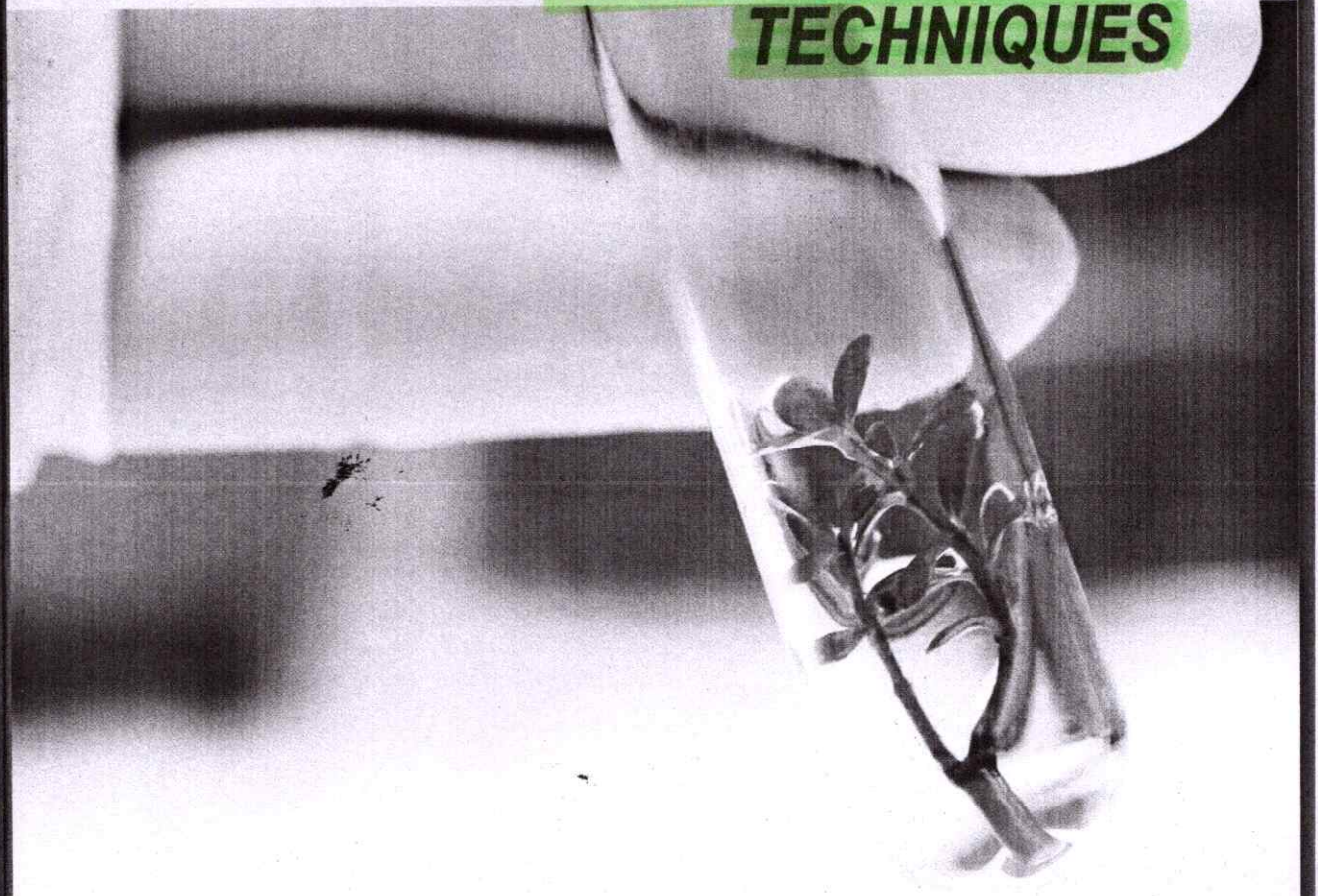
LABORATORY MANUAL IN Plant TISSUECULTURE TECHNIQUES



BIJU DHARMAPALAN

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MACFAST

Laboratory Manual In
Plant Tissue Culture Techniques

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Contents

Chapter 1

INTRODUCTION

Plant tissue culture, also referred to as cell *in vitro*, axenic, or sterile culture is an important tool in both basic and applied studies, as well as in commercial application . Plant tissue culture is the aseptic culture of cells, tissues, organs and their components under defined physical and chemical conditions *in vitro*. The theoretical basis for plant tissue culture was proposed by Gottlieb Haberlandt in his address to the German Academy of Science in 1902 on his experiments on the culture of single cells. He opined that, to his knowledge, no systematically organized attempts to culture isolated vegetative cells from higher plants have been made. He experimented with isolated photosynthetic leaf cells and other functionally differented cells and was unsuccessful, but nevertheless he predicted that one could successfully cultivate artificial embryos from vegetative cells. He, thus, clearly established the concept of totipotency, and further indicated that the technique of cultivating isolated plant cells in nutrient solution permits the investigation of important problems from a new experimental approach. On the basis of that 1902 address and his pioneering experimentation before and later, Haberlandt is justifiably recognized as the father of plant tissue culture. The first true plant tissue cultures were obtained by Gautheret in cambial tissue of *Acer pseudoplatanus*. He also obtained success with similar explants of *Ulmus campestre, Robinia pseudoacacia,* and *Salix capraea* using agar-solidified medium of Knop's solution, glucose and cysteine hydrochloride.

The 1940s, 1950s, and 1960s proved an exciting time for the development of new techniques and the improvement of those already available. The application of coconut water allowed for the culture of young embryos and other recalcitrant tissues, including monocots. Callus cultures of numerous species, including a variety of woody and herbaceous dicots and gymnosperms, as well as crown gall tissues, were established as well . It was recognized at this time that cells in culture underwent a variety of changes, including loss of sensitivity to applied auxin or

Using the formula:

V1= (0.01 M x 100mL)/1M = 1 ml, i.e. you need 1 ml of the stock, mixed with 99 ml water to have 100 ml of solution.

Molecular weight

It is sum of weight of atoms in the chemical formula of a compound.

For example,

Molecular weight of sucrose $C_{12}H_{22}O_{11}$ = 342.3 grams

Molarity

Number of moles per unit volume.

For example, a 1 M solution would mean 1 mole of the substance present in 1 litre of the solution

For preparing molar solutions, it is necessary to know molecular weight to calculate how much of a chemical to weigh out to make a stock solution:

(MW/desired molar concentration) x the number of litres desired = amount to be dissolved

Normality (N)

The normality of a solution is the number of gram equivalents of the solute per L of the solution.

A solution having one g equivalent of the solute per L of solution is called 1N solution

Normality = Amount of a substance in g/L of solution / Eq.wt of substance

For preparing 0.1N Na_2CO_3 (Eq. wt.=53) solution , dissolve 5.3 g Na_2CO_3 in a final volume of 1L of solution

LABORATORY MANUAL IN PLANT TISSUE CULTURE TECHNIQUES

Biju Dharmapalan ● Arun K Das

About the Book

The **Laboratory Manual In Plant Tissue Culture Techniques** discusses basic techniques used in plant biotechnology laborartory. The book is divided into two parts. In the first part the theoretical aspects of various techniques have been explained and in the second part common experiments that are carried out in plant tissue culture lab is explained. The manual will be useful for B.Sc. and M.Sc. students

Biju Dharmapalan is the Head ,School of Biosciences,MACFAST. He is also the editor of Journal of Science Technology and Management. Before joining MACAFST he had worked at Mar Ivanios College, Trivandrum; TBGRI Palode and CPCRI Kasargod. His research interest is in the field of Plant Tissue Culture and Phytochemistry. He has over 17 years of experience in teaching /research and is the author of several text books and popular science articles. His name is listed in the Data Base of Science Communicators in India, published by Vignan Prasar, DST, Govt. of India and also as Mentors/Resource persons for Summer / Winter Camps and other INSPIRE initiatives of DST, Govt. of India.

Arun K Das is the Assistant Professor, School of Biosciences, MACFAST. He has completed his Ph.D from the Madurai Kamaraj University. Before joining MACFAST he had worked in the Department of Plant Sciences at Madurai Kamaraj University. He has published several research papers in National and International Journals. His field of interest is Plant Tissue Culture and Bioprospecting. He has been the resource person for various National Level Training programmes

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