

Plant Biochemistry and Biotechnology

Course No: Bot 301

Full Marks: 100

Nature of the Course: Theory

Pass Marks: 35

Year: B.Sc. III year

Lectures: 150

Objectives: The general aim of this course is to provide fundamental knowledge of plant biochemistry and biotechnology.

Unit A: Plant Biochemistry **75**

- 1. Introduction:** a) Plant biochemistry as a science (b) Relationship between plant biochemistry and plant sciences (genetics, cell biology, molecular biology, plant physiology and other branches of bio sciences) **2 hrs**

- 2. Bio-molecules** **37 hrs**
 - (a) Water:** molecular structure, properties, water as a solvent; Ionization of water, pH scale, buffer. 6 hrs
 - (b) Functional groups** found in bio-molecules: general concept 3 hrs
 - (c) Carbohydrates:** Definition, classification
Monosaccharides: classification (based on functional group; based on no of C atoms), Properties (Esterification, oxidation, reduction, osazone formation, cyanohydrin reaction, furfural formation, enolization); derivatives of monosachharides (de-oxy derivatives, acids, alcohols, amino sugars, sialic acid); Biological importance

Disachharides, Polysaccharides and their biological importance (1+6+3 hrs)
 - (d) Lipids:** Definition, structure of glycerol and fatty acids
Properties of fatty acids, kinds of lipids (Glycero lipids; lipids without glycerol; complex lipids), properties of lipids, Biological roles of lipids (1+ 4 hrs)
 - (e) Proteins:** Definition, Amino acids and their classification (essential, semi essential and non essential; polar and non polar; acidic, basic or neutral)
Properties of Amino Acids: Amino acids as zwitterions, Amino acids as electrolytes, Reactions of amino acids (ninhydrin reaction; Sanger's reaction; Edman's reaction, Dansyl Chloride reaction; Phosgene reaction; decarboxylation; Esterification; Acylation)
Peptide bonds and Polypeptide; Structure of polypeptides- primary/secondary/tertiary and quaternary structures

Classification of proteins, Functions of proteins. (2+5+3+3 hrs)

3. **Enzymes:** Properties and chemical structure, Mechanism of action, Denaturation, Factors affecting enzyme action, Allosteric protein and feedback inhibition. Classification and nomenclature **7 hrs**
4. **Plant pigments:** Structure and functions of chlorophylls, carotenoids, anthocyanins, phycobillins **4 hrs**
5. **Vitamins and their role in plants:** Fat soluble vitamins (A, E and K); Water soluble vitamins (Coenzyme A, Vitamins B1, B2, B6, C, and H) **5 hrs**
6. **Introduction to Bioinformatics** **5 hrs**
Definition, role of computer in bioinformatics, Branches of bioinformatics, Aim of bioinformatics, Scope and research areas in bioinformatics, Biological data-DNA sequence, protein sequence, macromolecular structure. Databases in bioinformatics, basic bioinformatics tools- Databases search (Entrez, SRS), Blast, Fasta.

Unit B: Plant Biotechnology **75**

1. Overview of Biotechnology (1+3+10 hrs)= **14 hrs**

Introduction : A) Origin and History of biotechnology, B) Scope and importance of biotechnology: a) Biotechnology in Medicine, b) Biotechnology in food industry, c) Biotechnology in agriculture, d) Biotechnology in Fermentation technology e) Biotechnology in environmental engineering; C) Achievements of biotechnology: a) Gene cloning, b) Recombinant DNA technology, c) *In vitro* culture technology, d) Genetically engineered drugs, e) Diagnosis of diseases f) Biosensors g) Biofertilizers h) Mutation Breeding, i) Enzyme technology , j) DNA finger printing, h) Monoclonal antibody

2. Plant tissue culture technique and application (2+3+2+10+15+3) = **35 hrs**

A. Introduction to plant tissue culture.

B. Basic principles and techniques of *In vitro* culture: Totipotency, Basic requirements for growing plants in vitro: Laboratory organization, composition and selection of nutrient media, sterilization, culture room

C. Types of plant tissue culture and their applications: a. Seed culture, b. organ culture (Meristem culture, root culture, shoot culture), c. Embryo culture, d. Anther Culture e.

Ovule culture, f. Endosperm culture, g. Callus culture h. Protoplast culture i. cell suspension culture

D. Cryopreservation : a. Principles, **Method of cryopreservation-** i) Preparation of material for deep freezing, ii) Cryoprotectors, iii) Freezing programmes, iv) Storage strategies, v) Assessment of successful cryopreservation. b. **Uses of cryopreservation** –i) Cryopreservation of Semen, ii) Cryopreservation of ova and embryo, iii) Maintenance of plant germplasm for long duration, iv) Organ explants, v) Zygotic and immature embryos, vi) Shoot tips, vii) Callus, viii) Cell suspensions x) Protoplasts, c. **Significance of cryopreservation**

3. Plant- Microbe Interaction

12 hrs

A. Biological Nitrogen fixation: mechanism and its importance

B. Symbiotic and asymbiotic organisms for soil fertility and crop improvement.

C. Biofertilizers (Symbiotic associations): i) Rhizobium- Formation of nodule, Nitrogen fixing organism found in nodules, Structure and function of nodule, Mechanism of Nitrogen fixation by Nodules, Rhizobial biofertilizers, Rhizobium biofertilizers in forest trees. ii) Blue Green Algae (BGA)-Mass production and application of Blue Green Algae, Azolla – anabaena symbiosis, Asymbiotic associations, Non symbiotic nitrogen fixing bacteria, Non symbiotic bacterial biofertilizer- a) Azotobactor, b) Azospirillum, c) Carrier formulation, iii). Frankia biofertilizer, iv) Mycorrhiza-Types of mycorrhiza, benefits from Mycorrhizas to plants, establishment of Mycorrhiza associations in vitro

4. Gene transfer in plants:

(10+1+3 hrs) = **14 hrs**

A) Concept of gene cloning: Basic requirements for gene cloning in plants; gene isolation and cloning; Concept of vectors; marker and reporter genes and their roles in plant transformation; identification and analysis of cloned genes (colony hybridization, immunological detection, PCR, blotting)

B. Gene transfer techniques in plants: i) gene transfer methods: (Direct/vector less and indirect/ vector mediated); transformation of mitochondria and chloroplasts iii) GM crops: applications and limitations iv) General concept of molecular farming from transgenic plants, v) ethical issues in plant genetic engineering

Text and Reference books

Plant Biochemistry

1. Bhattarai, T. 2005. Experiments on Plant Biochemistry and Plant Biotechnology. Bhundipuram Prakashan, Kathmandu
2. Bhattarai, T. 2007. Plant Physiology. Bhundipuram Prakashan, Kathmandu.
3. Jain, J.L. 2004. Fundamentals of Biochemistry. S Chand and Company Ltd. New Delhi
4. Lehninger, A.L., Nelson, D.L. and Cox, M. 2004. Principles of Biochemistry. 4th edition. McMillan Limited, USA (Indian Reprint)
5. Rastogi, S.C. 1993. Biochemistry. Tata McGraw-Hill Publishing Company Ltd. Delhi

Bioinformatics

1. Attwood, T.K. and Parry-Smith, D. J. 2001. Introduction to Bioinformatics. Prentice Hall Inc.
2. Ghosh, Z. and Mallick, B. 2008. Bioinformatics –principle and applications. Oxford University Press, India.
3. Mount, D. W. 2001. Bioinformatics Sequence and Genome Analysis. Cold Spring Harbor Laboratory Press, New York.

Plant Biotechnology

1. Altman, A. and Hasegawa, P. 2012. Agricultural Biotechnology. Academic Press.
2. Bhojwani S. S. 1990. *Plant Tissue Culture: Applications and Limitations*, Elsevier Science Publishers.
3. Bhojwani S.S. and Razdan, M. K. 1996. *Plant Tissue Culture: Theory and Practice*. Elsevier Science Publishers.
4. Chawala H.S. 2009. *Introduction to Plant Tissue Culture*. Third Edition. Oxford and IBH
5. Crispeels, M.J. and Sadava, D.E. 2006. Plants, Genes and Crop Improvement. American Society of Plant Biologists, USA.
6. Debergh, P.C. and Zimmerman, R.H. 1990. Micropropagation. Kluwer Academic Publ. Dordrecht.
7. Dodds, j.H. and Roberts, L.W. 1995. *Experiments in Plant Tissue Culture* (3rd Edition). Cambridge University Press. Cambridge, UK.
8. Dubey R.C. 2009. A text Book of Biotechnology S Chand and company Limited.
9. Gamborg O.L. and Phillips G.C. 1995. *Plant Cell, Tissue and Organ Culture – Fundamental Methods* (Lab. Manual). Springer-Verlag.
10. Greene J.J. and Rao V.B. 1998. *Recombinant DNA Principles and Methodologies*. Marcel Dekker.
11. Lal R. and Lal S. 1995. *Genetic Engineering of Plants for Crop Improvement*. CRC Press.
12. Pierik R.L.M. 1997. *In vitro Culture of Higher Plants*. Kluwer Academic Publisher, Netherlands.

13. Punia M.S. 1999. *Plant Biotechnology and Molecular Biology: A Laboratory Manual*. Scientific Publishers, India.
14. Razdan M. K. 2003. *Introduction to Plant Tissue Culture*. Agritech Publications.
15. Satyanarayan U. 2005. *Biotechnology*. 1st ed. Arunabha Sen books and Allied P. Ltd.

Plant Biochemistry and Biotechnology

Course No: Bot 302

Full Marks: 50

Nature of the Course: Practical

Pass Marks: 20

Year: B.Sc. III year

Lectures: 75

Objectives: The general aim of this course is to provide Practical knowledge of plant biochemistry and biotechnology

Unit A: Plant Biochemistry

1. Preparation of solutions: Concept of normality, molarity, percentage, ppm (parts per million) and their inter-conversions
2. Preparation of buffers (acetate and phosphate) of different pH; measurement of pH by using pH meter and different indicators
3. Qualitative test of carbohydrates (mono-, di- and polysaccharides)
4. Quantitative estimation of sugars by anthrone method.
5. Qualitative tests of lipids and fatty acids (use of sudan dye; determination of acid value, saponification value and iodine number)
6. Qualitative test of proteins (Ninhydrin test; biuret test; millon's test)
7. Quantitative estimation of proteins
8. Qualitative test of nucleic acids (DNA and RNA)
9. Quantitative estimation of nucleic acids
10. Bioassay of plant hormones
11. Extraction and quantification of different plant pigments using colorimeter/spectrophotometer
12. Effect of different factors on enzyme catalyzed reactions (Substrate concentration; enzyme concentration; temperature, pH, inhibitors)
13. Separation of plant pigments by paper/thin layer chromatography.

Unit B: Plant Biotechnology

1. To study about the sterilization Technique

i Heat

ii. Radiation

iii. Chemicals

iv. Filtration

1. Study and operation of an Autoclave
2. Study and operation of A Hot Air Oven
3. Study and operation of Laminar Air Flow Cabinet
4. To perform the surface sterilization in plants (explants) .
5. Preparation of 1 litre Murashige and Skoog (1962)(MS) medium.
6. Preparation of Stock solution for MS medium.
7. Preparation of 1.litre of MS medium using stock solutions.
8. Culture technique in tissue culture: root tip culture, shoot tip culture, meristem culture, Pollen culture, anther culture, embryo culture, and seed culture.
9. Regeneration of plantlets by *in vitro* culture.
10. Isolation and inoculation of *Rhizobium* and *Azetobacter*
11. Extraction of Genomic DNA by CTAB method

Evolution and Biogeography

Course No: Bot 303

Full Marks: 50

Nature of the Course: Theory

Pass Marks: 17.5

Year: B.Sc. III year

Lectures: 75

Objectives: The general aim of this course is to provide fundamental knowledge of evolution and biogeography

Course Contents

Evolution (Lectures: 35)

Unit 1: Basic concepts: (i) Introduction: what is evolution?, basic concepts of micro- and macro-evolution, convergent and divergent evolution, molecular evolution, evolution and adaptation, co-evolution; (ii) History of evolutionary thought (pre-Darwin, Darwin and post-Darwin periods), modern synthesis; (iii) Natural selection and evolution: introduction, types of natural selection (**Lecture: 4+6+3 = 13**).

Unit 2: Variation and evolution: (i) Sources and pattern of variation: sources of variation (recombination, gene flow, mutation); patterns of variation (geographic, ecological and genetic patterns); study of population variation (basic techniques and tools); (ii) Gene pool concept: introduction, gene/allele frequency and change, genetic drift, Hardy-Weinberg Principle and evolution; (iii) Speciation: biological species concept, isolation mechanisms, modes of speciation (allopatric, parapatric and sympatric) (**Lecture: 8+7+7 = 22**).

Biogeography (Lectures: 40)

Unit 3: Basic concepts: (i) Introduction: definition and scope of biogeography, brief history of biogeography, relationship with other sciences; (ii) Biogeographic regions: concept of biomes and biogeographic regions; phytogeographic (floristic) regions of the world [basic characteristics: geographic coverage and major floristic elements) of six kingdoms – Boreal (Holarctic), Paleotropical, Neotropical, South African (Capensic), Australian, and Antarctic] (**Lecture: 3+7 = 10**).

Unit 4: Historical biogeography: (i) History of earth: geological time scale, plate tectonics, continental drift; (ii) Modes of biogeographic distribution: dispersalist and vicariance biogeography, endemism; (iii) History of evolution: fossil records and origin of life, concept of molecular clocks for dating the history, phylogenetic inference; (iv) History of biological diversity: rise of flowering plants (early diversification and past interactions), late Cretaceous and Cenozoic changes, Pleistocene glaciations and biological changes (**Lecture: 3+3+6+6= 18**).

Unit 5: Ecological biogeography: (i) Current patterns of biodiversity distribution: basic factors, processes and conditions controlling biodiversity distribution; major gradients in biodiversity distribution; (ii) Biodiversity hotspots: introduction and concepts, distribution of hotspot areas around the world; (iii) Island biogeography theory: basic concept, conservation application (**Lecture: 6+3+3 = 12**).

Selected Readings

Briggs D. and Walters M. 1997. Plant Variation and Evolution, Third Edition. Cambridge University Press.

Lomolino M.V., Riddle B.R. and Brown J.H. 2006. Biogeography. Sinauer Associates, Inc., Sunderland, Massachusetts, USA (Third edition).

Suggested Further Readings

Cox C.B. and Moore P.D. 2009. Biogeography: an Ecological and Evolutionary Approach. Blackwell Publishing (seventh edition).

Futuyma D. 1997. Evolutionary Biology. Sinauer Associates, Sunderland, MA, USA.

Huston M.A. 1994. Biological Diversity: The Coexistence of Species on Changing Landscapes. Cambridge University Press, UK.

Katy Human 2006. Biological evolution: An anthology of current thought. The Rosen publishing group, Inc.

Maxtoshi Nei and Sudhir Kumar (2000). Molecular Evolution and phylogenetics. Oxford University Press.

Roderic D M Page and Edward C Holmes 1998. Molecular Evolution: A phylogenetic approach. Blackwell Science Ltd.

Stebbins George Ledyard 1971. Process of Organic evolution. Prentice Hall of India.

Takhtajan A. 1986. Floristic Regions of the World. University of California Press.

Whittaker, R.J. and Fernández-Palacios, J.M. 2007. Island Biogeography: Ecology, Evolution, and Conservation, 2nd edn. Oxford University Press, Oxford.

Medicinal and Aromatic Plants

Course No: Bot 304

Full Marks: 50

Nature of the Course: Theory

Pass Marks: 17.5

Year: B.Sc. III year

Lecture Hours: 75

Objectives: The general objective of this course is to provide basic and applied knowledge on the medicinal and aromatic plants (MAPs) of Nepal

Course contents

Unit 1: Overview and importance: (i) Introduction: definitions of Non-timber forest products (NTFPs) and medicinal and aromatic plants (MAPs); (ii) historical perspectives and current use: with particular focus on the importance of MAP in traditional medicinal practices (Ayurvedic, Unani, Amchi/Tibetan and homeopathy); (iii) Future prospects: introduction to bioprospecting; MAPs, traditional knowledge and drug development; issues of IPR and biopiracy (**Lecture: 1+3+3 = 7**).

Unit 2: Diversity, distribution and trade potentials: (i) Diversity and distribution patterns and NTFPs and MAPs in various climatic zones of Nepal, factors affecting their distribution; (ii)

Trade potentials of NTFPs and MAPs in Nepal, major trends in NTFPs/MAPs trade, role of NTFPs/MAPs in the promotion of peoples' livelihoods (**Lecture: 3+3 = 6**).

Unit 3: Conservation status and sustainable use: (i) Conservation status of MAPs: major conservation issues and threats, MAPs of Nepal in IUCN RED List, CITES appendices and government protection list; (ii) Conservation and sustainable use: major principles, opportunities and constraints, dimensions of MAP sustainability; (iii) *in-situ* and *ex-situ* conservation strategies, alternatives to wild harvest, eco-certification processes, community-based management; (iv) national policies and programs for the promotion of MAP-sector in Nepal (**Lecture: 2+4+4+2 = 12**).

Unit 4: Pharamcognosy: (i) Concept and scope of pharamcognosy, crude drug production of MAPs; (ii) Overview of techniques for the extraction of major phytochemicals from MAPs, distillation technology for essential oils, quality control; (iii) Herbal cosmetics, potentiality of herbal drugs and herbal based industries (**Lecture: 2+3+3 = 8**).

Unit 5. Cultivation technologies of commercially important MAPs: (i) introduction: Opportunities and constraints in the cultivation and production of MAPs in Nepal; (ii) Importance (traditional and commercial uses), active constituents, distribution, climatic and soil requirements, cultivation technologies (propagation and nursery techniques, transplantation of seedlings and rooted cuttings, irrigation techniques, disease and pest management), harvesting and post harvest (processing and value addition) technologies, and major trade issues of the following commercially important MPAs prioritized by the Government of Nepal for economic development in the lower, mid and high altitudes: *Asparagus racemosus*, *Azadirachta indica*, *Neopicrorhiza scrophulariiflora*, *Paris polyphylla*, *Phyllanthus emblica*, *Piper longum*, *Rheum australe*, *Sapindus mukorossi*, *Swertia chirayita*, *Taxus wallichiana*, *Valeriana jatamansii*, *Zanthoxylum armatum* (**Lecture: 1+24 = 25**).

Unit 6. Harvesting technologies of commercially important MAPs: (i) Harvest and post-harvest technologies (harvesting period, and methods of sustainable harvest, processing and value addition), marketing and values chain analysis, major trade issues, active constituents and uses of some commercially important MAPs – *Cordyceps sinensis*, *Dactylorrhiza hatagirea*, *Hippophae* spp., *Morchella* sp., *Nardostachys grandiflora* and *Podophyllum hexandrum* (**Lecture: 12**).

Unit 7.Field (herbal farm/herbal industry) visit and report writing (**Lecture: 5**).

Suggested Readings

- Agrawal S.S. and Paridhavi M. 2012.*Herbal Drug Technology*. Universities Press (India) Private limited, Hyderabad, India.
- ANSAB, 2003.*Commercially Important Non-Timber Forest Products (NTFPs) of Nepal*. Asia Network for Sustainable Agriculture and Bioresources, Kathmandu, Nepal (in Nepali).
- Balick M.J. and Cox P.A. 1997. *Plants, People, and Culture: the Science of Ethnobotany*. Scientific American Library, New York, USA.
- DPR 2067 B.S. *Nepalko Aarthik Bikaskalagi Prathamikta Prapta 30 Jadibutiharuko Pahichan Pustika*. Department of Plant Resources, Ministry of Forest and Soil Conservation, Government of Nepal, Kathmandu.
- DPR 2007. *Medicinal Plants of Nepal*. Bulletin of the Department of Plant Resources No. 28. Department of Plant Resources, Ministry of Forest and Soil Conservation, Government of Nepal, Kathmandu.
- DPR 2060-2061 B.S. *Jadibuti Sankalan, Sanrakshan, Sambardhan Bidhi*. Jadibuti Parichaya Mala 1-5. Department of Plant Resources, Ministry of Forest and Soil Conservation, Government of Nepal, Kathmandu.
- Farooqi A.A. and Sreeramu B.S. 2010. *Cultivation of Medicinal and Aromatic Crops*. Universities Press (India) Private limited, Hyderabad, India.
- Ghimire S.K., Pyakurel D., Nepal B., Sapkota I.B. and Parajuli R.R., Oli B.R. 2008. *A Manual of NTFPs of Nepal Himalaya*. WWF Nepal Program, Kathmandu.
- Ghimire S.K., Sapkota I.B. Oli B.R. and Parajuli R.R. 2008. *Non-Timber Forest Products of Nepal*. WWF Nepal Program, Kathmandu.
- Gurung K. 2009. *Essential Oils in Nepal: a Practical Guide to Essential Oil and Aromatherapy*. Himalayan Bio-Trade Pvt. Ltd., Kathmandu.
- Gyawali R. 2013. *Handbook of Pharmacognosy*. Nabodit Hamro Pustak Bhandar, Kathmandu.
- Handa S.S. 2008. An overview of extraction technique of medicinal and aromatic plants. In: *Extraction Technologies for Medicinal and Aromatic Plants* (Eds. S.S. Handa, S.P. Singh Khanuja, G. Longo, D.D. Rakesh). International Centre for Science and high Technology, Italy.
- Jha P.K., Karmacharya S.B., Chettri M.K., Bania C.B. and Shrestha B.B. eds. 2008. *Medicinal Plants in Nepal: an Anthology of Contemporary Research*. Ecological Society (ECOS), Kathmandu.

Manandhar N.P. 2002. *Plants and People of Nepal*. Timber Press. Portland, Oregon.

Rajbhandary S. and Ranjitkar S. 2006. *Herbal Drugs and Pharmacognosy: Monographs on Commercially Important Medicinal Plants of Nepal*. Ethnobotanical Society of Nepal (ESON). Kathmandu.

Thomas Y., Karki M., Gurung K. and Parajuli D. eds. 2002. *Himalayan Medicinal and Aromatic Plants, Balancing Use and Conservation*. Proceedings of the Regional Workshop on Wise Practices and Experiential Learning in Conservation and Management of Himalayan Medicinal plants (December 15-20, 2002, Kathmandu, Nepal). Ministry of Forests and Soil Conservation, His Majesty's Government of Nepal, Kathmandu.