BIOINFORMATICS: AN EMERGING TOOL FOR BIOLOGICAL SCIENCE

Abstract

Recent advances in technology have accelerated the understanding of the genetic basis of phenotypes. With these developments, genomics has altered the way that biological challenges are thought about on a genome-wide (genome-wide) scale, revealing a large amount of information and creating a myriad of opportunities. One of these recently developed fields is bioinformatics, which uses the concept of computer Science, mathematics, molecular biology and statistics to store, retrieve & analyze biological data. Even though it is still in its infancy, it has quickly emerged as one of the field with the quickest growth rates and established itself as a crucial part of any biological research program. It is becoming more well-known because of its capacity to quickly and affordably analyze vast amounts of biological data. A biologist can use a variety of web- and/or computer-based tools provided by bioinformatics to extract valuable information from biological data, the majority of which are free to use. This introductory chapter aims to provide overall picture on basics and advancement in the field of bioinformatics benefitting readers in various fields of Biological science.

Keywords: Genomics, Genome-wide, Bioinformatics, database, molecular biology

Authors

Rabiya Parveen
Department of Plant Breeding and Genetics
Bihar Agricultural University
Sabour, Bhagalpur, Bihar, India
rparveen181@gmail.com

Mankesh Kumar
Department of Plant Breeding and Genetics
Bihar Agricultural University,
Sabour, Bhagalpur, Bihar, India

Zafar Imam
Department of Plant Breeding and Genetics
Bihar Agricultural University
Sabour, Bhagalpur, Bihar, India

Digvijay Singh
Department of Plant Breeding And Genetics, Bihar Agricultural University
Sabour, Bhagalpur, Bihar, India

Swapnil
Department of Genetics And Plant Breeding
Centurion
University of Technology and Management
Paralakhemundi, Odisha, 761211, India

Abhinav Kumar
Department of Plant Breeding And Genetics, Bihar Agricultural University
Sabour, Bhagalpur, Bihar, India

I. INTRODUCTION

Due to enormous advancements in the domains of genomics and molecular biology, the amount of biological information has greatly increased during the genomic era. Since its inception in the 1980s, the field of bioinformatics has expanded quickly in tandem with the expansion of genome sequence data. In order to develop methods for the retrieval, storage and analysis of biological data, multiple diverse fields of study—including computer Science, mathematics, molecular biology and statistics—were combined to form the interdisciplinary field of study known as bioinformatics [32]. The amount of molecular data collected from multiple levels of organization of an environmental sample or organism has significantly increased as a result of the quick adoption of omics techniques, their expanding power, and more affordable costs. With the development of Next-Generation Sequencing (NGS) technologies, the sequencing of nucleic acids underwent yet another revolution, ushering in a new era for omics techniques.

Paulien Hogeweg is credited with the creating the term "bioinformatics" (1979). Since the establishment of SWISS- MODEL server and the introduction of user-friendly interactive automated modeling about 18 years ago [37], this field has expanded tremendously. Since then, using databases and informatics at the backend to process biological data at a much faster rate has been crucial to the biological sciences. Large-scale biological data management, analysis, and manipulation fall under the purview of the field, which includes all computational tools and techniques [27]. To organize, store and index the massive amount of sequence data generated by various sequencing techniques, electronic databases are required. The databases also require specific tools so that researchers may access, analyze, and add fresh or updated sequencing data. In order to identify the structural quirks and relations of molecular sequences that are essential for structural biology & the creation of pharmaceuticals, bioinformatics tools can be employed for restoration, folding, pattern recognition, molecular modeling and simulation. [40]. It is difficult to manually examine the enormous, molecular sequence, genome-derived studies of raw "Big Data." [30].

II. DEVELOPMENT OF BIOINFORMATICS

The networking of computers and the collection of data on genes and proteins marked the beginning of the development of bioinformatics. In 1956, 51 amino acid residues of bovine insulin were described as the first protein sequence. A few years after the first protein sequence became accessible; the first bioinformatics database was built. Physical chemist Margaret Dayhoff, who lived in America from 1925 to 1983, was a pioneer in the use of computational techniques in the field of bioinformatics. She gathered all the sequence data that was available and created the "Atlas of Structure and Protein Sequence," the first bioinformatics database. David J. Lipman, a former director of NCBI, referred to Dayhoff as "the mother and father of bioinformatics" because of her contribution to the creation of algorithms that can recognize and shows the structures for use in X-ray crystallography as well as computational approaches for protein sequence assessment that allow us to deduce the evolutionary relationships between kingdoms [21,50]. Dayhoff also created one-letter amino acid code that is still in the use at present day, as well as three-letter abbreviation (for example lysine as Lys, serine as Ser) (Table 1). For the first time, one-letter code was used in the Dayhoff and Eck's ‘Atlas of Protein Sequence and Structure’ (1965) [9], the first biological sequence database.

Table 1: Symbols used to represent amino acid in Protein Sequences

Single letter code	Amino acid	Three letter code
A	Alanine	Ala
B	Asparagine or Aaspartic acid	Asx
C	Cystine	Cys
D	Aspartic acid	Asp
E	Glutamic acid	Glu
F	Phenylalanine	Phe
G	Glycine	Gly
H	Histidine	His
I	Isoleucine	Lie
K	Lysine	Lys
L	Leucine	Leu
M	Methionine	Met
N	Asparagine	Asn
P	Proline	Pro
Q	Glutamine	Gln
R	Arginine	Arg
S	Serine	Ser
T	Threonine	Thr
V	Valine	Val
W	Tryptophan	Trp
X	Any amino acid	Xaa
Y	Tyrosine	Tyr
Z	Glutamine or Glutamic acid	Glx

The foundation of the Indian Institute of Science in 1990 and the Bioinformatics Institute of India in 2002 helped in the field of bioinformatics to expand it in India during the 1980s. GN Ramachandran is considered as the god father of Indian bioinformatics. The Department of Biotechnology gave a plan in 2004 to make India a centre for bioinformatics worldwide.

George Bell and associates began compiling DNA sequences into GenBank in 1974 in order to provide immunology research with a theoretical foundation. Between 1982 and 1992, Walter Goad's team [32] produced the first version of GenBank. As a result of their efforts, the most widely used DNA sequence databases GenBank [17], "The European Molecular Biology Laboratory (EMBL) [45], and DNA DataBank of Japan (DDBJ) [8] were made in 1979, 1980 and 1984 respectively. The most significant development in DNA sequence databases was the introduction of web-based search engines, which allow investigator to locate and evaluate the target DNA sequences. These pioneer inventions were made by David Lipman, David Benson and their associates, who also developed the software programs "GENEINFO" and "Entrez" [32]. Researchers may quickly search database-indexed sequences and compare them to the sequence they were querying by utilizing this application. Software is now easily accessible through web-based interface of NCBI database's web-based interface [46]. Comparison, analysis and visualization of molecular sequences have become more sophisticated, and a variety of techniques have helped to advance bioinformatics in this field.

III. BRANCHES OF BIOINFORMATICS

Gene products have come into emphasis since the first draught of the human genome was finished [23, 49] instead of genes themselves. Genetic information is given a functional relevance in functional genomics. When analysing and interpreting biological data, information is taken into account at different levels, including the genome, proteome and transcriptome. Transcriptomics is the study of the messenger RNA transcripts generated by a cell, whereas proteomics is the study of the total number of proteins (proteome) expressed by a cell (transcriptome).

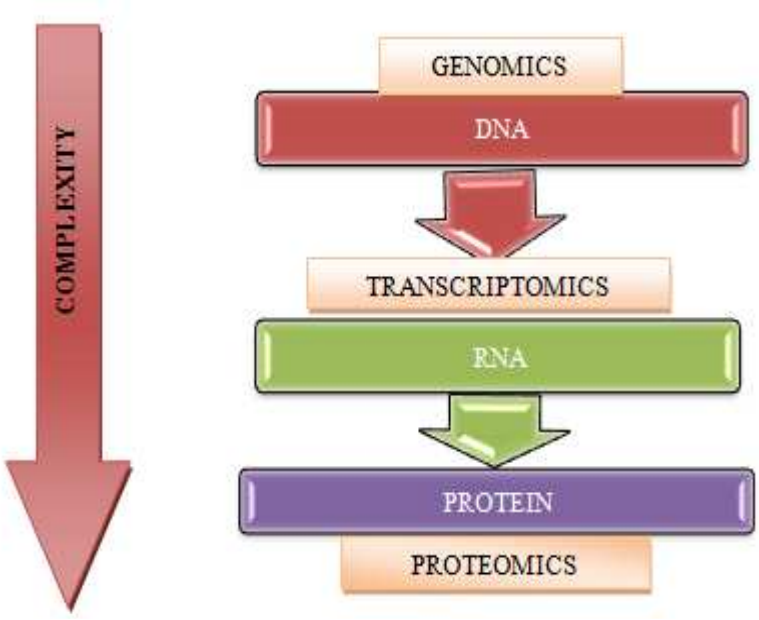


Figure 1: Flow diagram representing complexity of genomic data processing (Source: Bayat A, 2002)

Gene is considered as the basic unit of heredity responsible for the transmission of traits from one generation to the next and is stored in a genome as DNA molecules. Generating detailed physical and genetic maps of the genome in order to designate segments with ever higher resolution and sequentially organize the segments is the primary goal of genome sequencing. [18]. A method known as the direct shotgun methodology is also used for genome sequencing [48]. The goal of this method is to split the genome into overlapping, random fragments and then use computer algorithms to sequence the fragments and put these sequences together. Analysis of genomic sequences reveal that every organism possesses an array of genes essential for basic metabolic processes and also the genes whose products determine the specialized function of an organism. Complete genome sequencing confer better understanding of a particular gene and protein expression based on the information obtained by evaluating the genome sequences. Complete genome sequencing offers a foundation upon which to construct knowledge about the expression of genes and proteins [29], but it is not always adequate to describe all of the protein constituents of an organism. In proteomics, the amino acid sequence of a protein is analyzed to determine its three-dimensional structure and to link it to the protein's specific function. Applications of bioinformatics in the field of proteomics included the study of amino acid sequences, the discovery of protein binding partners, the detection of polymorphism, splice variants, and

post-translational modifications. The major technologies in the field of proteomics include two-dimensional gel electrophoresis, mass spectrometry, and protein microarrays. Understanding and extracting information from the data generated by these instruments requires the use of bioinformatics techniques. The functional study entails metabolic pathway reconstruction and simulation, protein-protein interaction prediction, protein sub-cellular localization prediction, and gene expression profiling [42, 52]. These aspects of bioinformatics analysis are not isolated, but often interact to produce integrated results.

IV. DATABASES

Biological databases are collections of biological sciences gleaned from high throughput experimentation techniques, published literature, and computational analysis. Storage and management of biological data and information in computer accessible forms is the primary goal of biological databases [2]. An integrated collection of computer software known as a "database management system (DBMS)" allows access to the information in the databases. By using this software users get access to all of the data stored in the databases. The data that is stored can be used as primary source as well as for future use. As a result of the range of information that they store, databases are divided into three categories: *primary*, *secondary*, and *composite*.

1. **Primary Sequence Database(s)** are those that serve as repositories for raw sequence data and may be freely accessed via the Internet contain information on the sequences or structure alone (WWW). For example, the DNA Databank of Japan (DDBJ) for genome sequence, the Nucleotide Sequence Database operated by the European Molecular Biology Laboratory (EMBL), and GenBank, maintained by the National Center for Biotechnology Information (NCBI).

   It gathers distinctive data from the lab and makes these data available to the users without any change. Each data when entered has their unique accession number through which data can be later retrieved.

2. **Secondary sequence database(s)** consists of information that was obtained via the study of data found in primary databases, such as conserved sequences, a protein family's active sites, or conserved secondary motifs [14,19]. The primary database is subjected to computational algorithms, and the secondary database contains useful and instructive data. Some databases, including SCOP made at Cambridge University, CATH created at University College London, eMOTIF created at Stanford, etc., are built and hosted by individual researchers in their own labs.

3. **Composite database(s)** contains a range of primary database sources, eliminating the need to look up information at several places. Nucleotide and protein databases are provided by the National Center for Biotechnology Information (NCBI), on its vast, highly accessible network of computer servers.

Table 2: List of Important Databases

Database	Description	References
Nucleotide Databases		
DNA Databank of Japan (DDBJ)	It is one of the largest databases for nucleotide sequences and a member of the International Nucleotide Sequence Databases (INSD).	[8]
European Molecular Biology Laboratory(EMBL)	Repository of DNA and RNA sequences that is complementary to GenBank and DDBJ	[45]
GenBank	It belongs to the international nucleotide sequence databases (INSD). NCBI's primary nucleotide sequencing database in the USA	[17]
Protein Databases		
Uniprot	One of the largest collection protein sequences	[47]
Protein DataBank	A significant source of information on proteins, giving details on the complex assemblages of nucleic acids, proteins, and their empirically confirmed structures	[5]
Prosite	Gives details on the active sites, conserved domains, and protein families.	[43]
SWISS-PROT	A division of the UniProt knowledge repository where protein sequences have been manually annotated.	[6]
InterPro	Resource that includes information on conserved domains, protein families and active sites.	[41]
SCOP	Relationship between Familial and Structural Proteins	[16]
Genome Database		
Ensemble Plants	A comprehensive database that provides genome-scale data for an increasing number of sequenced plant species.	[15]
Protein Information Resource (PIR)	Non-redundant, comprehensive, and annotated protein sequence database	[51]
Phytozome	A comparative hub for comparison and research of plant genome and gene family information.	[20]
Miscellaneous databases		
The Arabidopsis Information Resource (TAIR)	For the model plant Arabidopsis thaliana, TAIR keeps a collection of molecular and genetic information.	[44]
Kyoto Encyclopedia of Genes and Genomes (KEGG)	The KEGG is a knowledge repository that connects genomic information with advanced functional data to analyse genes' activities systematically.	[25]

V. GENE IDENTIFICATION AND SEQUENCE ANALYSES

Understanding the various characteristics of a biomolecule, such as a protein or nucleic acid that give it its particular function is referred to as a sequence analysis. The sequences of the associated compounds are first acquired from open databases. After refinement, if required, they are subjected to various tools that enable prediction of their more

precise features. The analysis can be used to recognize introns, exons, transit peptide or an open reading frame (ORF), as well as to identify specific variable regions that can be used as an indicator for diagnostic purposes. It can also be used to identify promoter, terminator or un-translated regions involved in the expression regulations. There are many tools designed for this purpose, some of which are crucial (enlisted in Table 3 with function).

Table 3: Tools for Primary Sequence Analysis

Tools	Description
BLAST (Local Sequence Alignment)	It is a search tool that is used to identify DNA or protein sequences.
Clustal Omega (Global Sequence Alignment)	This program can be used to conduct multiple sequence alignments.
HMMER	With the use of this tool, homologous protein sequences can be searched in the relevant databases
Open Reading Frame (ORF) Finder	This tool can be used to find ORF for putative genes.
ProtParam	used to forecast proteins' physicochemical qualities
Prokaryotic Promoter Prediction (PPP)	tool used to determine the promoter sequences that are present upstream of the gene
JIGSAW	To identify the genes, and to find out the splicing sites in the selected sequences
Genscan	makes use of genomic sequences to determine the intron-exon locations
Softberry Tools	Along with the prediction of structure as well as function of RNA and proteins, some technologies are specialized in the annotation of bacterial genomes, plants and animals.

VI. SEQUENCE ALIGNMENT

The increased accessibility of data produced by NGS technologies has processed alignment, which is widely utilized and crucial for comparing different biological sequences [12]. Comparing two or more nucleotide sequences (DNA or RNA) or amino acid sequences (peptides or proteins) entails looking for specific features or patterns that are ordered in the sequences as well [24,28]. The identification of point mutations, the creation of evolutionary trees, the categorization of genes and proteins, biological function prediction, the classification of genes and proteins, and other activities all rely on sequence similarity. Indicators of how conserved a given section or sequence motif is throughout lineages can be made using the degree of similarity between amino acids at a specific place in a protein's sequence.

Sequence alignment seeks to reduce mismatches and gaps while maximizing the number of matches. Given an alignment and a scoring system, the alignment with the highest alignment score is the best alignment. A scoring system includes two components. The first is a match/mismatch matrix that details how many points are added for matches and subtracted for mismatches. The second is gap penalty which specifies how gaps are penalized by deducting points. Three scores are given in the simplest scenario: (1) the cost of aligning a

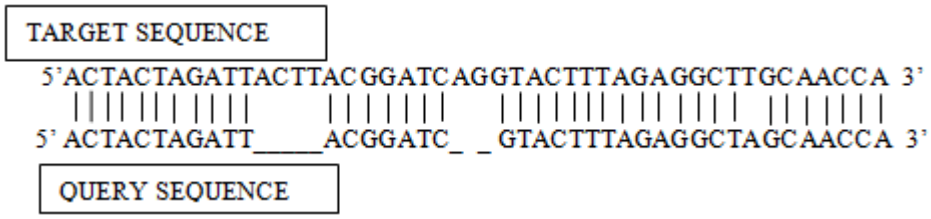
trait in one sequence with a gap in the other sequence (2) The advantage of aligning a pair of sites that have the same character (state) in both sequences. (3) The cost of aligning a pair of sites that include different characters.

Sequence Alignment Types:

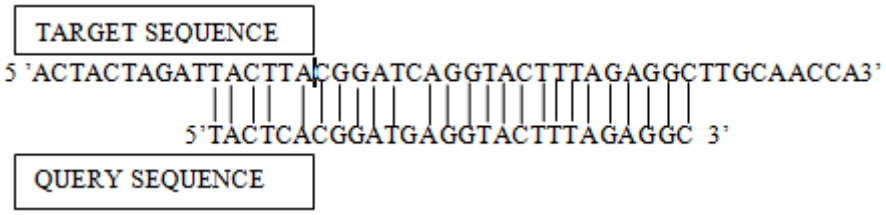
Based on Number		Based on Length
1	Pairwise Sequence Alignment	Global Sequence Alignment
2	Multiple Sequence Alignment	Local Sequence Alignment

1. **Pairwise Sequence Alignment (PSA):** Pairwise sequence alignment is a form of sequence alignment, where we compare only two sequences. A type of sequence alignment called pairwise comparison compares just two sequences. This procedure entails determining the best alignment between the two sequences, scoring according to how similar or distinct they are, and determining the importance of this score. Each pair of sequences is aligned by PSA once. The rapid search for matches to a query sequence in large DNA and protein databases is one particular application of pairwise sequence alignment .Algorithms based on dynamic programming are substantially slower than well-known heuristic algorithms as those from the FASTA [36] or BLAST families. It is the most basic type of alignment and can be carried out using either a *global* or *local* sequence alignment approach.

    - **Global Sequence Alignment:** The ideal sequences for global alignment are those that are closely related and have the same length. Here, the alignment is carried out from the start of the sequence to the end in order to find the optimal alignment. Saul B. Needleman and Christian D. Wunsch created 'The Needleman-Wunsch algorithm', also referred to as dynamic programming for sequence alignment in 1970 (33). Global alignments are generally used to compare two proteins with similar functions or homologous genes with the same function. The global sequence alignment is described in the algorithm for aligning nucleotide or protein sequences.



    - **Local Sequence Alignment:** Smith and Waterman were the one who first proposed the fundamental local alignment method (1981b). Similar to the Needleman-Wunsch algorithm, this method is likewise based on dynamic programming, but with more options to start and terminate at any location (38). It identifies nearby areas with a high degree of resemblance. Any two sequences can be locally aligned because local alignment identifies sequence stretches with a high level of similarity without taking the alignment of the other sequence regions into account.



2. **Multiple Sequence Alignment:** Multiple sequence alignment, or MSA, is a technique used to align three or more biological sequences (protein or nucleic acid) of comparable length. The results allow us to study the evolutionary relationships between sequences and the inference of homology. A known phylogenetic tree was required for early alternate methods for multiple alignments. Progressive alignment is the method for multiple sequence alignment that eventually gained a lot of traction [12,13]. In progressive alignment, one typically begins by creating all feasible pairwise alignments (there are n (n-1)/2 pairs for n sequences). Using a distance-based procedure like the un-weighted pair group method with arithmetic mean (UPGMA) or neighbor joining, these pairwise alignments are utilized to estimate a phylogenetic tree. A pairwise approach is used to match the most comparable sequences to one another using the tree as a guide. On the basis of the phylogenetic tree's structure, one then gradually adds sequences to the alignment, one sequence at a time.

   Higgins created CLUSTAL series of programs, which employs a progressive algorithm, is one of the most effective MSA solutions because it uses heuristic methods with approximate approaches [12].

VII. DYNAMIC PROGRAMMING

The application of dynamic programming results in the best alignment of two sequences. It discovers the alignment in a more quantitative way rather than just applying dots by awarding specific values for fits and mismatches (Scoring matrices). The greatest scores in the matrix can be used to precisely locate alignment.

1. **Substitution matrices :** Sequence alignment using pairwise and multiple sequence alignment techniques is scored using substitution matrices. Since all bases experience equal amounts of mutation, the score matrices used for nucleotide sequence alignment are quite simple. A match is given a positive or higher value, whereas a mismatch is given a negative or lower value. The matrices can be scored using these scores based on assumptions. Point Accepted Mutation (PAM - Dayhoff 1978) and Blocks Substitution Matrix are two common protein substitution matrix models (BLOSUM - Henikoff and Henikoff 1992).

2. **PAM matrices:** Margaret Dayhoff invented the PAM matrix, also known as the Point Accepted Mutations matrix. PAM matrices are computed using differences in proteins that are closely related to one another. For every 100 amino acid residues, one PAM unit (PAM1) indicates one allowable point mutation, implying that only 1% of the original structure is changed.

3. **BLOSUM:** Henikoff and Henikoff's created BLOcks SUbstitution Matrix in 1992, which utilizes conserved regions. Actual percent identity values make up these matrices. Blosum 62 means there is 62 % similarity.

4. **Gap (-)** representing one or more nucleotide indel events.

The algorithm that was utilized can be categorized as either optimum or heuristic [38]. The best alignment is the ideal outcome, but the heuristic, while not delivering an optimal result, displays the best alignment within a specific time window of analysis.

VIII. BLAST

The Basic Local Alignment Search Tool (BLAST) is the most popular similarity search tool. Region of similarity between sequences are discovered via BLAST. The software compares protein or nucleotide sequences and determines the statistical importance of matches. BLAST can be used to infer functional and evolutionary relationship between sequences besides identifying members of gene families.

The Smith-Waterman method (1981) was the source of the specialized local alignment algorithm known as BLAST, which displays a maximum alignment score of two sequences [1]. To search the sequences in the database, BLAST uses a heuristic based on the *k*-tuple approach in addition to the dynamic programming resulting from the aforementioned algorithm [24]. The *k*-tuple method restricts the search to words that are more significant, which are words with a length of 3 or 11 characters for amino acids and nucleotides, respectively. BLAST is a group of software tools that can be used for a variety of tasks depending on the sequence type of interest and the database being searched [39]. Table 4 lists a few applications that are accessible by BLAST.

Table 4: Description of BLAST family programs

Program	Query	Subject
BLASTx	nt*	aa
BLASTn	nt	nt
BLASTp	aa	aa
tBLASTn	aa	nt*
tBLASTx	nt*	nt*

nt: nucleotide, aa: amino acid, *Translated for all possible sequences [1]

The value of the score (Score bits) and the E-value are the two parameters through which the BLAST results are displayed. When the alignment score and database size are taken into account, the E-value is a statistical value that shows the likelihood that the alignment did not happen at random [39, 1]. However, the algorithm assigns the score based on the similarities and differences between the input sequences and the database [1].

IX. PHYLOGENETIC ANALYSES

Due of the shared requirement of determining sequence similarity, phylogenetics and sequence alignment are closely related fields [35]. Sequence alignments are frequently used in the study of phylogenetics to build and understand phylogenetic trees, which are used to categorize the evolutionary relationships between homologous genes found in the genomes of different species. Sequences' evolutionary separation from one another is qualitatively connected to how much they differ from one another. The simplest approaches use distance matrices, like un-weighted pair group method with arithmetic mean (UPGMA) or neighbor joining (NJ).

Table 5: Description of Tools to study Phylogenetic Relationship

Tools	Description
MEGA (Molecular Evolutionary Genetics Analysis)	constructs phylogenetic trees to investigate evolutionary proximity
PAML	A collection of applications for phylogenetic analyses of DNA or protein sequences using maximum likelihood.
PHYLIP	A package for phylogenetic studies
TreeView	Software that allows t switch between different views of the phylogenetic trees.
itol (Interactive Tree of Life)	An online tool for managing, displaying, and annotating phylogenetic trees.

X. APPLICATION OF BIOINFORMATICS

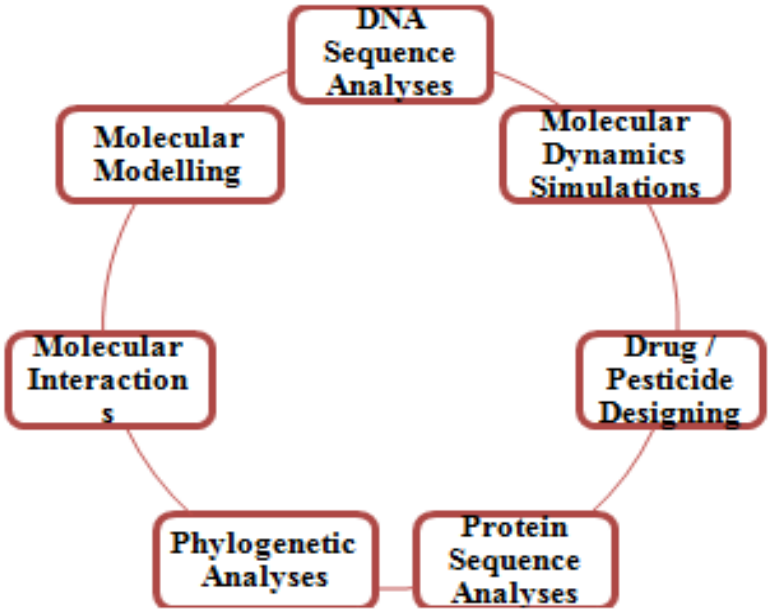


Figure 2: Basic bioinformatics tools of biological sciences. (Source: Mehmood et al 2014)

Table 6: Application of basics Bioinformatic tools in various areas of Biological Sciences

DNA Sequence Analyses	• BLAST • Clustal X • Gene Prediction • Regulatory Elements • Promoter Analyses • Intron - Exon finding • Primer Designing • Codon Usage Optimization • Virtual Translation
Molecular Dynamic Simulations	• Protein-DNA Simulations • Drug-DNA Simulation • Protein-Ligand Simulation
Drug / Pesticide Designing	• Target identification • Target validation • Lead identification • Lead optimization • ADMET prediction
Protein Sequence Analyses	• amino acid composition, Molecular mass, pI • Motifs and Domain search • Single peptide identification • Secondary structure analyses
Phylogenetic Analyses	• Reconstruction of evolutionary history • Tracking gene flow • Identification of conserved regions
Molecular Interactions	• Protein-Protein docking • Finding inhibitors and activators of protein • Protein-DNA interactions • Transcriptional factors identification • Interaction between Protein and Ligand

XI. CONCLUSION

The emerging discipline “Bioinformatics” is the solution for the current demand in the every field of plant research. Sequence analysis, data mining, gene discovery, the development of phylogenetic trees, the prediction of protein structure and function, interaction networks, and many more computational approaches are used in this field. The field of bioinformatics will play a significant role in plant research. If plant science could be summed up in one word, it would be "integration," barring any unforeseen circumstances. The link that will enable all of these forms of integration will come from bioinformatics. For a better understanding of functional and expression-related issues in a specific gene family, specific cellular process, or any specific plant disease, the increasing number of databases in combination with tools offering a targeted dataset and extensive annotation to the omics technology is highly helpful. The outcomes of genetic research will revolutionize the medical

industry. As a result of genomic status, diagnostic methods will change quickly and may now concentrate on the association between genotypes and complicated phenotypes with the use of bioinformatics. This will advance the discipline of bioinformatics since the future generation of biologists will need to be equally at ease using a computer workstation as they are using laboratory benches.

XII. REFERENCES

[1] Amaral AM, Reis MS and Silva FR (2007). O programa BLAST: guia prático de utilização. 1st edn. Embrapa Recursos Genéticos e Biotecnologia. EMBRAPA, Brasília.
[2] Attwood TK, Gisel A, Eriksson, NE, Bongcam-Rudloff E (2011) Concepts, Historical Milestones and the Central Place of Bioinformatics in Modern Biology: A European Perspective". *Bioinformatics - Trends and Methodologies*. InTech.
[3] Bayat A. (2002) Bioinformatics: Science, Medicine and the Future. British Medical Journal.; 324; 1018-22
[4] Benton D. (1996) Bioinformatics-principles and potential of a new multidisciplinary tool. *Trends Biotech*; 14:261-312.
[5] Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, et al. (2000) The Protein Data Bank. Nucleic Acids Res 28: 235-242.
[6] Boeckmann B, Bairoch A, Apweiler R, Blatter MC, Estreicher A, et al. (2003) The SWISS-PROT protein knowledgebase and its supplement TrEMBL in 2003. Nucleic Acids Res 31: 365-370.
[7] Bostan H, Chiusano ML. (2015) NexGenEx-Tom: a gene expression platform to investigate the functionalities of the tomato genome. BMC Plant Biol.;15(1):48.
[8] DataBank of Japan [Internet].(2016). http://www.ddbj.nig.ac.jp. Accessed: 2016-03-10
[9] Dayhoff MO. (1965) National Biomedical Research Foundation. *Atlas of Protein Sequence and Structure*, Vol. 1. Silver Spring, MD: National Biomedical Research Foundation.
[10] Dayhoff, M.O., Schwartz, R.M., Orcutt, B.C., (1978). A model of evolutionary change in proteins. In: Dayhoff, M.O. (Ed.), Atlas of Protein Sequence and Structure. National Biomedical Research Foundation, Washington D.C., pp. 345-352.
[11] Daugelaite J, O' Driscoll A and Sleator RD (2013). An overview of multiple sequence alignments and cloud computing in bioinformatics. *Int. Sch. Res. Not.* e615630. doi:10.1155/2013/615630.
[12] Feng D-F, Doolittle RF (1987) Progressive sequence alignment as a prerequisite to correct phylogenetic trees. J Mol Evol 25: 351–360
[13] Feng D-F, Doolittle RF (1990) Progressive alignment of protein sequences. Methods Enzymol 183:375–387
[14] Finn RD, Bateman A, Clements J, Marco Punta, Penny C Coggill, et al. (2014) Pfam: the protein families database. Nucl Acids Res 42: D222-D230.
[15] Flicek P, Amode MR, Barrell D, Beal K, Brent S, et al. (2012) Ensembl 2012. Nucleic Acids Res 40: D84-90.
[16] Fox NK, Brenner SE, Chandonia JM (2014) SCOPe: Structural Classification of Proteins--extended, integrating SCOP and ASTRAL data and classification of new structures. Nucleic Acids Res 42: D304-309.
[17] GenBank [Internet]. 2016. http://www.ncbi.nlm.nih.gov/genbank. Accessed: 2016-03-10
[18] Gibson G and Muse SV (2002) A Primer of Genome Science, Sinauer, Associates, Sunderland, MA, USA.
[19] Gonzalez S, Binato R, Guida L, Mencalha AL, Abdelhay E4 (2014) Conserved transcription factor binding sites suggest an activator basal promoter and a distal inhibitor in the galanin gene promoter in mouse ES cells. Gene 538: 228-234.

[20] Goodstein DM, Shu S, Howson R, Neupane R, Hayes RD, Fazo J, Mitros T, Dirks W, Hellsten U, Putnam N, Rokhsar DS. (2012) Phytozome: a comparative platform for green plant genomics. Nucleic Acids Res. 2012 Jan;40(Database issue):D1178-86.
[21] Hagen JB (2000). The origins of bioinformatics. *Nat. Rev. Genet.* 1: 231-236
[22] Henikoff, S., Henikoff, J.G., (1992). Amino acid substitution matrices from protein blocks. Proc. Natl. Acad. Sci. U S A 89, 10915-10919.
[23] International Human Genome Sequencing Consortium. Initial sequencing and analysis of the human genome. *Nature (*2001) 409:860-921.
[24] Junqueira DM, Braun RL and Verli H (2014). Alinhamentos. In: Bioinformática da biologia à flexibilidade molecular (Verli H, ed.). SBBq, São Paulo, 38-61.
[25] Kanehisa M (2002) The KEGG database. Silico Simulation of Biological Processes 247: 91-103.
[26] Kerr MK, Martin M and Churchill G (2000) Analysis of variance for gene expression in microarray data. J Computer Biology 7: 819-837.
[27] Luscombe NM, Greenbaum D, Gerstein M, (2001) What is bioinformatics? A proposed definition and overview of the field. Methods Inf Med.; 40(4):346-58.
[28] Manohar P and Shailendra S (2012). Protein sequence alignment: A review. *World Appl. Program.* 2: 141-145.
[29] Mehmood MA, Sehar U, Ahmad N (2014) Use of Bioinformatics Tools in Different Spheres of Life Sciences. J Data Mining Genomics Proteomics 5: 158.
[30] Molecular Modelling [Internet]. 2016://en.wikipedia.org/wiki/Molecular modelling. Accessed: 2016-03-10
[31] Moody G. Digital Code of Life: How Bioinformatics is Revolutionizing Science, Medicine, and Business. London: Wiley, 2004.
[32] Mount WD.)2004) Bioinformatics: sequence and genome analysis. 2nd ed. New York: Cold Spring Harbor Laboratory Press;. 692 p.
[33] Needleman Saul B and Wunsch Christian D. (1970) "A general method applicable to the search for similarities in the amino acid sequence of two proteins". Journal of Molecular Biology 48 (3): 443–53.
[34] Ng PC; Henikoff S (May 2001). "Predicting deleterious amino acid substitutions". *Genome Res*. 11 (5): 863–74
[35] Ortet P, Bastien O. (2010) Where Does the Alignment Score Distribution Shape Come from? *Evolutionary Bioinformatics*.
[36] Pearson WR and Lipman DJ (1988) Improved tools for biological sequence comparison. *Proceedings of National Academy of Sciences U S A* **85**(8): 2444-2448. DOI:10.1073/pnas.85.8.2444
[37] Peitsch MC (1996) ProMod and Swiss-Model: Internet-based tools for automated comparative protein modelling. Biochem Soc Trans 24: 274-279.
[38] Polyanovsky, V. O.; Roytberg, M. A.; Tumanyan, V. G. (2011). "Comparative analysis of the quality of a global algorithm and a local algorithm for alignment of two sequences". *Algorithms for Molecular Biology*. 6(1): 25.
[39] Prosdocimi F, Cerqueira GC, Binneck E and Silva AF (2002). Bioinformática: Manual do usuário. *Biotec. Cienc. Des.* 12-25.
[40] Protein Folding [Internet]. 2016. https://en.wikipedia.org/wiki/Protein_folding. Accessed: 2016-03-10
[41] Quevillon E, Silventoinen V, Pillai S, Harte N, Mulder N, et al. (2005) InterProScan: protein domains identifier. Nucleic Acids Res 33: W116-120.
[42] Rao, V. S., Das, S.K.., Rao, V.J and Srinubabu, G. (2008). Recent developments in life sciences research: Role of Bioinformatics. African Journal of Biotechnology7 (5): 495-503.
[43] Sigrist CJ, de Castro E, Cerutti L, Cuche BA, Hulo N, et al. (2013) New and continuing developments at PROSITE. Nucleic Acids Res 41: D344-347.
[44] Swarbreck D, Wilks C, Lamesch P, Berardini TZ, Garcia-Hernandez M, Foerster H, Li D, Meyer T, Muller R, Ploetz L. (2008) The Arabidopsis Information Resource (TAIR): gene structure and function annotation. *Nucleic Acids*;36: D1009–D1014.

[45] The European Molecular Biology Laboratory [Internet]. 2016. http://www.embl.org. Accessed: 2016-03-10
[46] The National Center of Biotechnology Information (NCBI) [Internet]. (2016). http://www.ncbi.nlm.nih.gov. Accessed: 2016-03-10
[47] UniProt Consortium (2008) The universal protein resource (UniProt). Nucleic Acids Res 36: D190-195.
[48] Venter JC (2001) The sequence of human genome. Science 291: 1304 – 1351.
[49] Venter JC, Adams MD,Myers EW, Li PW, Mural RJ, Sutton GG. (2001) The sequence of the human genome. *Science*; 291:1304-51.
[50] Verli H (2014). O que é Bioinformática? In: Bioinformática da biologia à flexibilidade molecular (Verli H ed.). SBBq, São Paulo, 1-12.
[51] Wu CH, Yeh LS, Huang H, Arminski L, Castro-Alvear J,. (2003) The Protein Information Resource. Nucleic Acids Res 31: 345-347.
[52] Xiong, J. (2009). Essential bioinformatics: introduction and biological databases. Cambridge University press, USA. http://www.cambidge.org /catalogue.

