which hinder the path of normal meiosis and are the cause of changes in the morphology and genetic constitutions of the plant. The evolution of vascular plants is dependent upon the variation in chromosome numbers which may be caused due to genomic mutations especially polyploidy (auto or allopolyploidy) (Soltis *et al.* 2009, Bedini *et al.* 2012). There are number of research papers on the phenomena of polyploidy, emphasizing its origin, impact and role in speciation (Stebbins 1985, Ramsey and Schemske 1998, Otto and Whitton 2000, Cifuentes *et al.* 2010, Jiao *et al.* 2011). The autotetraploids are generally characterized by the presence of quadrivalents due to homology of 4 sets of chromosomes, whereas, in allopolyploids there is normal pairing because of existence of two separate sets of chromosomes. On the other hand in segmental allotetraploids due to the partial homology of two genomes there is low frequency of quadrivalent formation. In the present study *W. somnifera* shows normal bivalent formation in all the PMCs, without any quadrivalent formation which indicates its allotetraploid behavior. However, the absence of quadrivalents does not confirm that it is an allotetraploid because there are many artificially produced autotetraploids where there is only bivalent formation because the formation of quadrivalents depends upon many other factors such as localization of chiasmata, small size of chromosomes, and presence of some suppressor genes etc., which does not allow the pairing between the homologous chromosomes (Morrison and Rajhathy 1960, Gottschalk 1978). On the other hand in *W. somnifera* the meiosis is abnormal with the presence of spindle abnormalities which indicates the absence of multivalents and also indicates that it might be hybrid or more probably due the presence of specific genes which interfere in the pairing and functioning of spindle (Baum *et al.*1992, Risso-Pascotto *et al.* 2003; Kumar and Singhal 2008; Singhal and Kaur 2009). The basic function of the spindle is to attach at kinetochore and separate the chromosome or chromatids at anaphases (Wadsworth *et al*. 2011), these attach to the centromeres (Qu and Vorsa 1999) and rearrange the chromosomes on the equatorial plate and bring them together at metaphase-I (Qu and Vorsa 1999). But, if due to some factors (genetic or environmental) the spindle activity fails then chromosomes are unable to line up in the equator and then separate at Anaphases of the meiosis, which leads to abnormal meiotic course. Earlier, a number of plants have been reported with abnormalities like irregular spindle activity, cytomixis and chromatin stickiness

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leading to abnormal microsporogenesis (Baum *et al*. 1992, Caetano-Pereira and Pagliarinini 2001, Kumar and Singhal 2008, Rai and Kumar 2010, Singhal and Kaur 2009). Abnormalities like lagging chromosomes, chromatin bridges and cytomixis which ultimately lead to abnormal microsporogenesis with the production of dyads, triads, polyads, tetrads with micronuclei, and sterile and fertile pollen grains.

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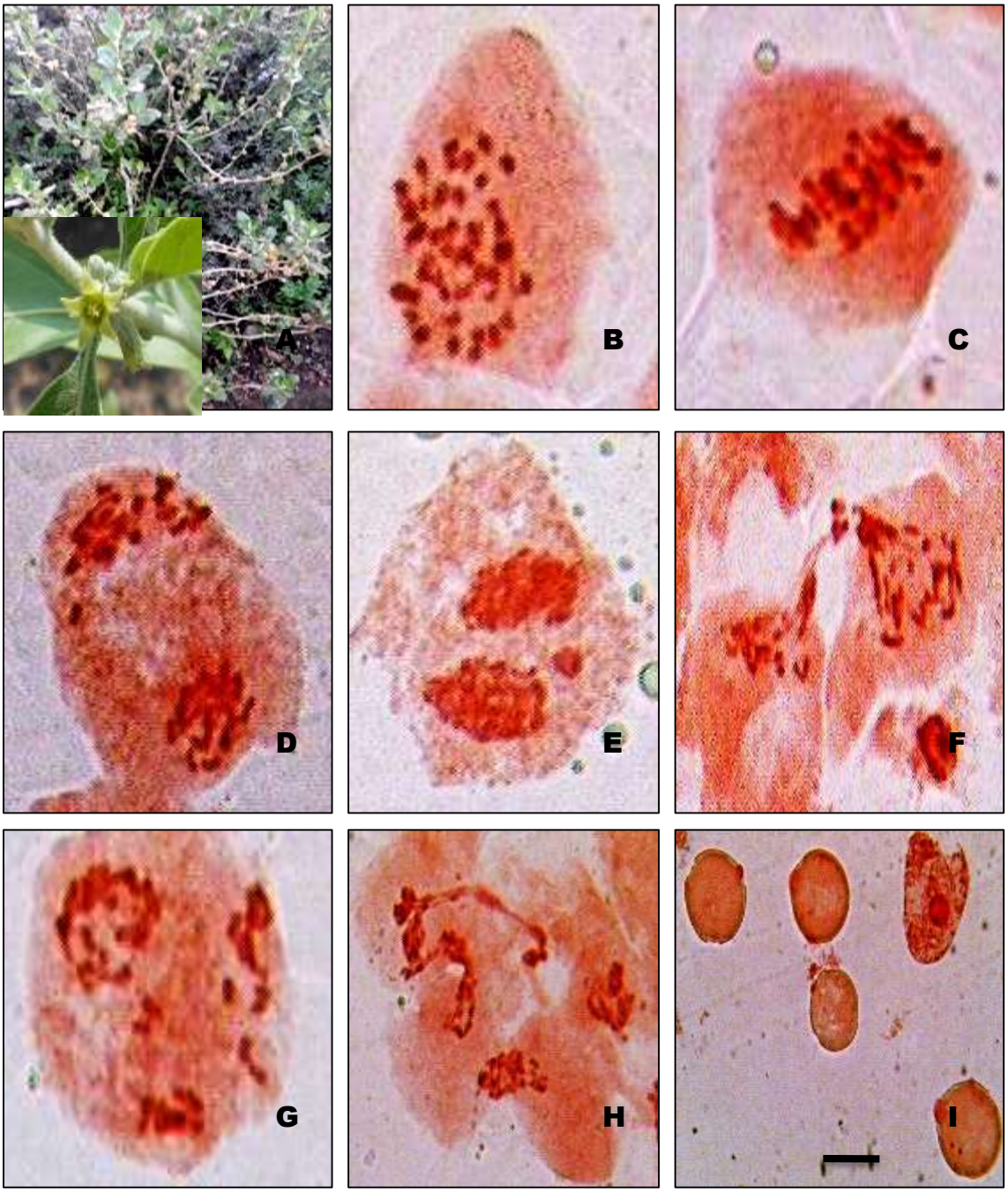

Figure (A-I): Microsporocytes showing different meiotic stages in *Withania somnifera* Dunal. A) Morphology of *Withania somnifera* Dunal. B) Diakinesis showing 24 bivalents (2*n* = 48) C) Metaphase I D) Anaphase I showing 24:24 chromosome segregation E) Laggard at telophase I F) Cytomixis G) Tripolar movement with laggards H) Bridge at telophase II I) Pollens (Scale bar = 10μm).

Table 1: Place of collection, meiotic chromosome count, ploidy level and pollen fertility in *Withania somnifera* L.

Name of taxon	Place of collection	Meiotic chromosome Number (*n*)	Ploidy level	Pollen fertility
Withania somnifera L.	VU Campus, Ujjain	*n* = 24	4*x*	97.52

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Table 2: Chromosome association (Mean and range) and distribution of chromosomes at anaphase I in *Withania somnifera* L.

Species	No. of Cells analyzed	Ring bivalents		Rod bivalents		Anaphase separation
		Mean	Range	Mean	Range	
Withania somnifera	25	17.2	13-22	6.8	2-11	24:24

Table 3: Average chiasma frequency per PMC, terminalized, untereminalized and terminalization coefficient of *Withania somnifera* L.

Taxon	No. of cells analyzed	Chiasma/cell Mean ± SD	Terminalized	Untereminalized	Terminalization Coefficient
Withania somnifera	25	45.8 ± 3.25	41.20 ± 3.12	4.60 ± 0.80	0.90

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Engineering Microfluids, Biosensors and Chip Analysis

By Lekshmi Gangadhar

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Abstract- Biosensors is a type of devices that detect the biological signal and transfer to a measurable electrical signal. It encompasses the mixture of biological entities including deoxyribonucleic acid, ribonucleic acid, protein and enzyme based sensor to the electrochemical transducers to identify and perceive few biological analyte including Ab-Ag interaction. Some of the types includes, DNA and protein based biosensor materials have been pondered herein to highlight their obligatory uses in innumerable areas. The discovery of new diagnostic methods brings further attention to the implementation of point-of - care. This poses a big challenge for us to establish a novel material in electroanalytical approaches which can be precisely sensed animal studies. With the development of NT, biosensors depends on NMs are demonstrated enormous possibilities of more effectively diagnosing and detecting disease based biological markers. Further, Micro-Electro-Mechanical Systems (MEMS), high performance liquid chromatography (HPLC) and chip based analysis for DNA and protein are discussed in this article.

Keywords: *biosensors, DNA-protein, chip analysis, HPLC.*

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Engineering Microfluids, Biosensors and Chip Analysis

Lekshmi Gangadhar

Abstract- Biosensors is a type of devices that detect the biological signal and transfer to a measurable electrical signal. It encompasses the mixture of biological entities including deoxyribonucleic acid, ribonucleic acid, protein and enzyme based sensor to the electrochemical transducers to identify and perceive few biological analyte including Ab-Ag interaction. Some of the types includes, DNA and protein based biosensor materials have been pondered herein to highlight their obligatory uses in innumerable areas. The discovery of new diagnostic methods brings further attention to the implementation of point-of - care. This poses a big challenge for us to establish a novel material in electroanalytical approaches which can be precisely sensed animal studies. With the development of NT, biosensors depends on NMs are demonstrated enormous possibilities of more effectively diagnosing and detecting disease based biological markers. Further, Micro-Electro-Mechanical Systems (MEMS), high performance liquid chromatography (HPLC) and chip based analysis for DNA and protein are discussed in this article.

Keywords: biosensors, DNA-protein, chip analysis, HPLC.

I. Introduction

Biochemical and biological methods plays a crucial role in medicine, genetics and biotechnology have a very important role. Nevertheless, translating biochemical data directly into electrical signal is very complicated, the biosensors (BS) can transform these signals and the biosensors over this effort. Nowadays, the use of these goods has increased dramatically thanks to improved techniques and tools. It is a technique which assimilates an organic detection component to transducer. Scientist transferred glucose oxidase to semipermeable membrane of an amperometric O_2 electrode surface, mentioned the first biosensor in 1962, to measure glucose level immediately in a sample [1, 2]. It explains "make electrochemical sensors (pH, potentiometric, polarographic or conductometric) smarter" via introducing "enzyme transducers as sandwiches embedded in the membrane".

It's an instrument comprises of mainly two features:

- A biological receptor is a restrained biotic direct effect, for instance, enzyme, deoxyribonucleic acid sample and Ab that identifies the analytes like, enzyme substrate, complementary deoxyribonucleic acid and Ag. While Abs and oligonucleotides are broadly used, proteins in biosensors are the most frequently utilized biological sensing components.
- A transducer is employed for translating biological signal that comes after analytes contact with a biological receptor to an electrical. The strength of the signal being generated is straightly/contrariwise proportional to samples concentration. Biosensors are also produced using electrochemical transducer. Such systems deal certain merits including, less cost, simple design and tiny size [1].

II. Basic Principle of BS

The principal parts of biosensor include, intermediary matrices amid the identification coating and transducer plays a crucial part of describing the selectivity, etc. of BS [3]. Figure 1 exemplifies the schematic representation of the basic principle of BS. It involves 2 major transduction mechanisms including electrochemical and optical sensors, depends on the intensity of light/ electrical circulation which show a significant part in an existing BSs. Amongst, the electrochemical based sensors exhibits a large prospective, furthermost appropriate in a perspective of therapeutic usage. While changing diverse NMs, it will deal a variability of biological particles to recognized by abundant sensitivity and specificity.

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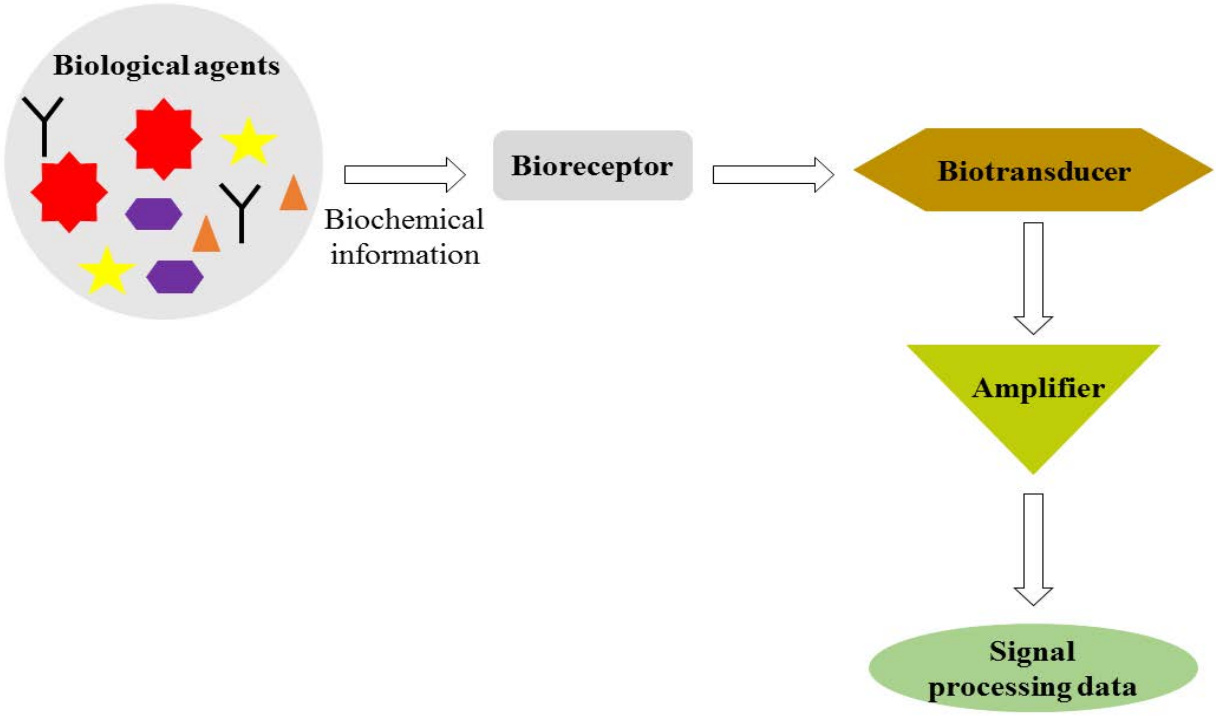

Figure 1: Fundamental principle of Biosensor

In case of BS, transducer transforms biotic incident to an electrical signal. There are 2 greatest widely utilized factors in electrochemical detecting are ampero- and potentio-metric. The circumstance of these sensor, systematic idea acquired via biological recognition method is transferred to potential whereas in amperometric sensor, continuous voltage related with oxido-reduction of electrochemically active classes are examined [4]. Hence, they are used expansively in illness diagnostics for the identification of appropriate receptor markers, Abs and sequencing of deoxyribonucleic acid or living cells.

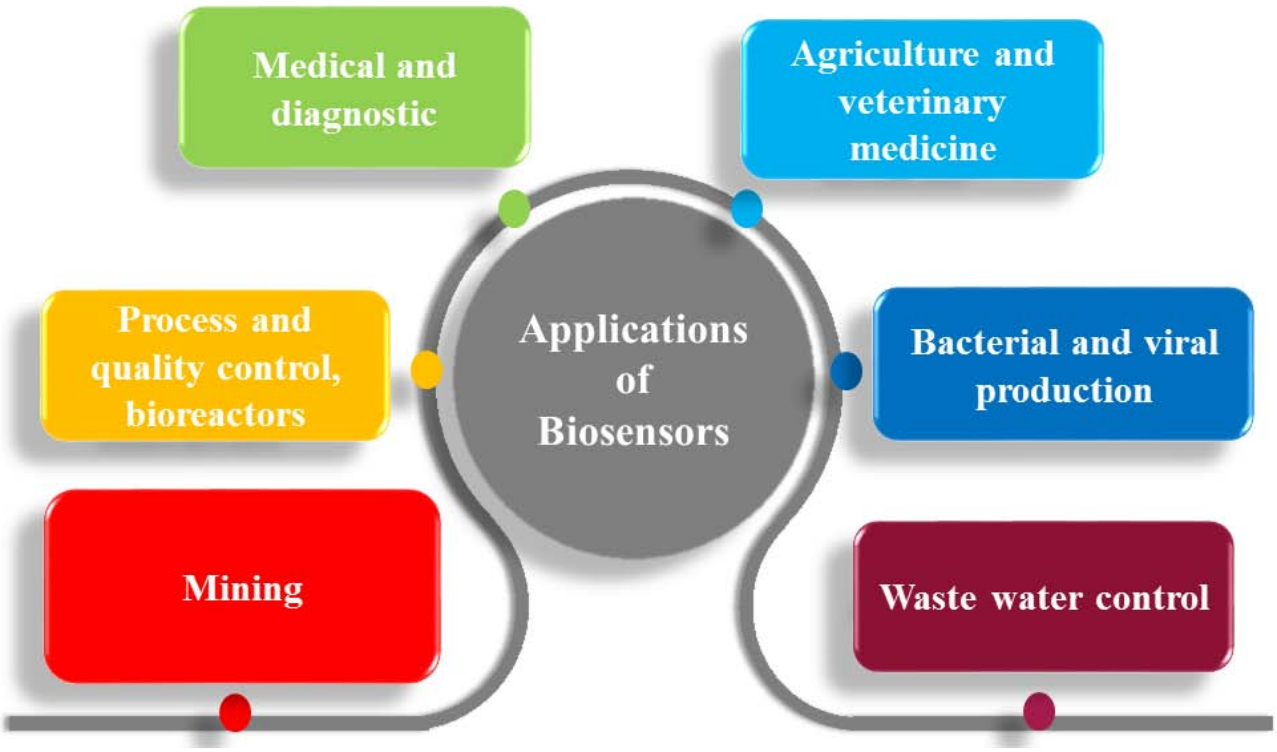

Figure 2: Applications of Biosensors in diverse fields

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The diverse applications of biosensor arenas in illustrated in Figure 2. The discovery of new diagnostic methods brings further attention to the implementation of point-of - care. This poses a big challenge for us to establish a novel material in electroanalytical approaches which can be precisely sensed animal studies. With the development of NT, biosensors depends on NMs are demonstrated enormous possibilities of more effectively diagnosing and detecting disease based biological markers. Significant progress in this respect have been decided to make with the use of various types of NMs includes, metal NPs [5], magnetic and carbon NMs, etc. [6, 7] to develop the electrochemical signal of biological catalytic events that occur on the surface of the electrode.

Further, NMs are characterized by outstanding features, including higher surface area to volume ratio, better electro-catalytic for instance carbon based materials and improved adsorption capacity like Au NPs. This further results in the construction of biochemical based sensors that possesses enhances selectivity with specificity [8]. Moreover, nano-structures including, nano-wires, tubes, particles and quantum dots have discovered hugely for biological sensing, meanwhile their dimension is compared to the biochemical organisms.

NMs were used in changing electrochemical transducers and enhance the electron transfer in analytical utilization, deliver biologically compatible micro-environment to biological molecules. Currently, attempts are made to utilize NS altered electrodes for observing particular biological organisms *in vivo* which unlocks the probability to identify a particular biomolecule in living organisms [9]. Hence, real-time watching of certain analytes including $C_6H_{12}O_6$ can be executed [10].

III. Engineering Micro Fluids

Microfluidic technique is recognized as the micrometer-level fluid regulation. Multiple research possibilities currently represent a major study to meet the vast majority of biomedical tasks. With the improvement in the area of microelectronics, different methods and techniques have been presented which allow scientists to develop and manufacture separate device structures for drug test and a segregation of the biomolecules [11]. In the semiconductor industry, photolithography and etching procedures have historically been employed to design and create NSs on glass. The method is full of expensive, need high instruments along with speedy prototyping banned. A significant breakthrough was the breakthrough of a replica molding brought out the invention of softer lithography [12]. Glass materials are perceived as costly and it was only possible for single system output [13, 14].

Polydimethylsiloxane also called as PDMS based production are described as PDMS to manufacture fluid structures dedicated to cellular biology study also for typo-graphing molecular study. Figure 3 stated the classical method for progressing a nano-based liquid instrument. The subsequent strategy utilized to PDMS manufacture is beading strategy [15, 16]. Photolithography strategy for the creation of molds requires both a spin coater and a dedicated UV lamp.

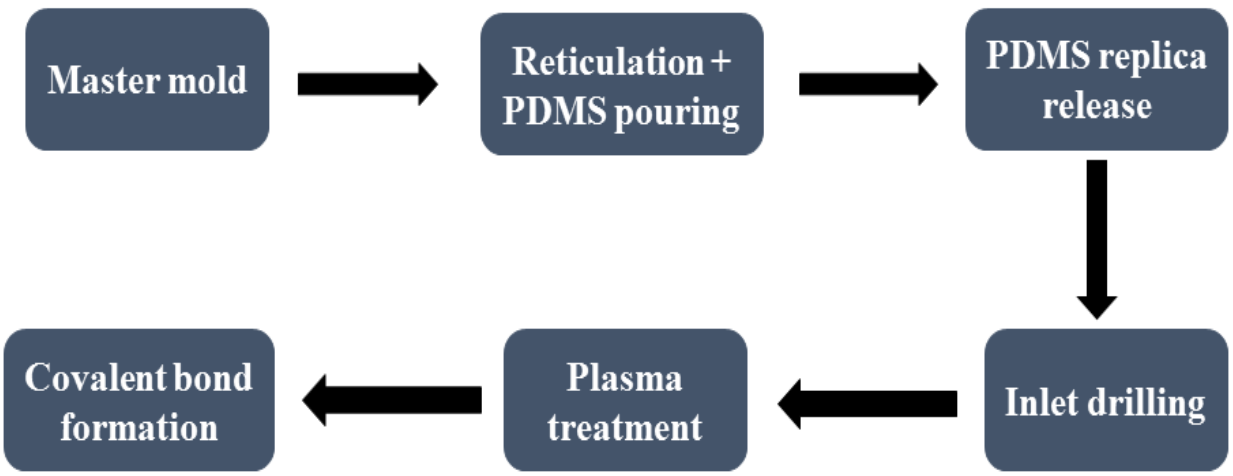

Figure 3: Visual representation of single layer microfluidics scheme generation

Manufacturing of monolayer microfluidic devices is achieved using traditional soft methods. Both combination of low Young's PDMS modulus enables integration of micro-valves during the manufacture of multilayer instruments [17, 18]. The lightness and limited surface energy make metal accumulation on PDMS surface complicated to attain. The desirability of glass substrates aids in establish hygienic techniques in Si-based microelectronics alongside feasible during PDMS manufacturing practice before plasma adhesion to metallic and dielectric strands obviously on the glass substratum [19].

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IV. Micro Fluids in Diagnosis

The advancement of point-of-care microbial medical methods remains a powerful field of clinical study and is an important precursor for a broad range of human infections to be treated with success. Particularly, numerous topical studies characterize the usage of CRISPR amplification techniques for premised on prompt detection of human papillomavirus, Zika and dengue viruses [20, 21]. Likewise, microfluidic methods have been studied in nowadays for clinical medical testing, with many platforms showing effectiveness for appropriate and prompt detection of infections by virus. A recent literature report showed that multiple viral infections can be identified in parallel by means of a microfluidic channel comprising various networks, virally, deoxyribonucleic acid can be enhanced and create a hydrogels. The creation of hydrogel viral infection was identified by injection of dye [22]. One disadvantage of this approach could be the fact that RNA virus identification needs to rely on past reverse transcription to generate noticeable cDNAs. Nevertheless, since DNA frameworks inside a microfluidic device are reliable for some time, this scheme can be well-matched detection must be planned and accessible. Furthermore, the technique mentioned can extended to diagnose a diverse range of virus and remarkably easy set-up can clamp possible for virus identification areas where refined investigational equipment is inaccessible and electricity is lacking. Confirmation of this technique for the discovery, employing sera from infected virus individuals are the further crucial process to identify its medical importance [23, 24]. Within the experiments, a system aimed at the identification of dengue virus by mass Ab detection has been exposed to increase thresholds for discovery compared to commonly employed treatment methods, like polymerase chain reaction and immunoassays coupled with enzymes [23]. Nevertheless, because adaptive Ab responses may take numerous days to achieve post-pathogenic exposure in an infected host and infection indicators to an individual's acute signs directly.

V. Commercial Trends in Miniaturization

Several gas sensor reports have been written, but too many of them are unique to a particular form of sensor owing on a variety of solutions employed or focused on a particular usage. More metal oxide semiconductor gas devices have been examined by numerous regarding the origins of both gas-sensitive products utilized and metal oxide semiconductor heating structures addressed the miniaturization of the detector and its heat source from the size of macro to nano [25-27]. In Arafat et al. study, one dimensional nano-structure metal oxides were discussed examining their performance [25]. Another report revealed that, gas sensors depends on polymers and reviewed the sensing mechanisms, properties that effect the enactment with few pros and cons [28]. Gaseous sensors depends on gravimetric identification was discussed and also zeolite based NMs for gaseous detectors pinpoint the probable NMs in gaseous sensing particularly in case of selectivity. Moreover, CNTs gas and vapor detectors attained in a significant equipment in words of specificity and selectivity. Ceramic based detectors which function at higher Ts and electrospun based NFs were also discussed with their specificity.

Moreover, Kong et al. stated that the electronic confrontation of semi-conducting single walled nano-tubes modifies when visible to gaseous particles, possessing a response time of an order of magnitude quicker than those depends on colloidal state detectors. They also has tiny size and work at room T with a huge specific sensitivity [29]. Also, Modi et al. reported that detectors have better sensitivity and specificity and were not influenced by numerous atmospheric conditions like T, gaseous flow and humidity possessing the electronic breakage of gaseous amalgamations at the corners of carbon nano-tubes, the cathode of the detector was Al whereas the anode was lined vertically with multi-walled carbon nano-tubes film [30].

VI. High Density and Lab-on-a-Chip Analysis

Micro-total-analysis systems also called as μTAS and the subdivision of devices are called as lab-on-a-chip or LOC. It is originated from a uses of soft and hard manufacturing methods for the fabrication of miniaturized devices that execute all or portion of a biological analysis. μTAS might be a hybrids of many chips, integral electricals and exterior supports, whilst LOC denotes more particularly to a micro-fluid based chip or other device which executes a well-structured analytical task [31]. Biological based chips are more generally including the LOC and microarray techniques. The main description of this word differs with diverse researchers and might be utilized interchangeably. Microarray based analysis can be widely divided into micro-fluidic or array based devices [32]. Deoxyribonucleic acid chips for the reading of DNA and RNA including both DNA LOC instruments and DNA based microarrays while receptor protein chips such as both LOC and protein microarrays. The main purpose of this LOC and μTAS instruments is to attain enhanced efficiency via tiny scales and to assume analysis that cannot be done expediently by another means.

Lab-on-a-chip, scheme/technologies were utilized to execute a mixture of evaluations on a mono miniaturized scheme for physiological and clinical

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enquiries. Several of the crucial sectors of usage of these technologies are part of the fields of study in life sciences including genomics and pharmaco-genomics, vaccine development or point-of-care computing. All instruments involve multiple analytical process, for instance, preparing for trial includes cell concentration and organizing, development and identification, cell lysing and polymerase chain reaction, cell development and impedance cell biology discovery methods as well as other identification strategies to ensure compliance at each chip stage. This enhances the identification precision and diminishes the probabilities of false positives.

Some of the micro-particles are isolated using DEP strategies and it was executed in early phases utilizing pin-wire electrodes, prohibiting DEP methodologies for only larger objects. In recent decades, microelectrodes with sizes as small as 0.5micrometer can be produced with the development of photolithographic methods, hence, producing very broad electric fields that are necessary for submicron-level manipulation particulate particles including deoxyribonucleic acid, protein and virus particles, etc. The benefits of composition of DEP and specific encapsulate of an Ab have been illustrated [33].

VII. Nano HPLC System

Nano based high performance liquid chromatography also called as Nano HPLC is an imperative qualitative and quantitative procedure, commonly employed for the valuation of therapeutic and biotic samples. HPLC is a proven approach utilized in standardized laboratories over the past couple of years. The development of packaging materials utilized to accomplish the isolation was one of the main reasons for the progress of this strategy [34]. After a few modifications Nano Liquid Chromatography was developed in high performance liquid chromatography by Karlsson and Novotny in the year of 1988. Biological implementations and mainly proteomics study have driven the massive spike in miniaturized liquid chromatography systems [35]. Several interpretations premised on column diameter and carrier gas flow rates are being reported in the literature [36, 37].

In genomics and proteomics the analysis of nucleic acids and proteins includes successful analytical methods such as microchip-based liquid chromate-graphy also called nano-liquid chromatography. Lab-on-a-chip system enhances the efficiency of many various sampling tasks on a singular instrumental device in conjunction with computerized data analysis in one procedure. The micro-manufactured chip consists of a board, frits or filters, an injector and a sensor, manufactured in a process consistent with those traditionally used to form circuit boards. The column can be loaded with endorses for distinct stationary phases to enable the impact of existing forms of nano-liquid chromatography such as, but not restricted to, normal and reversed phase, ion, size affinity and adsorption chromatography. A cross-channel compressor infuses a specimen plug at the section inlet with a nanolitre or picolitre-volume. A column outlet-integrated electro-chemical or permeability sensor monitors isolation signals. A self-aligned stream reinforcement method exploits the microfluidic system's P rating, enabling it to withstand large P ordinarily being used high-performance liquid chromatography. Utilizing ion-exchange nano-liquid chromatography, the on-chip specimen injection, isolation and identification of anion combination in water is clearly tested [38].

a) *Principles of HPLC*

Really no arrangement on the microscale chromatographic definitions. For micro-based columns of various [39], the concepts "microbore" and "capillary" liquid chromatography was utilized synonymously. Variations executed utilizing columns of 0.50 to 1 millimeter, as per the study [40]. Characterized as micro-liquid chromatography 100 to 500 millimeter columns which are defined as capillary-liquid chromatography, eventually, columns distinctions of 10 to 100 millimeter, are referred to as nano-liquid chromatography. This category comprises microchip phase separation, because there are 20 to 100 mm of nano-high performance liquid chromatography, columns happening chips. For this research, the nano-liquid chromatography category is employed for separations operating at movement charges of nL, common in 10–100 mm columns.

b) *Nano-HPLC instruments*

The traditional equipment in nano-LC was just miniaturised. Pumps, links, frames, injecting and sensing loops, the interface is sized to small volumes and reduced backpressure. These characteristics can have a significant influence on chromatography Nano-LC output and required power for a better separation.

i. *Pumps*

Nano-liquid chromatography pumps urge to provide repeatable nano-mass flow and stabilization mostly during isolation, and allow nano-scale differential elution. Nano-based chromatography could utilize 2 main systems: break and split less pumps, second group being widely viable.

Split systems segregate high flows from standard higher performance liquid chromatography pumps employing a pump-to-pump flow restrictor Thumbnail column. Such systems allow the normal high performance chromatography pumps to be used a nano-based flow control valve that is quickly assembled. Split systems, nevertheless, can result in adjustable split proportions and weak nano stream repeatability, reducing segregation reproducibility [41]. It is

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nearly hard to attain repeatable gradient elution, particularly to freshly made separating devices: the various apparent viscosity of the mixture solution can provoke back-pressure perturbations, thus restricting the eluent mode [42].

Split less technologies are typically commonly utilized in the nano-LC market. Such devices lower costs of solvents and have higher repeatable nano-flow speeds. Single-volume syringe pipes are stronger than split structures but sequential batch pumps, identical to traditional 2 piston reciprocating pumps for every platform. The sequential batch pumps was employed in isocratic as well as in isocratic directional filtration only at nano streams and the required changes successful promotional nano flow speeds.

ii. *Connections and Tubing*

Peak enlargement is a major constraint to the production of nano-based liquid chromatography. An analyte band spreading in the support is labeled as function and capillary longitude and the smaller the number, the poorer the contribution to distribution on column [43]. Lost pre- and post-column volumes can lead to major band expansion and essential in employing decreased columns. To reduce this contribution to band expansion, insufficient tubing and connections upsurge band expansion, hence the usage of small, close-fitting connections created with little volume tubing. Standard joints are made of either stainless steel or polyetheretherketone, the final is particularly fruitful for fused Si tubes. Spaces created by insufficient interactions could also facilitate smaller screen of the isolation. Study explored certain popular nano-LC isolation constraints namely contact issues and their realistic solution. Digest dispersions of bovine serum albumin were conducted, compared the relations are both insufficient and satisfactory [44].

iii. *Injections*

The approximate amounts of infusion for nano pillars could be measured as a function of both the length of the pipe, plate number, color intensity or any other variables but are usually a tiny nano-liters [40]. Tiny amounts implanted are a big issue in nano-based liquid chromatography, due to reduce of detection range but bigger injections quantities have an extending impact on the band, reducing the segregation efficiency, especially for badly preserved substances. Nevertheless, study showed that, enrichment benefit and a benefit in productivity by employing a poor fluid for the specimen, which encourages concentration of a specimen plug after insertion into a strong mobile medium [45].

iv. *Nano-based columns*

Though 10 millimeter columns are usable, 75 mm nano-based liquid columns are the most commonly utilized. The column offers a better compromise among detection range, envisages and reliability in breakups among nano-LCs [41]. Nano-based liquid systems divisions are normally produced of polyimide-coated fused silica capillaries which offer durability, good mechanical strength and a range of internal diameter, but stainless steel and Ti tubes are employed for nano-columns. It was lined with Si-based molecules, packed with a monolithic bed or, less generally, ceiling-coated with natural or synthetic molecules.

Monolithic column chromatography are specific cell or synthetic rods product created from within the capillary column. No fusion of monolithic columns and pore volume are needed for materials, greater mobile phase flow levels are possible, decreasing detachment Time [46, 47]. Could prepare monoliths by the usage of various synthesis paths, natural or synthetic and biocompatible products are useful alternatives for biologically specific applications study [48].

v. *Detection*

The nano-based chromatography detection kinds are identical to those used for HPLC extraction. Due to its less value, broad variety of suitability and usage of detection purposes, diode array detection is frequently utilized in nano-based chromatography. Because of the nano-based column's thin path length, detection and classification is nevertheless restricted when on-column identification is implemented. This is superseded by the usage of identification cells particularly configured which delivered a longer light paths [42]. Laser-stimulated fluorescence [49] and inductively joined plasma mass spectroscopy [50], are utilized in nano-based detection, but these are not quality sufficient to be implemented for simultaneous estimation.

Pharmaceutical and biotechnology implementations probably involve excellent detection performance, as well as a standardized technique of identification, like the one supplied by mass spectrometry. The nano-based flow from the column is suitable for mass coupling via different nano-spray interfaces, particularly electrospray ionization, which needs a tiny portion of eluent from the liquid chromatography column to be effective [51].

c) *Applications of Nano-based HPLC*

With extremely accurate tests, substances of biologically relevant must be established rapidly. Current revelations in analytical techniques and specimen process conditions in this field have driven biomedical analyzes for such important compounds to be identified. Nano-liquid chromatography analyzes are now used for medicinal and veterinary medicines, prevention of doping, treatment of diseases and quantitative confirmation of genetic markers and detection of proteome, the final are key fields of implementation, mainly due to the extremely small sample quantity available.

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Gene expression studies unquestionably react to the widespread uses of nano-based chromatographic extraction [52-57]. Nutrient synthesis of complicated tissue specimens is required for gene expression detection, prevention of diseases, and therapy options, primarily from blood and tissue specimens. HPLC-based approaches solve the traditional protein techniques issues, like gel electrophoresis and immuno analysis are constrained by several phases prior to analysis. Proteome complexity heterogeneity facilitates rapid and unchallengeable detection methods, encouraged by nano-based liquid system appearance combined with mass spectrometry. It will permitted the accurate identification of amino acid chains from receptors.

If not properly diagnosed, periodontitis can induce severe teeth failure and systemic complications. Study suggested distinguishing gingival specimen components from safe and periodontitic clinicians when looking for a common diagnostic tool for this inflammatory condition [58]. The researchers utilized nano-based liquid system of mass spectrometry for protein quantification and test validation immunoassays, 305 proteins were found across both sick and stable patients, 45 of which were clearly connected to periodontitis. Azurocidin was recognized as the main biomarker, and its scores were increased in patients with periodontitis, thus impeding osseous separation. The key outcomes of this research has been the suggestion for an effective treatment parodontitis by precise observation of stages of azurocidin by nano-based liquid and mass spectroscopy in complicated oral specimens, trying to prevent undiagnosed illness health problems.

Nano-based liquid and mass spectroscopy has been used to conduct proteomic analyzes of synovial fluid from rheumatoid arthritis clinicians. Osteoarthritis and rheumatoid arthritis also are damaging articular infections, defined by a deterioration of protection cells in the cartilage skin cells, accompanied by inflammatory disorders. Study recognized peptides which are associated with both articular and other peptide disorders are unique from each [59].

The definition of biological markers is endogenous markers of a particular genetic state, generally a peptide or carbohydrate. These can be monitored and assessed laboratory experiments for standard or dysfunctional procedures. Genetic markers are particularly linked in the biological sciences to safe or diseased states. A biomarker might be material injected through an individual for estimation its component is basic or unhealthy [60, 61]. Nano-based chromatography plays a significant part in the analysis of biomarkers. The small level of analyte from tissue specimens needs extremely sensitive strategies of segregation and nano-liquid chromatography combined to that aspect is easily presented by mass spectrometry.

Study used nano-liquid chromatography mass spectroscopy to test the synthesis of the polyphenol in human cancer cell lines [62]. The polyphenols were present in extra virgin olive oil, and the anti - cancer efficacy of their derivatives has been confirmed. The researchers computed the polyphenol derivatives by the tumor cells and per the time taken and suggested that such genetic variants can easily calculated by mass spectroscopy. Finding genetic markers of brain trauma in cerebrospinal fluid Sjo ¨ din et al. was proposed [63]. After a posttraumatic time frame by nano-based liquid chromatography mass spectroscopy they evaluated some molecules which might determines the degree of brain trauma. The auto sampler was held at 10°C to avoid the receptor degradation. The biomarkers were enhanced and measured over a wide frequency range by using a marketing ligand. Nevertheless, the chromatographic maximum throughput was far too long sometimes by using gradient elution, definitely owing to the increase communication between the solid phase and the nutrients analytes.

A biomarker of oxidative damage status, 8-isoprostaglandin F2 alpha, has been measured from human urine for its confirmation of some diseases, such as diabetes, cancer and Alzheimer's disease [64]. The researchers used a nano-high performance liquid chromatography based on microchips and this method required a phase of enhancement beforehand the chromatographic investigation. This advancement encouraged an increment of the mass spectroscopy signal, in percentage to an exponential rise of the composition injected. The framework developed was tested and established to be a successful tool for the production of isoprostaglandin. This enrichment encouraged a rise in the mass spectroscopy sensor in percentage to an exponential rise in the concentration injected.

Liquid chromatography is good introduced in diverse matrices as a framework for analyzing of pharmaceutical targets. From the development of new drugs to the quality control of dosage forms, authenticated liquid chromatography techniques were used effectively by the pharmaceutical companies, in research institutes and for drug released assessment in rubbish water [65]. Even though nano-based liquid chromatography would be utilized rather than typical liquid chromatography, at modern the less acceptance of this novel method is associated with the huge initial cost of acquisition. Nevertheless, the usage of nano-liquid chromatography is growing slowly due to the obvious decrease in the quantities of solvents needed and associated wastewater costs.

Eight specific dose of penicillin in medicines were calculated [66]. The researchers also detected these medications in dairy and tissue samples, confirming the predictive algorithm's validity in various biological vectors. For segregation iterations a filled C18

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column was formulated with higher repeatability. Polymeric fusion were assessed to loading the columns, and frits premised on polystyrene were selected leads to improved segregation resolution than most other polymer frits evaluated. The efficiency of nano-based chromatography was contrasted by utilizing both ultra violet and mass spectrometry identification for the direct estimation of the penicillin substances. The process was tested employing both devices, and detectable penicillin medication was detected in certain industrial specimens of tissue.

Researchers conducted the right to an effective of 18 sulfonamides, antimicrobials utilized for human and animal treatments [67]. Nano-based systems with ultra violet and mass spectroscopy identification are used to measure the covalently linked sulfa medicines. The multi-residual evaluation was carried on a C18 core-shell column in less than 40 min at a flow rate of 190 nL per min, which was chosen for better chromatographic precision and segregation quality compared to two other stationary phase choices. The researchers noted that ultra violet and mass spectroscopy detection offered good identification and confirmation of the strategies permitted the usage in sulfa based medications study from the dairy specimens [68].

Hair samples were obtained from participants in a detoxification institute for the study of cocaine, amphetamine, morphine and associated substances [68]. The researchers designed a simplified and established nano-liquid system, utilizing special nano-based chip liquid chromatography equipment as an option to incomplete immunoassay strategies. We also dramatically decreased the sample collection phases and the sample quantity needed which is lower than ten percent of the standard quantity. Whilst it is an excellent screening device, nano-based liquid systems are not commonly used to detect and quantify illicit drugs, possibly owing to the unavailability of nano-liquid instruments in standard labs for research. Due to their broad dissemination in investigative centers, gas and traditional liquid chromatography are the key analytical options, while immunoassay studies are perhaps the most general analytical techniques for preliminary drugs. Nano-LC is hitherto rarely utilized to analyze the enzymes [69, 70]. The column chromatography of nano-based chromatography often modifies enzyme configurations and reduces their catalytic performance [71]. Other highly advanced methods, like capillary electrophoresis, are favored to nano-liquid systems, as they do not allow modification of the protein's actual shape. Repeatability in nevertheless nano-based liquid chromatography is greater than capillary electrophoresis [72], definitely since this flow is much steadier than the oxygen diffusion produced within the capillary electrophoresis.

Study showed the latest kinetic enzymatic trials and capillary column with its segregation modes were extensively utilized for enzyme study, whilst in current years nano-based liquid chromatography was utilized in certain articles [72]. The usage of biological affinity columns is one way to resolve the boundaries of enzyme analysis in nano-based liquid systems. These distinct stationary particulate or monolithic phases restrain the enzyme in an available configuration without substantial loss of actual enzymatic activity [73]. Biological affinity columns for nano-based liquid systems can be successfully formed from biopolymers, not only for proteins but for other biomedical analysis [74].

Currently, the miniaturization of analytical techniques plays a crucial role in experimental science growth, which is supported by research in diverse fields. Techniques for therapeutic purpose must be capable of detecting and evaluate in smaller amounts of biological processes drugs present. The methods involved should have outstanding detection rate and indisputable recognition, notably for these small concentrations compounds, as supported by nano-based liquid chromatography hyphenations.

The biggest drawback to broader usage of nano-based liquid systems at the current moment is the massive price of analytical techniques. Nevertheless, this constraint is solved by the fast production of new devices, extending the nano-based chromatography to regular laboratories and sectors. The science of currently accessible nano-based liquid columns still seems to be a determining factor especially in comparison to the many and durable columns synthetic liquid chromatography columns, covering a broad range of different options. Choosing to focus on properties of nano-pillars including, monolithic and sub-2 mm particulate bit different, stationary phase processing is still an area which is only beginning its growth. Nano-LC seems to have the ability to do so in the immediate future take centralized role in biological molecular studies as an alternative to electrophoresis and immunoassays.

The massive spike in miniaturized liquid chromatography systems has lately been biochemical implementations, chiefly proteomics study, were driven. Protein or protease combinations must be analyzed and the information derived quantities and the smaller amounts of specific analyte are not consistent with solid sample common liquid chromatographic-systems. Nano-based liquid chromatography is an option to traditional liquid chromatography and therefore provides more chemical analytical choices. Nearly half of the samples analyzed by the modern fluid chromatography is analyzable using a miniaturized methodology. In case of capillary electrophoresis alongside chromatography in this context, it also enhance nano-based chromatographic-systems and start competing as miniaturized differentiations of the two liquids.

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Nano-based liquid chromatography advances are associated with multiple strengths this methodology provides over traditional high performance liquid chromatography analysis. Some potential benefits are, the considerable reduction in the intake of mobile and stationary phases, such as harmful reagents, the tiny proportion required, the highly efficient relocations all whilst keeping the very same actions in preservation, fast coupling to mass spectrometry. Among the most significant benefit at the moment is lower waste production, in consistent with the guidelines of green chemistry [75, 76]. The observational equipment utilized in nano-based liquid systems, nonetheless, is still quite costly, limiting their pervasive use. In addition, valuable technical information on in addition, serious technological information is necessary about the nano-liquid chromatography information to escape scientific problems, in particular those concerning Instrumental environments. In recent years, the amount of research methodology is a systematic on nano-based liquid systems implementations has increased; nevertheless, in such articles, neither the technical concepts nor those relating to equipment are published. This analysis thus contains the fundamental features of the nano-based liquid chromatography methodology and some current pharmaceutical and biomedical applications, especially biochemical analysis in a most commonly found field. Also described is proteomic evaluation, which correlates to the great use of this liquid chromatography.

VIII. Conclusion

This article highlights the improvements in the growth of BSs, its applications in diagnostic fields were discussed. Further, micro-electro-mechanical systems and their characteristics in genomics were briefly noted. Eventually, liquid chromatography and the nano chip based analysis miniaturization topics were reviewed. Conclusively, we need that future research would discover and determine the sensors accessible for versatile sensing applications.

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Floral Biology, Field Controlled Pollination and Hybrid Obtention of *Dioscorea Dumetorum* (Kunth) Pax) in Cameroon

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Abstract- *Introduction-* There is very little literature on the floral biology, synchronization of maturity of male and female flowers, flowering phenology and controlled pollination of *Dioscorea dumetorum (D. dumetorum).* However, the genetic improvement of this yam species, which is widely consumed in Cameroon and has exceptional nutritional qualities, cannot be achieved without knowledge and understanding of these biological attributes or phenomena. The aim of this study was to (1) - examine the sexual reproductive biology of *D. dumetorum*; (2) - study the phonology of flowering in this plant species; and (3) - develop a pollination method in order to obtain hybrid seedlings.

Keywords: *yam, dioscoreadumetorum, floral traits, flowering behavior, hybridization, field pollination, seed germination, cameroon.*

GJSFR-C Classification: *DDC Code: 632.96 LCC Code: SB933.3*



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Floral Biology, Field Controlled Pollination and Hybrid Obtention of *Dioscorea Dumetorum* (Kunth) Pax) in Cameroon

Gabriel Mahbou Somo Toukam [α], Eddy Leonard Mangaptche Ngonkeu [σ], Godswill Ntsomboh-Ntsefong [ρ], Marie Noel Ateko Tiokeng [ω] & Emmanuel Youmbi [¥]

Abstract- Introduction- There is very little literature on the floral biology, synchronization of maturity of male and female flowers, flowering phenology and controlled pollination of *Dioscorea dumetorum (D. dumetorum).* However, the genetic improvement of this yam species, which is widely consumed in Cameroon and has exceptional nutritional qualities, cannot be achieved without knowledge and understanding of these biological attributes or phenomena. The aim of this study was to (1) - examine the sexual reproductive biology of *D. dumetorum*; (2) - study the phonology of flowering in this plant species; and (3) - develop a pollination method in order to obtain hybrid seedlings.

- *Materials and Methods:* Forty seven cultivars of *D. dumetorum* collected in the main yam growing regions of Cameroon were used; (1) - In order to study the floral biology and synchronization of maturity of male and female flowers, two repetitive observations of floral characteristics of yam were undertaken. Data were collected on the stages of stem emergence, first bud emergence, first flower opening and flowering pattern within a day; (2)- For flowering study, the flowering records of the 47 cultivars collected over a three-year period; (3)- For the determination of the best pollination method, three methods were tested. Materials used include syringe, wire brush, bamboo splinter, scissors, pencil, notepad, alcohol, vials, razor blade, and roll of thread.
- *Data analysis:* Five types of data analysis were done in this study. (1) - ANOVA, for quantitative data, when the hypothesis of normality of data is confirm by Kolmogorov test, including data related to (a) - flower morphology. These are: inflorescence Number (InfN), Inflorescence Length (InfL), Flowers Numbers (FlwN), Flower Length (FwrLg), Flower Diameter (FlwD) and Density; (b) - data related to flowering pattern including Days to Emergence (DE); Days to First Bud emergence (DFB), Days to First Flower Opening (DFFO), Days to First Bud emergence to First Flower Opening (DFB-DFFO), Number of Flowered Plants (NFP) and Maturation Time (MT) over three years evaluation. (2) - Mean separation. Means were separated using LSD or DUNCAN according to the number of means to separate. This was done at 5% threshold and (3) – Relationship establishment with Pearson correlation coefficient. In the case of abnormality of data, Mann Whitney test according to Monte Carlo method at 99% confidence interval was performed. (4) – For qualitative data, Binomial logistic regression analysis was performed with Monte Carlo permutation in order to establish the relation between flowering, year and sex and lastly (5) - Kendall correlation coefficients at 5%.
- *Results and discussion:* This study has confirmed that *D. dumetorum* is a gynodioecious plant. The female fruit is a spike and the male fruit a panicle. The spike is on average 10 times longer than the panicle. The mean number of inflorescences (16) is comparable between male and female and male cultivars. The floral density is fifteen times greater on male cultivars; this could be interpreted as a strategy of this yam species to favor fruit bearing by female flowers. Sex influences most of the floral traits. There is an average lag of 16 days between the maturation of male and female flowers, with the males maturing earlier. Flowering monitoring of 47 *D. dumetoum* cultivars confirmed the erratic nature of flowering to the extent that the chance of flowering of a cultivar in one year to the next can be multiplied by 4. Sex could also influence flowering. Pollination with a syringe is an efficient method that allows up to 64% fructification. These fruits produce viable seeds that germinate according to a sigmoid curve, which starts to slope on day twenty-five and reaches its peak on day forty-four, marking the end of germination. This information is useful for strategic planning of genetic improvement of *Dioscorea dumetorum* by sexual means.

Keywords: yam, dioscorea dumetorum, floral traits, flowering behavior, hybridization, field pollination, seed germination, cameroon.

I. Introduction

Dioscoreaceae (yam) of the order *Dioscoreales* and genus *Dioscorea*, constitute a family of monocotyledonous flowering plants with about 750 known species in 9 genera (Xu and Chang, 2017) distributed in 59 sections (Govaerts and al ; Coursey, 1967). True seeds are seldom used probably due to variation of flowering in yam. Several yam species are grown for their edible tuberous roots, which constitute a staple food or traditional medicine for most communities. For the same amount of raw materials consumed, the energy value of yam is generally lower than that of other roots, tubers or starchy fruit, but the

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coverage of protein requirements, minerals and vitamins is more complete (Trèche, 1998). Like other root or tuber crops (cassava, irish potato, sweet potato), yams are generally vegetatively propagated from seed tubers or sections of tubers, rhizomes and bulbils. However, yams can also reproduce by seed. This is the most successful way to improve the species.

Eight yam species are commonly grown and consumed in West and Central Africa. They include *D. dumetorum*, *D. esculenta*, *D. bulbifera*, *D. schimperiana*, *D. semperflorens, D. alata* and *D. burkilliana*. Among them, *D. dumetorum* (bitter yam) is the most nutritious (Sefa-Dedeh and Afoakwa, 2002). Bitter yam belongs to the section *Lasiophyton* which contains 51 edible species originating from tropical Africa. It occurs in both wild and cultivated forms (Degras, 1993), resulting in a huge unexplored genetic diversity that could benefit the genetic improvement of this orphan species.

A major constraint to the international and even local development of this yam species with high nutritional potential is caused by its storage ability. Postharvest hardening of the tubers of this yam species begins within 24 h after harvest and renders them unsuitable for human consumption (Sefa-Dedeh and Afoakwa, 2002; Siadjeu and al, 2021). Hardening is manifested by the loss of culinary quality due to a combination of factors resulting from normal but inadvertently deleterious reactions leading to textural changes (Medoua, 2005). Therefore, *D. dumetorum* is consumed exclusively during its limited harvest period. Thus, only freshly harvested tubers are cooked and sold to consumers. Ground work has been done in our previous endeavors towards the genetic improvement of *D. dumetorum*. This includes studies on the genetic diversity using morphological markers (Siadjeu and al, 2015); genetic diversity and population structure (Siadjeu and al, 2018); the influence of some agronomic traits on yield indicating the optimal seedling weight at 300 gr, (Mahbou and al, 2015), or the influence of the cultivar on postharvest hardening of trifoliate yam (Siadjeu and al, 2016) and more. recently, a candidate gene controlling the post-harvest phenomenon has been detected (Siadjeu and al, 2021). A second major constraint for the amelioration of *Dioscorea* spp. could be due to erratic flowering and poor seed production and fruiting (Mondo and al, 2020) as noted on related species *Dioscorea alata* and *Dioscorea cayenensis-rotundata* complex. A third constraint that may need to be addressed in breeding is the asynchronous flowering of male and female plants as noticed in *Dioscorea cayenensis-rotundata* complex (Zoundjihékpon and al, 1997). True seeds are being used successfully in breeding programs (Girma and al, 2014), such as the case of *D. alata* and *D. cayenensis-rotundata* (Lebot, 2009) in addition to tuber sets. Hybridization of yam has become feasible due to a better understanding of the reproductive biology of the cultivated species (Asiedu and al, 1998). However, unlike other yams, little information is available concerning the complete description of the flowers and flowering pattern of *D. dumetorum*.

The improvement of a species by conventional means requires (1) - mastery of its floral biology ; (2) - an understanding of factors that could influence anthesis, i.e. the blossoming of flowers; (3) knowledge of phenology of biological phenomena leading to flowering; (4) - the development of a viable pollination method leading to viable seeds for the production of seedlings. The aim of this study is to give answers to these four concerns by gathering information, which could be useful for the genetic improvement of this orphan crop species.

a) Plant materials, methods and study area

The plant material used in this study consists of 47 cultivars of *D. dumetorum* yam collected in three regions of Cameroon (Table 1). In order to study floral biology, a total of 20 plants of each cultivar were planted in two repetitions of 10 plants / block. The experimental site is located at the Boukue School Farm (FEBO) in the West Region of Cameroon, Hauts- Plateaux-Department, Baham District. Location, climate and soil characteristics are indicated in previous studies (Mahbou and al, 2021).

b) Field planting

The yam tubers ware planted in the field at 1 m spacing in each row, and 1 m between rows. The tubers were arranged in a randomized complete block with a plot of two replicates of ten tubers per accession. Each plant was supported by an individual stake of *Phyllostachys* to induce good canopy development. Tuber sets were planted in April 2018, 2019 and 2020. Given the acidity of the soil, each plant received 0.5kg of poultry manure and 100 gr of Tropicote (21% N + 25% CaO) and 50 gr of NPK (20/10/10) each year.

c) Biology of sexual reproduction, observations and description of inflorescences and flowers of D. dumetorum

Observations were made twice from mid-June to November in 2018 and 2019. Five plants of each cultivar were randomly selected to observe inflorescence and flower behaviour. Observations included Inflorescence Number (InfN), Inflorescence Length (InfLg), Number of Flowers (NumF), Flower Length (FlwLg), Flower Diameter (FlwD) and Flower Density calculated as a ratio of number of flowers per centimeter of inflorescence. The description of the floral structure was done with the help of a magnifying glass (x8, x10, x16) and camera. The analysis of variance (ANOVA), followed by the comparison of means was carried out using Duncan's method at the 5% threshold. Pearson correlation coefficient was calculated to determine the relationship among traits.

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d) Phenology of biological phenomena leading to flowering

i. *Flowering pattern within a year and anthesis*

In order to study flowering pattern, data was collected including number of Days to Emergence (DE), Days to First Bud Emergence (DFB), Days to First Flower Opening (DFFO), Days to First Bud emergence to First Flower Opening (DTB-DFFO), and Number of Flowered Plants (NFP). For anthesis, observations included those made on a weekly basis within a day (number of newly opened flowers per hour in one day, and number of newly opened flowers per week in one month) on the one hand and those made annually on a three-year period on the other hand. The dimensions (length and diameter) of the reproductive system were measured using electronic calipers and ruler. These parameters were recorded until senescence. Data represented the mean of five inflorescences per plant.

Quantitative data recorded in this section (InfN, InfLg, NumF, FlwLg, FlwD, DE, DFB, DFFO, DFB-DFFO, and NFP) were subject to exploratory statistics to test the normality of the distribution using the Kolmogorov test. In case of normality of data, ANOVA analysis were done and means separated using Duncan Multiple Range Test at 5% threshold. In case of abnormality of the distribution, comparison was done with the Mann Whitney test according to Monte Carlo method at 5% significance level. Statistical analyses were performed using IBM/SPSS statistics 20 software.

e) Flowering rate of different D. dumetorum accessions as a function of time

Data on the flowering status of 47 cultivars were collected during three years. Analysis of data was realized by the binomial logistic regression analysis with Monte Carlo permutation in order to establish the relation between flowering, year and sex and lastly at 95% confidence interval and Kendall correlation coefficients at 5% threshold to evaluate possible relations among year, sex and flowering.

f) Synchronism of maturation of male and female flowers

In order to evaluate the synchrony of maturation of the male and female flowers of the different accessions with respect to the possibilities of crossbreeding, we use time to maturity (TM). TM is defined as the time (measure in days) to maturity in flowering plant, considered to be the period from emergence to the maximum opening of male and female flowers. This was recorded over a three-year period during the years 2018, 2019 and 2020. Data were analyzed using a T test at 5% significance level for time to maturity (TM). For flowering response as a function of year and sex of the plant, Binomial logistic regression was performed.

g) Evaluation of the best pollination method

To evaluate the possibilities of controlled sexual hybridization, three controlled pollination methods were tested. Bamboo splinter pollination, wire brush pollination and syringe/pollen solution pollination as illustrated on figure 3. Two controls (positive control and negative control) allowed us to evaluate the performance of these pollination techniques with respect to fruiting. The analysis of variance (ANOVA), followed by the comparison of means were carried out using LSD method at the 5% threshold based on fructification performance.

II. Results

a) Biology of sexual reproduction of D. dumetorum

In general, significant variations were observed on all the morphological parameters between male and female accessions, showing that there was a significant source of variation that could be used in the improvement programs of *D. dumetorum* based on the selection of objectives and to mitigate the problems observed relative to flowering in yams.

b) Structure of the female flowers

The female inflorescences are spikes (Figure 1A). They are formed in the leaf axils at the level of the flowering axes. Each flowering node has 1 or 2 inflorescences. The inflorescences are oriented downwards in relation to the branches. The perianth is of a single bell-shaped leaflet forming a large three-angled capsule, corresponding to three lobes. The corolla is absent, the calyx consists of three sepals and the pistil consists of a trilocular inferior ovary, three simple styles and a central trifurcate or trigonal stigma (divided into three branches) (Figure 1B). Each lobe of the ovary contains two apically placentating ovules (Figure 1C). The flowers are piliferous and purple or violet in colour and have two lanceolate bracts.

c) Structure of the male flowers

The male inflorescences are panicles (formed by clusters of spikelets) (Figure 2A). Morphologically, the immature inflorescences are very different from the mature ones. They appear to be single spiked at first but after full development, panicles are observed. The flowers are sessile, spherical and protected by bracts. The flowers consist of a calyx with 3 sepals and a corolla with 3 petals, arranged regularly and almost similar in size and appearance (Figure 2B). The sepals are white and the petals greenish. The stamens are 6 in number and each consists of a very short, capillary filament and a single anther. The anthers at maturity take on different colours depending on the accessions. They are white (Muyuka 3, Mabondji sweet white 1), yellowish (Ibo sweet 2) and purple (Bayangam 1). This result confirms that *D. dumetorum* belong to the narrow 10% of

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flowering plants that are said to be doiecious, a prevalent mating system in *Cucurbitacea* family (Ainsworth C, 2000) (Figure 1 & 2), more precisely *D dumetorum* is gynodioecious.

d) Morphology of female flowers

The number of inflorescences (InfN) varied from 4.60 ± 2.60 (Bamendjou 2) to 33.7 ± 21.6. (Country yam 1) with a mean of 14.6 ± 8.2. The length of the inflorescences (InfLg) varies from 40 ± 17 mm (Bambui 1) to 215 ± 69 (Ibo sweet 1) mm with a mean of 92 ± 46. The number of flowers (FlwN) per inflorescence varied from 11.3 ± 2.1 (Bambui 1) to 18.1 ± 2.8 (Bamendjou 2) with a mean of 15.1 ± 2.4. The length of the flower (FlwLg) varies from 2.20 ± 0.4 (Baigon 1) to 13.6 ± 1.8 mm (Ibo sweet 1) with a mean of 10.7± 3.0. The diameter of the flower (FlwD) varies from 1.4 ± 0.2 mm (Baigon 1) to 5.7 ± 0.6 mm (Mankon 1) with a mean of (4.2 ± 1.2). Density varies from 0.7 ± 0.3 (Ibo Sweet 1) to 3.4 ± 1.9 (Bambui 1) with a mean of 2.1 ± 0.7. This is illustrated in figure 3. The results obtained show that the length of the female flowers is greater than the diameter. In general, the diameter of a flower is proportional to its length. Cultivars with the longest flowers are those with the widest flowers.

e) Morphology of male flowers

The number of inflorescences (InfN) varies from 3.0 ± 2.0 (Lysoka sweet 1) to 30.5 ± 21.6 (Mabondji sweet white 1) with a mean of 17.7 ± 9.9. The length of the inflorescences (InfLg) varies from 12 ± 4 mm (Lysoka sweet 1) to 78 ± 8 (Mabondji sweet white 1) mm with a mean of 34 ± 20. The number of flowers (FlwN) per inflorescence varies from 20.2 ± 6.4 (Lysoka sweet 1) to 158.9 ± 61.7 (Mabondji sweet white 1) with a mean of 95.7 ± 43.3. The length of the flower (FlwLg) varies from 0.95 ± 0.12 (Buea sweet 1) to 1.25 ± 0.04 mm (Mabondji sweet white 1) with a mean of 11.0± 1 mm. The diameter of the flower (FlwD) varies from 1.07 ± 0.05 mm (Bayangam 1) to 1.48 ± 0.06 mm (Mabondji sweet white 1) with a mean of (1.3 ± 0.1). For density, it varies from 20.1 ± 9.6 (Lysoka sweet 1) to 49.4 ± 8.3 (Bayangam 1) with a mean of 31.5 ± 11.3. Density varies from 20.1 ± 9.60 (Lysoko Sweet 1) to 49.4 ± 8.3 (Bayangam 1) with a mean of 31.1 ± 11. Statistical analysis showed that there is no significant difference ($p = 0.518$) between the inflorescence number (InfN) of male and female cultivars. Sex strongly influenced ($P < 0.000$) InfLg, NumF, FlwLg, FlwD and Density independently of cultivar. This is illustrated in figure 3 where it can be observed that the male inflorescences are 15 times denser as compared to the female inflorescences. The female flower is approximately 10 times longer, 3 times wider and its inflorescences 3 times longer than the male (Figure 3). Indeed, in dioecious clonal plants, the reproductive effort required to set seeds is responsible for the larger investment in sexual reproduction by females (Mizuki and al, 2005). This result can be explained under the principle of Bateman as mentioned in (Willson, 1994) and (Queller, 1994) suggesting that males invest more in blooming to increase the chances of success of pollination while females invest more in seed production.

The relatively large size of the female flower (106.3 mm) is probably an evolutionary adaptation of this species to produce a high number of seeds for each pollination event. This theory is shared by (Bawa and al, 2019) who suggested that single or few seeded fruits may be correlated with small flowers. In contrast, those with multiple ovaries or many seeded fruits (like *D. dumetorum*) may be associated with large flowers with specialised pollination mechanisms. Furthermore, both combinations of traits, small flowers and one or few seeds per fruit, and large flowers and many seeded fruits, have persisted in monocots longer than other combinations of traits.

f) Flowering patterns of female and male flowers within the year

Female plants emerge 5 days earlier (DE, $p = 0.027$), than male plants. Similar work by (Zoundjihékpon and al, 1997) found instead a 25-day earliness of emergence of male plants over females with cultivars of the *Dioscorea cayenensis-rotundata* species complex. These authors worked with a sample of 13 plants. Despite the delay in emergence, the flower bud appears 15 days (DFB, $p = 0.017$) earlier in male plants. The time from the appearance of the first flower bud to the opening of the first female flower is on average 20 days before for female flowers (DFB-DFFO, $p < 0.0001$). There is no effect of sex on DFFO ($p=0.356$) and NFP ($p=0.118$) (Table 3). All of this is illustrated on figure 4.

We also note a strong positive correlation (0.74; $p=2.19 \times 10^{-04}$) between flower length and inflorescence length. The longer the inflorescence, the longer the flower and the fewer flowers it will have (-0.75; $p = 1.33$ x 10-04). The longer the inflorescence, the lower the flower density (-0.63; $p = 3.03 \times 10^{-03}$). The longer the flower, the lower the flower density (-0.83; $p = 5.61 \times 10^{-06}$) (Table 4).

The opening of the female flowers in *D. dumetorum* occurs gradually during maturation. They become receptive after a certain point in the maturation process. Once opened, they do not close again until they turn brown. These flowers do not have a characteristic strong smell. The receptive female flower has prominent, colourful and spotless stigmas, is well open, the stigmas are clearly visible, separate and distinct. It remains in this morphological state for 5 to 7 days after which it turns brown and fades if not pollinated. A weekly count for more than a month of new receptive open female flowers in each variety shows that their number varies according to the variety; the number of open flowers is very irregular over time (Figure 5). The

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total number of opened flowers per hour between 9 a. m. and 5 p.m. was 4-6 days for male flower. For all accessions considered, maximum opening was observed between 11 a.m. and 2 p.m. Flowers opened in the morning (9 a.m.) and started to close by 5 p.m. in the evening (Figure 6). Zoundjihékpon and al 1997 (*up cit*) obtained semilar result in their work with *Dioscorea cayenensis-rotundata*. They reported a cultivar on which flowering last 8 days, with peak of flowering occuring between the fourth and eighth day.

g) Flowering pattern over the three-year evaluation

Flowering monitoring over three years demonstrates the erratic nature of flowering in *D. dumetorum*. Of the 47 accessions used in this study, 30 are flowering (i.e. 63.83%), including 9 males (19.15%) and 21 females (44.68%). The remaining 17 (36.17%) accessions did not flower during the three years evaluation. they are said to be non-flowering. Of the 63.83% of accessions that flowered, 19.15% flowered continuously over the three years. The cultivars Muyuka 3, Bayangam 2 and Bamendjou 2 flowered in the first two years and did not flower in the third year. Conversely, the cultivars Country yam 2, Muyuka 3, Bana 1 and Bangang 1 did not flower in the first two years of the evaluation and did flower in the third (Table 5). The flowering rate of cultivars is influenced by the year ($p= 0.03$). An increasing number of cultivars flowered from year to year (Figure 7). A cultivar planted in the second year of trial has 3.67 more chances to flower than one planted a year before (Table 6). It can also be stated, but with little certainty ($p = 0.09$), that sex influences flowering. Its erratic character seems to be more pronounced in male plants than in females. However, this hypothesis remains to be consolidated. Positive Kendall correlation coefficient was noted between year and flowering (0.22; $p = 13 \times 10^{-03}$) and between sex and flowering (0.18; $p = 46 \times 10^{-03}$) (Table 7). The presence of non-flowering cultivars in the yam collection may limit sexual breeding in them. Similar results showing the absence of flowering in some yam accessions was highlighted by (Yolou and al, 2015). In the collection, the rate of female clones exceeding that of males excludes the problem of scarcity of female clones which is a limiting factor for sexual breeding of yam (Segnou and al, 1992; Asiedu and al, 1992). The temporary flowering discontinuity observed in some accessions in the collection was also highlighted by other workers (Zoundjihékpon, 1993) on the *D. cayenenses-rotundata* complex. This is one of the characteristics of yams that according to Segnou et al (1992) hinders their improvement by sexual means. The presence of some continuous flowering accessions in the collection reduces this problem of lack of flowering continuity over the years. The absence and discontinuity of flowering is thought to be due to the disturbance caused during the domestication process. Indeed, all accessions of *D*. dumetorum would be potentially flowering like other angiosperms. However, the variation in the environment during domestication probably repressed the expression of the genes responsible for flowering. This repression was probably slight in the case of accessions with discontinuous temporary flowering, or accentuated in the case of non-flowering accessions during the three years of the study. This result is in agreement with the ideas of some workers (Asiedu and al, 1992) who believe that the time lag between the flowering of male and female plants limits fertilisation in yam.

h) Maturation synchrony of male and female flowers

Data of maturation time are illustrated in figure 8. Using a Student's t test, we found that the time to maturity of the male flowers is on average 16 days shorter than that of the female flowers ($p = 0.001$) over a three-year period. This consistency does not change over the years. For pollination to take place, not only must the flowering period of the male and female flowers be synchronised, but also the opening period. Our results show that the male flowers open 1 week after the female flowers (figure 4). As a consequence, there is no synchronism of either flowering initiation or flower opening in yams. Indeed, this situation, which leads to a much-reduced availability of both viable pollen and receptive female flowers, has been reported in *D. rotundata* (Zoundjihékpon et al., 1997). Pollination requires not only synchronism in male and female flowering periods but it also requires that flowers open at the same time during the day. Our results showed that female flowers (Figure 5) opened gradually during their maturation and were completely open at full maturity (anthesis). It could not have a flower opening peak hour during the day as reported on other yam species (Zoundjihékpon *et al.,* 1997; (Li and al, 2014). Male flowers started opening during the morning and closed in the evening (Figure 6). Flower opening peaked from the 4th to 6th day between 11 a.m. and 2 p.m. According to (Hamon and Koechlin, 1991), pollination due to insects is higher between 10 a.m. and 12 p.m. Moreover Zoundjihékpon *et al.* (1997) reported that in yams, the availability of pollen was highest in the morning when the natural pollinators (thrips, *Larothrips dentipes*) are present and active. Thus, an overlap of flower opening was considerable between male and female accessions.

i) Pollination method trial

Results are indicated on figure 9. Significant effects were obtained while comparing different pollination technique ($p < 0.05$) with respect to fructification. No significant difference between the fruiting rates of the positive control (1%) and the negative control (0%) flowers was noted. The best result was obtained with the pollen solution (64%). which is highly superior to wire brush (9%) and the bamboo

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splinter (9%) methods. This confirms that the fruits formed on the artificially pollinated plants were indeed controlled and not subject to parthenogenesis. The plants in the positive control had a fruiting rate of 1.11% and did not differ significantly from those in the negative control. They provide information on the efficiency of natural pollination, which in this case is low. This failure of natural pollination could be due to the absence of pollinating agents in our experimental site. These are insect pollinators such as *Larothrips dentipes, Chirothrips sp., Haplothrips sp. and Xylocopas brasilianorum*. Their absence could be due to environmental conditions that do not favour their development. The presence of insecticides sprayed in the experimental field possibly prevented the development and even the presence of these pollinating insects. The superiority of the pollination method involving the pollen solution over the pollination methods with the bamboo splinter and the metal brush could be due to the insufficient dehiscence of the anthers in the latter two methods. The crushing of the anthers during the preparation of the pollen solution must have caused the rupture of the anther and the release of the pollen into the solvent (rainwater). This pollen was certainly deposited directly on the stigma of the female flowers, using the syringe, and must have relatively favoured fertilisation and therefore fruiting. However, pollination with the bamboo splinter and the metal brush allowed the deposition of anthers on the stigma of female flowers; these anthers probably had not undergone sufficient dehiscence after their deposition in order to release the pollen. This lack of dehiscence in these two pollination methods possibly limited fertilisation and therefore fruiting, which was significantly lower than that observed in the case of pollination with the pollen solution. This fruiting rate of 64% is much higher than those obtained in Nigeria on *D. rotundata* (41.3%) (Akoroda, 1985); on *D. alata* (19.7%) in Cote d'Ivoire (Ehounou and al, 2019) and are comparable to that obtained on *D. alata* (74%) (Abraham and al, 1990) in India.

j) Fructification and germination of D. dumetorum seeds

Artificially pollinated flowers developed into fruits (Figure 10). The germination kinetics of these seeds as a function of time is shown in Figure 11. During germination in *D. dumetorum*, the cotyledons remain buried in the soil, so germination is said to be hypogeal (Figure 12). The germination curve of *D. dumetorum* seeds as a function of time has a sigmoidal shape. *D. dumetorum* seeds start germinating after 23 days; this is the germination delay. After 23 days, the germination rate increases exponentially and most seeds germinate after 40 days. At 45 days after germination, the seeds do not germinate anymore; this is reflected in the horizontal shape of the curve, which marks the end of germination. The fruiting rate of the negative control flowers is almost zero because of the lack of pollination of their flowers before covering them with cloth.

The seeds of *D. dumetorum* germinated because they each contained an embryo resulting from the fusion of the antherozoite and the oosphere. The germination of *D. dumetorum* seeds is hypogeal because this species is a Monocotyledon. Their germination time is longer than that of *D. cayenenses-rotundata* which germinates from day 13 (Zoundjihékpon, 1993). The same is true for the average germination time of *D. cayenenses-rotundata* which is relatively shorter (20 days). These differences in germination time are thought to be due to genetic variability between these two yam species (*D. cayenenses-rotundata* and *D. dumetorum*).

III. Conclusion

This work consisted in evaluating the possibilities of artificial hybridization of sweet yellow yam (*D. dumertorum*) through controlled pollination in the field. The studies were conducted in West Cameroon, more precisely at the Boukué School Farm in Baham. The effect of temporary flowering discontinuity and the lack of synchronisation in the flowering of male and female flowers on the possibilities of controlled hybridisation of 47 accessions of *D. dumetorum* was evaluated. On average, female flowers (21 days) bloom quickly compared to male flowers (26 days). In addition, the male flowers bloom 2 weeks before the female flowers. However, the females open 1 week before the males. This shows that there is no synchronisation between the flowering of male and female flowers, however this problem could be solved by staggered sowing. Thus, the slow flowering individuals will be sown before the fast flowering ones by respecting the flowering time lag during sowing. Female flowers of *D. dumetorum* open gradually during maturation and are fully open at full maturity. Male flowers, in contrast open only when they reach maturity. They open in the morning and close at the end of the day. The opening peaks between 11 am and 2 pm correspond to the favourable pollination period in *D. dumetorum*. From this study on floral biology it appears that *D. dumetorum* can be improved by sexual reproduction (Akoroda, 1985). Of the sweet yellow yam pollination techniques studied (bamboo splinter, wire brush and pollen solution injection techniques), pollen solution injection technique performed best with respect to fruiting rate. The fruits formed contained seeds which germinated after 24 days to produce hybrids. For breeding purposes, accessions with continuous temporary flowering may be preferred as progenitors. The pollination method with pollen solution is recommended for controlled crosses. Based on our results, the two main potential problems

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(temporary flowering discontinuity and the lack of synchronisation) in yam breeding in general can be overcome by sexual reproduction through breeding of *D. dumetorum*.

Our work contributes to a better control of hybridisation by sexual reproduction in sweet yellow yam. It aims at the genetic improvement of the *D. dumetorum* species through field crosses. For a more efficient and reasonable implementation of these results, it will be necessary to (1)- Develop a method of pollen conservation ; (2)- develop an approach to stimulate non-flowering accessions with growth hormones for instance; (3) and characterize hybrids obtained using the best controlled pollination technique with a proper cross-breeding plan in order to enhance the breeding programme of *D. dumetorum*in Cameroon.

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Table 1: Geographic origin of *D. dumetorum* cultivars used in this study

No.	Cultivar code	Local name	Place of collection	Latitude (N)	Longitude (E)
1	Alou 1	Meleuch	Alou	05°31.240	009°56.479
2	Babadjou 1	Neliola	Mbouda	05°37.488	010°15.668
3	Babungo 1	Leuch	Babungo	06°03.984	010°26.823
4	Bafut 1	Nelegha	Bafut	06°05.187	010°07.094
5	Baigon 1	Reiue	Baigon	05°34.183	010°40.489
6	Bambalang 1	Leuch	Bambalang	05°53.644	010°30.814
7	Bambui 1	Neleghe-ndongbeu	Bambui	06°00.797	010°13.635
8	Bamendjou 1	Chechio	Baham	05°20.063	010°22.118
9	Bamendjou 2	Torguem	Baham	05°20.063	010°22.118
10	Bamokonbou 1	Nelio	Bafoussam	05°28.781	010°25.197
11	Bana 1	Chinchi	Bafang	05°09.365	010°10.147
12	Banga bakundu sweet 1	Sweetyam	Muyuka	04°17.314	009°24.451
13	Bangang 1	Neliola	Bangang	05°34.303	010°09.133
14	Bangang 2	Nelio	Bangang	05°34.303	010°09.133
15	Bangangté 1	Peule	Bangangté	05°08.379	010°31.406
16	Bangou 1	Torgai	Bangangté	05°08.379	010°31.406
17	Batibo 1	Aysoh	Batibo	05°50.144	009°53.467
18	Bayangam 1	Chechio	Bayangam	05°17.930	010°26.446
19	Bayangam 2	Torguem	Bayangam	05°17.930	010°26.446
20	Bekora 1	Sweetyam	Bekora	04°36.114	009°05.704
21	Bekora 2	Sweetyam	Bekora	04°36.114	009°05.704
22	Buea sweet 1	Sweetyam	Muea	04°10.149	009°18.149
23	Buea sweet white yam1	Sweetyam	Muea	04°12.295	009°19.912
24	Country yam 1	Country yam	Muyuka	04°17.314	009°24.451
25	Dschang 1	Lilio	Dschang	05°26.637	010°03.404
26	Ekona Muyuka sweet white1	Sweetyam	Muea	04°12.740	009°19.554
27	Fenkam-Foto 1	Chechio	Bafoussam	05°28.781	010°25.197
28	Fongo-Tongo 1	Nloh-nelek	Fongo-Tongo	05°30.118	009°59.976
29	Fonkouankem 1	Torga	Bafang	05°09.365	010°10.147
30	Fundong 1	Alim	Fundong	06°16.790	010°17.075
31	Guzang 1	Ndong-mbeck	Guzang	05°49.983	009°55.278
32	Ibo sweet 1	Ibo sweet	Banga Bakondu	04°24.103	009°26.522
33	Ibo sweet 2	Ibo sweet	Banga Bakondu	04°24.103	009°26.522
34	Ibo sweet 3	Ibo sweet	Banga Bakondu	04°24.103	009°26.522
35	Kumbo 1	Reeg	Kumbo	06°12.386	010°40.478
36	Lysoka sweet 1	Lysoka sweet yam	Lysoka	04°11.306	009°18.745
37	Mabondji sweet white 1	Sweet yam Mabondji	Mabondji	04°33.745	009°11.806
38	Mabondji sweet yellow 1	Sweet yam Mabondji	Mabondji	04°33.745	009°11.806
39	Mankon 1	Ndong-nebengha	Mankon	05°58.172	010°08.541
40	Mbongue sweet 1	Sweetyam	Muyuka	04°17.314	009°24.451
41	Muyuka 1	Sweetyam	Muea	04°10.149	009°18.149
42	Muyuka 2	Sweetyam	Muea	04°10.149	009°18.149
43	Muyuka 3	Sweetyam	Muyuka	04°17.314	009°24.451
44	Muyuka 4	Sweetyam	Muea	04°10.149	009°18.149
45	Muyuka 5	Sweetyam	Muea	04°10.149	009°18.149
46	Nkwen 1	Zolik	Nkwen	05°57.717	010°10.078
47	Penda-bokosweet 1	Penda-boko sweet1	Muea	04°10.149	009°18.149

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Table 2: Mann-Whitney statistics table based on Monte Carlo Permutation method and 99% confidence interval indicating the sex influence ($p < 0.001$) on Inflorescence number (InfNum); Inflorescence length (InfLg); Flower number (FlwNum); Flower length (FlwLg); Flower diameter (FlwD) and Flower density (Density).

	InfN	InfLg	FlwN	FlwLg	FlwD	Density
Mann-Whitney U	44	5	0	0	2	0
Wilcoxon W	122	49,5	78	45	47	78
Z	-0.711	-3.521	-3.839	-3.838	-3.695	-3.840
Monte Carlo Sig. (2-tailed)	0.518	0.000	0.000	0.000	0.000	0.000

Table 3: Mann-Whitney statistics table based on Monte Carlo Permutation method and 99% confidence interval indicating the sex influence on DE (Days to Emergence; $p = 0.027$), DFB (Days to Emergence of the First Bud; $p = 0.017$) and DTB-DFFO (Days to first bud emergence to First Flower Opening; $p < 0.027$)

.	DE	DFB	DFFO	DFB-DFFO	NFP
Mann-Whitney U	14	12	29	0	21
Wilcoxon W	80	33	95	66	87
Z	-1.94	-2.11	-0.40	-3.33	-1.24
Monte Carlo Sig. (1-tailed)	0.027	0.017	0.356	0.000	0.118

Table 4: Pearson correlation coefficients between morphologic traits using Pearson

	InfN	InfLg	NumF	FlwLg	FlwD
InfN	1				
InfLg	0.18	1			
	4.38E-01				
NumF	0.33	-0.41	1		
	1.50E-01	7.16E-02			
FlwLg	0.00	0.74***	-0.75***	1	
	9.97E-01	2.19E-04	1.33E-04		
FlwD	-0.05	0.63**	-0.71***	0.95***	1
	8.21E-01	2.81E-03	5.02E-04	1.52E-10	
Density	0.05	-0.63***	0.84***	-0.83***	-0.78***
	8.30E-01	3.03E-03	3.53E-06	5.61E-06	4.48E-05

Table 5: Different cultivars having flowered (F) or not (NF) during the three-years evaluation

	Year 1	Year 2	Year 3
Male cultivars			
Muyuka 3	F	F	F
Mabondjisweet white 1	F	F	F
Banga bakundu sweet 1	F	F	F
Ibo sweet 2	F	F	F
Bayangam 1	F	F	F
Buea sweet 1	NF	F	F
Lysoka sweet 1	NF	F	F
Ibo sweet 3	NF	F	F
Country yam 2	NF	NF	F
Female cultivars			

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Dschang 1	NF	F	F
Bamendjou 1	F	F	NF
Muyuka 3	NF	NF	F
Bamendjou 2	F	F	NF
Bangang 2	F	F	F
Bayangam 2	F	F	NF
Ibo sweet 1	F	F	F
Bamokonbou1	F	F	F
Bangou 1	F	F	F
Fonkouankem 1	NF	F	F
Bana1	NF	NF	F
Guzang 1	NF	F	F
Mankon 1	NF	F	F
Bambui 1	NF	F	NF
Bangang1	NF	NF	F
Country yam 1	NF	F	F
Penda-boko 1	F	NF	F
Bekora 2	F	NF	F
Baigon 1	NF	F	F
Fongo-tongo 1	F	NF	NF
Mabondji sweet yellow 1	F	NF	NF

Table 6: Binomial logistic regression based on Wald method, indicating a significant effect of the year on the flowering pattern (p = 0.03). A cultivar planted in year 2019 has 3.67 times more chances to flower than one planted in 2018

		B	S.E.	Wald	df	Sig.	Exp(B)	95,0% C.I.for EXP(B)	
								Lower	Upper
Step 1[a]	Year			5,335	2	0,069			
	Year(1)	1.3	0.60	4.74	1	0.03	3.67	1.14	11.81
	Year(2)	0.39	0.62	0.38	1	0.54	1.47	0.43	4.99
	Sex(1)	-0.99	0.58	2.92	1	0.09	0.37	0.12	1.16
	Constant	-1.15	0.47	5.9	1	0.02	0.32		

[a]*Variable(s) entered on step 1: Year, Sex.*

Table 7: Kendall correlation coefficient indicating a strong relation between flowering and sex (p = 0,047) and flowering and year (p = 0,013)

		Flower	Sex	Year
Flower	Correlation Coefficient	1		
	Sig, (1-tailed)			
Sex	Correlation Coefficient	0.18	1	
	Sig, (1-tailed)	0.046		
Year	Correlation Coefficient	0.22	0.000	1
	Sig, (1-tailed)	0.013	0.500	

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Figure 1: Floral traits: A: Female inflorescence, B: Opened female flower; C: sectionofthe female flower a: sepal; b: stigma; Cross-section, c: ovule; d: ovary

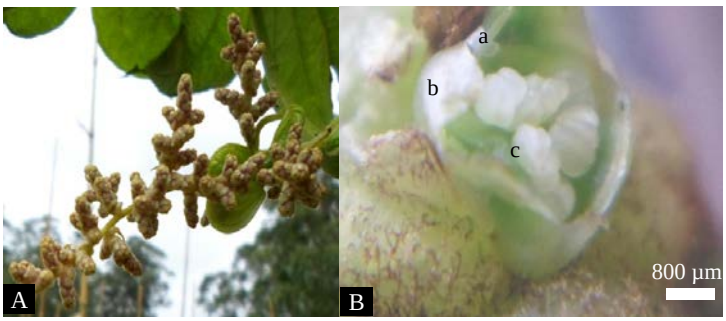

Figure 2: Floral trait, A: male inflorescence, B: opened male flower; a: sepal; b: petal; c: stamen

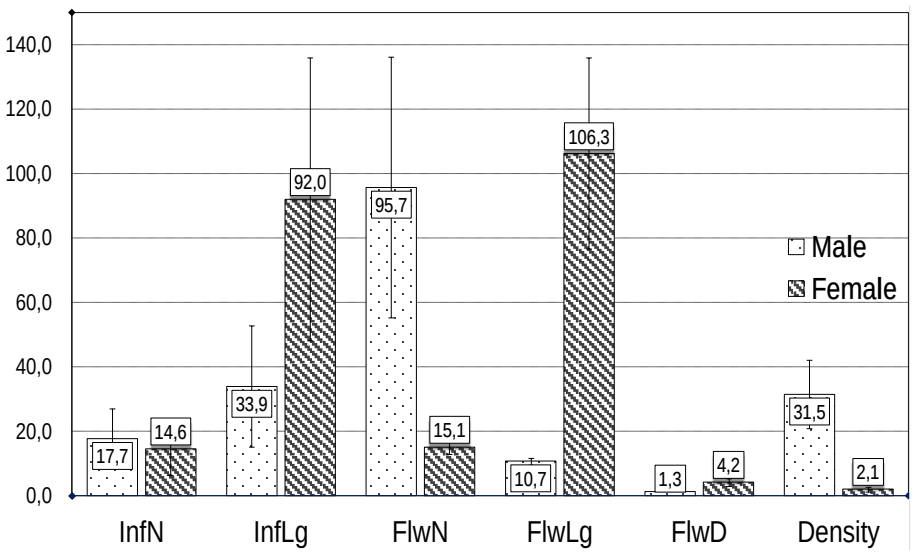

Figure 3: Male and female floral morphologic traits indicating the mean value of inflorescence number (InfN), Inflorescence length (InfLg), Flower number (FlwN), Flower length (Flwlg), Flower diameter (FlwD) and Density. Lengths are in milimeters. Bars are standard deviation. ANOVA indicades significant effect of sex on InLg, FlwN

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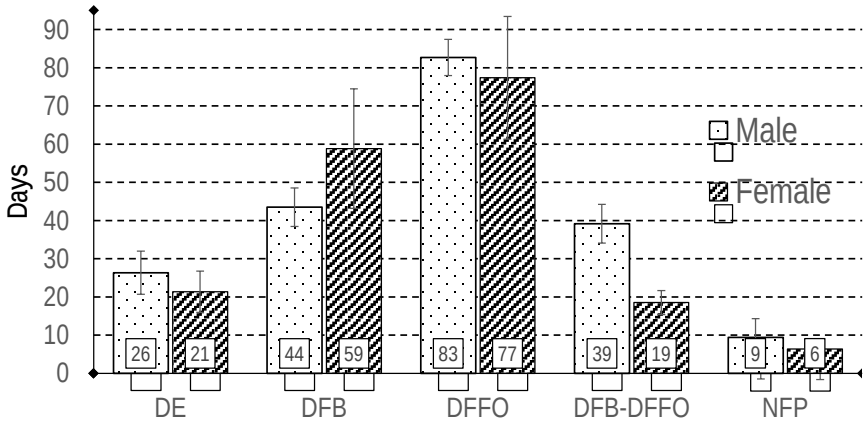

Figure 4: Flowering pattern of female and male flowers: Days to Emergence (DE), Days to first Bud Emergence (DBE), Days to First Flower Opening (DFFO), Days to emergence - First Bud emergence to First Flower Opening (DFB-DFFO) and Number of Flowering Plants (NFP). Bars indicate standard deviations.

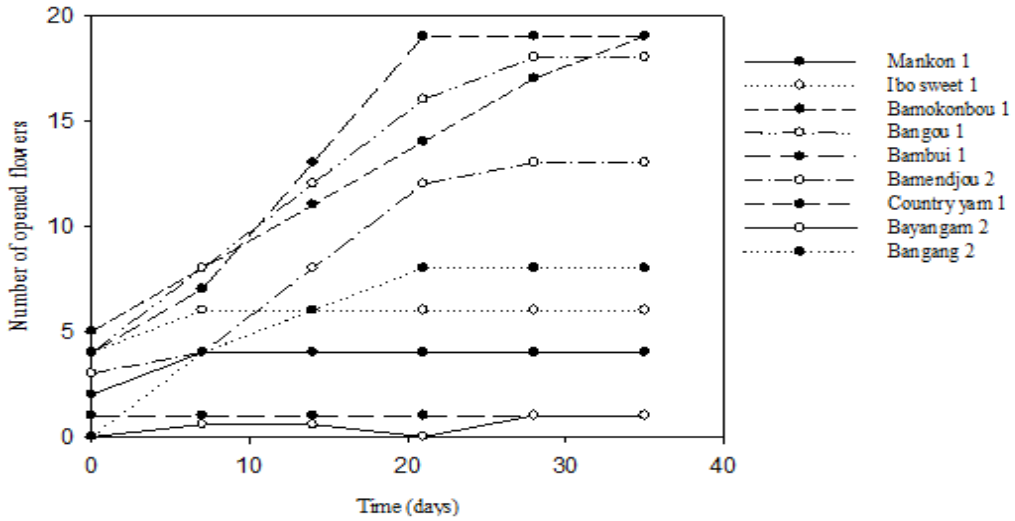

Figure 5: Number of newly opened flowers of female cultivars per week in one month.

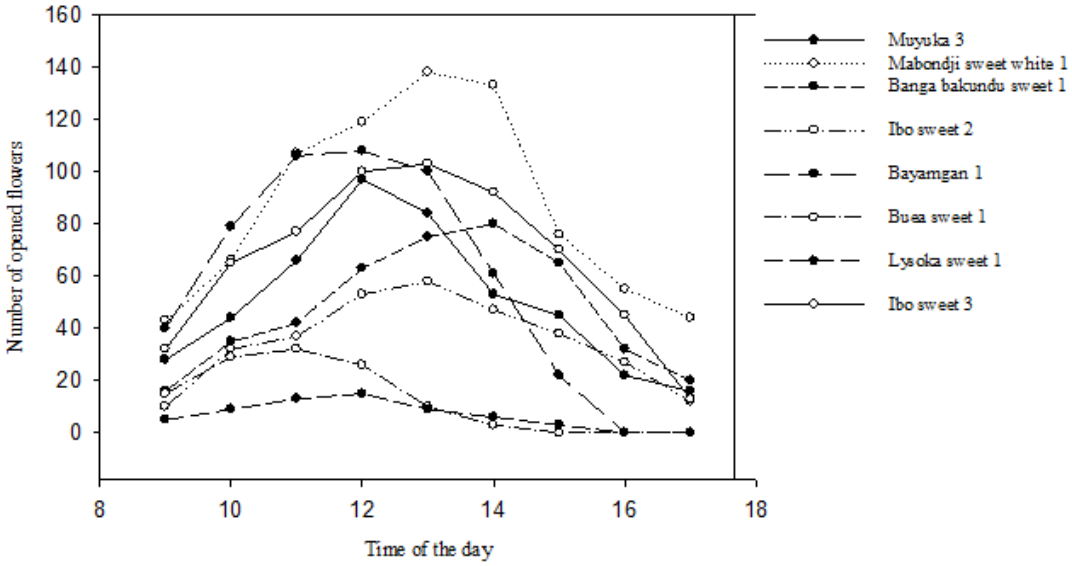

Figure 6: Number of newly opened flowers of male cultivars per hour in one day

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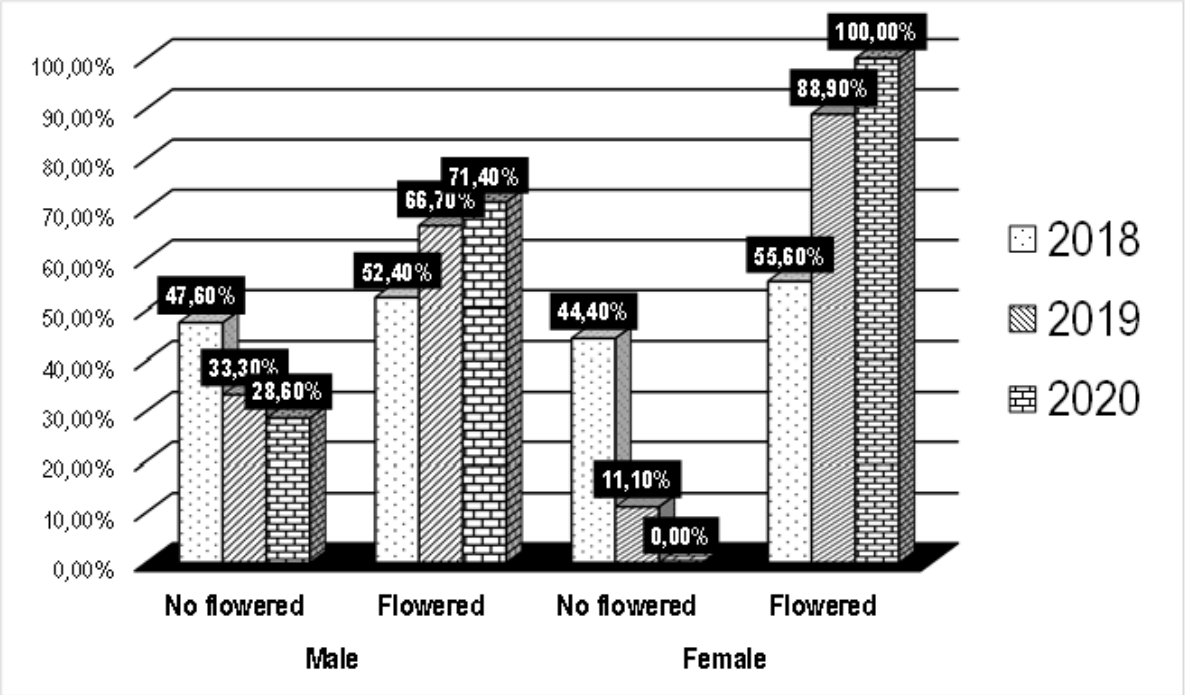

Figure 7: Flowering rate of *D. dumetorum* seed over time

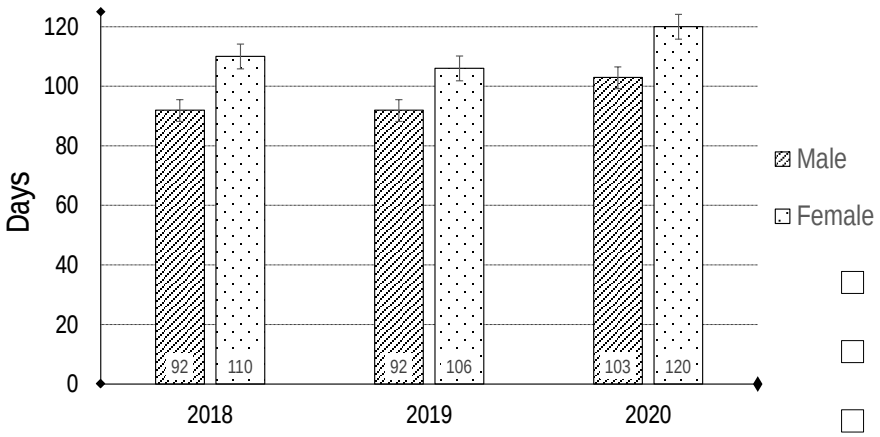

Figure 8: Maturation time of male and female flowers of *D. dumetorum* during the three years evaluation

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Figure 9: Illustration of pollination methods. Pollen collected on the anthers (a) and transferred to the stigma (b) during *D. dumetorum* pollination processes. This is done with the pollen solution (f), the bamboo splinter (e) or the metal brush (d). The pollination was followed by covering the pollinated female flowers with a white cloth (g).

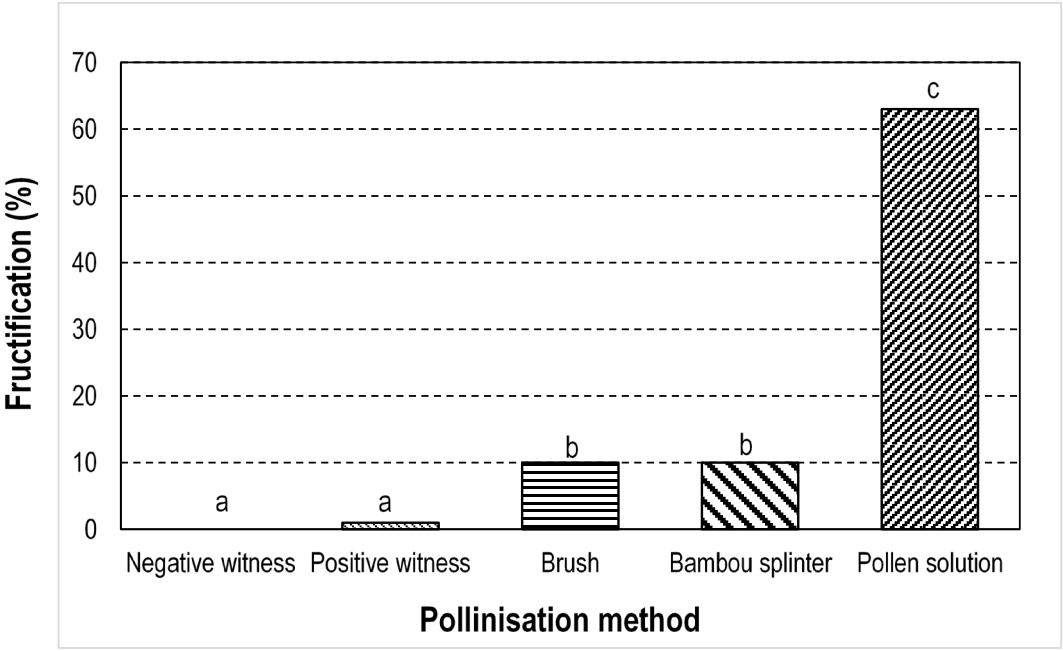

Figure 10: Fructification rate obtained using three pollination methods

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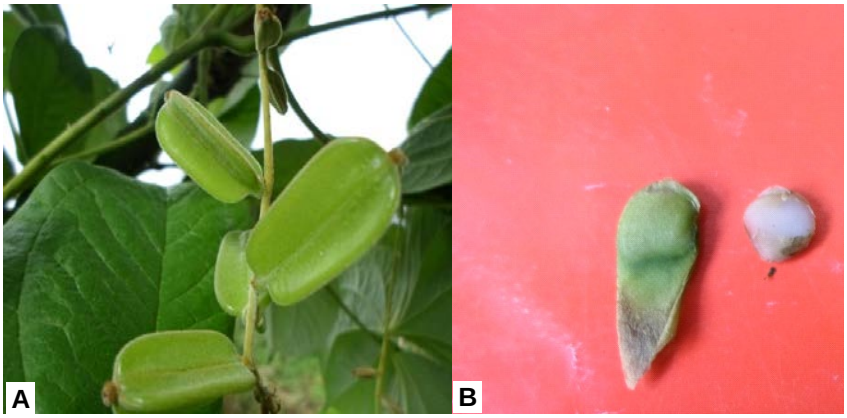

Figure 11: Fruit of *D. dumetorum*. A, Fruit; B, Seeds.

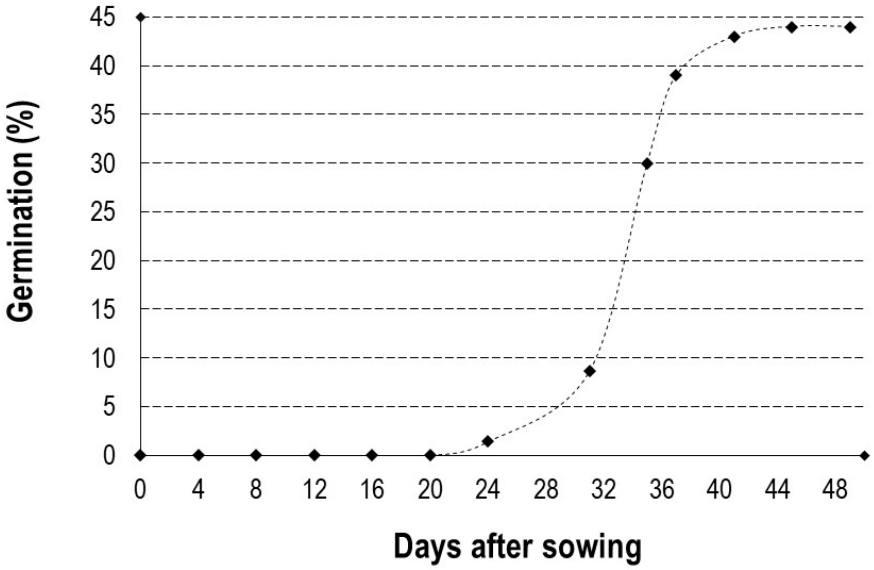

Figure 12: Germination rate curve of *D. dumetorum* seeds versus time



Figure 13: A- Mature seeds of *D. dumetorum*and B –hybrid seedlings at three stages of growth

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We typeset manuscripts using advanced typesetting tools like Adobe In Design, CorelDraw, TeXnicCenter, and TeXStudio. We usually recommend authors submit their research using any standard format they are comfortable with, and let Global Journals do the rest.

Alternatively, you can download our basic template from https://globaljournals.org/Template.zip

Authors should submit their complete paper/article, including text illustrations, graphics, conclusions, artwork, and tables. Authors who are not able to submit manuscript using the form above can email the manuscript department at submit@globaljournals.org or get in touch with chiefeditor@globaljournals.org if they wish to send the abstract before submission.

Before and during Submission

Authors must ensure the information provided during the submission of a paper is authentic. Please go through the following checklist before submitting:

1. Authors must go through the complete author guideline and understand and *agree to Global Journals' ethics and code of conduct,* along with author responsibilities.
2. Authors must accept the privacy policy, terms, and conditions of Global Journals.
3. Ensure corresponding author's email address and postal address are accurate and reachable.
4. Manuscript to be submitted must include keywords, an abstract, a paper title, co-author(s') names and details (email address, name, phone number, and institution), figures and illustrations in vector format including appropriate captions, tables, including titles and footnotes, a conclusion, results, acknowledgments and references.
5. Authors should submit paper in a ZIP archive if any supplementary files are required along with the paper.
6. Proper permissions must be acquired for the use of any copyrighted material.
7. Manuscript submitted *must not have been submitted or published elsewhere* and all authors must be aware of the submission.

Declaration of Conflicts of Interest

It is required for authors to declare all financial, institutional, and personal relationships with other individuals and organizations that could influence (bias) their research.

Policy on Plagiarism

Plagiarism is not acceptable in Global Journals submissions at all.

Plagiarized content will not be considered for publication. We reserve the right to inform authors' institutions about plagiarism detected either before or after publication. If plagiarism is identified, we will follow COPE guidelines:

Authors are solely responsible for all the plagiarism that is found. The author must not fabricate, falsify or plagiarize existing research data. The following, if copied, will be considered plagiarism:

- Words (language)
- Ideas
- Findings
- Writings
- Diagrams
- Graphs
- Illustrations
- Lectures

- Printed material
- Graphic representations
- Computer programs
- Electronic material
- Any other original work

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

Changes in Authorship

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

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

Acknowledgments

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

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Preparing your Manuscript

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

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

Manuscript Style Instruction (Optional)

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

Structure and Format of Manuscript

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

A research paper must include:

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

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

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

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

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



Format Structure

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

All manuscripts submitted to Global Journals should include:

Title

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

Author details

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

Abstract

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

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

Keywords

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

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

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

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

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

Numerical Methods

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

Abbreviations

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

Formulas and equations

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

Tables, Figures, and Figure Legends

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

Figures

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

Preparation of Eletronic Figures for Publication

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

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

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Tips for Writing a Good Quality Science Frontier Research Paper

Techniques for writing a good quality Science Frontier Research paper:

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

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

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

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

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



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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Informal Guidelines of Research Paper Writing

Key points to remember:

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

Final points:

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

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

The discussion section:

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

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

General style:

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

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



Mistakes to avoid:

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

Title page:

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

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

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

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

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

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

Approach:

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

Introduction:

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

The following approach can create a valuable beginning:

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

Approach:

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

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

Procedures (methods and materials):

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

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

Materials:

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

Methods:

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

Approach:

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

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

What to keep away from:

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



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Results:

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

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

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

Content:

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

What to stay away from:

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

Approach:

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

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

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

Figures and tables:

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

Discussion:

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

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

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

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

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

Approach:

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

Describe generally acknowledged facts and main beliefs in present tense.

The Administration Rules

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

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

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

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

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

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

Index

A

Amalgamations · 4
Amphetamine · 8
Anomura · 18

C

Calipers · 45
Carapace · 21
Clibanarius · 18
Clinacanthus · 36, 37, 39

D

Dehiscence · 48
Diogenidae, · 18

E

Elution · 5, 6, 7

H

Hitherto · 8, 18, 19

I

Immunoassay · 8
Iterations · 8

L

Longitarsus · 18
Lanceolate · 45

M

Mangrove · 18, 23, 24
Meiotic · 31, 33

P

Paguridae · 18, 22, 23, 24, 25
Plentiful · 36, 37
Pollination · 43, 47, 48, 49, 50
Pondered · 1
Posttraumatic · 7
Proteome · 7, 9

S

Splinter · 43, 45, 48, 49, 59
Stamens · 45
Staphylococcus · 36, 37, 38, 39, 40, 41
Synovial · 7, 11

V

Vacuolization · 39





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