Research Methods in Plant Sciences : Allelopathy

Volume 5

Plant Physiology

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PREFACE

Allelopathy is newly emerging multidisciplinary field of agricultural research. Till now much Allelopathy research work has been done in various fields of Agriculture and Plant Sciences. However, no standard methods are being used by various workers due to lack of compendium on the Techniques, hence, the results obtained are not easily comparable with each others. It is causing lot of problems to researchers working in underdeveloped/third world countries in small towns, where library and research facilities are not available. Therefore, to make available the standard methods for conducting allelopathy research work, this multi-volume book has been planned, with one volume each for each discipline. In all the conferences held since 1990's in India or in foreign countries, a need has always been felt for a Manual of Allelopathy Research Methods. Hence, Prof. S.S. Narwal has planned this multivolume book **Research Methods in Plant Sciences : Allelopathy.** This book series aims to provide basic information about various methods to research workers, so that they can conduct research independently without the requirement of sophisticated equipments. The methods have been described in a simple way just like "DO IT YOURSELF' book. Three volumes (Volume 1. Soil Analysis, Volume 2. Plant Protection and Volume 3. Plant Pathogens) of this book were released during the **IV International Allelopathy Conference, August 23-25, 2004 at Haryana Agricultural University, Hisar-125004, India.** The present book is 4th volume in the series. Many volumes are under preparation.

To elucidate a physiological process, one has to undertake quantative and qualitative analysis of a specific parameter. Various important physiological processes like seed germination, plant growth and development, photosynt hesis and respiration, senescence and abscission are included in this volume. To understand the basic mechanisms of various physiological processes, being affected by allelochemicals at the cellular level enzyme activity and metabolite studies are essential.

This volume is divided into 7 Sections. Section I. Seed Physiology, includes 5 chapters describing the structure of seed, optimum conditions for seed germination, physiological and biochemical changes at cellular level. Problems faced during seed germination can be overcome by studying dormancy, seed viability and seedling vigour. Bioassays using seed germination and seedling growth have also been explained. Section II. Growth and Development, of the test plant in term of leaf area, growth indices, senescence and abscission has three chapters. Once it is established that growth and development of the test plant is affected by allelochemicals, then next question arises: what is basis of such observed effect. Allelochemicals, present in soil or plant, can create chemical stress which may change the plant water status, plasma membrane properties, chlorophyll stability and waxes present on the organ surface. Methods to determine all these parameters are described in next 4 chapters in Section III. Stress Physiology. Changes in these parameters in the test plant can alter photosynthetic production by affecting the efficiency of light and dark reactions of photosynthesis. There are multiple sites in photosynthetic process which changes in response to chemical stress imposed by allelochemicals. These sites can be explored by estimating chlorophyll content, chlorophyll fluorescence, photosystems I and II activity, carbon dioxide exchange rate, activity of CO_2 fixing enzymes, intermediate metabolite level, photosynthate partitioning, respiration and finally the crop growth dynamics. Methods to determine extent of all these sites are explained in 7 chapters in Section IV. Gas Exchange Processes. The main cause of changed physiological process is at the gene level, for which estimation of nucleic acids is very critical. It is briefly explained in section V. Biochemical Estimation. Section VI. Microtomy and Histochemistry, has 7 chapters. Basic procedure to process the test plant material for microtomy, use of light and electron microscopy to study cellular changes, measurement of cellular dimensions, stomatal index and frequency, pollen viability and *in vivo* pollen germination and histochemical localization of important enzymes and metabolites are the core topics. Currently, tissue cultures are commonly used to study the precise effect of allelochemicals on callus growth and differentiation. To achieve these objectives techniques of tissue cultures is described under section VI. Tissue Cultures. In Section VIII. Annexures, seven Annexures have been included to facilitate growing of test plants under controlled environmental conditions, preparation of solutions and buffers.

This book will serve as ready reference in the laboratory or class room and help to solve many field problems of agriculture and allied field including allelopathy. Information provided can be use to determine the effects and mechanism of action of allelochemicals at the plant, organ and cellular levels. It will be useful for UG and PG students pursing allelopathic work, plant physiologists, agronomists and other plant science specialists. We have tried to provide appropriate solutions to the physiological problems, even with or without the most sophisticated laboratory instruments and equipments. The users of this book can select suitable methods, according to the available facilities.

We are indebted to the contributors, who have actually used all these methods in their fields of specialization for the last 10 to 25 years, in accepting challenging task to prepare chapters for the present book. All of them have made sincere efforts in presenting the procedure of various methods in very simple language, easily understood by beginners.

We will appreciate to receive valuable suggestions from the students and researchers, to make further improvements in future editions of this book, to make it more useful and meaningful.

S.S. Narwal
B. Politycka
C.L. Goswami

ABBREVIATIONS

Å	Angstrom 10^{-10}m
A	Leaf area
AA	Accelerated ageing
AA	Ascorbic acid
ABA	Abscisic acid
ABS	Photons absorbed by the antenna chlorophyll
AC	Alternate current
ADP	Adenosine diphosphate
AGR	Absolute growth rate
ATP	Adenosine triphosphate
b	Coefficient
B	Boron
B	Breadth
B5	Gamborg's medium
BA	Benzyladenine
BMD	Biomass duration
BP	Between paper
BSA	Bovine serum albumin
C_3	Calvin cycle or photosynthetic carbon reduction pathway
C_4	C_4 pathway
$CaCl_2$	Calcium chloride
CAM	Crassulaecean acid pathway
$CaSO_4$	Calcium sulfate
CCl_4	Carbon tetrachloride
CER	Carbon dioxide exchange (fixation) rate

CGR	Crop growth rate
$CHCl_3$	Chloroform
Co	Cobalt
$CoCl_2$	Cobalt chloride
CS	Cross section of leaf
CTI	Chlorophyll stability index
Cu	Copper
D	Density
d	Resolving limit
DAB	3,3' diaminobenzidine
DC	Direct current
DCPIP	Dichlorophenol indophenol
DDSA	Dodecenyl succinic anhydride
DEAE	Diethylaminoethyl
DG	Gorssheff & Doy's medium
DMP-30	Tridimethyl amino methyl phenol
DMSO	Dimethylsulfoxide
DNA	Deoxyribo- or se nulcleic acid
DOPA	Dihydrophenylalanine
dt	Change in time
DTC	Dimethyl dithiocabamate
DTT	Dithiothreitol
dw	Change in weight
E	Energy radiated
EC	Electrical conductivity
EC	Electron transport
EDTA	Ethylenediaminetetraacetic acid
ET	Evapotranspiration
F_0	Fluorescence in dark adapted leaf
F1,6-BP	Fructose1,6-bisphosphate
F-6-P	Fructose-6-phosphate
FAA	Formaline:acetic acid: alcohol

Fe	Iron
Fe_2SO_4	Ferrous sulfate
F_m	Maximum fluorescence under saturating excitation light
F_v	Variable fluorescence
G1,6-BP	Glucose1,6-bisphosphate
G-6-P	Glucose-6-posphate
GA_3	Gibberellic acid
H_2O	Water
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulfuric acid
H_3BO_3	Boric acid
HCl	Hydrochloric acid
$HClO_4$	Perchloric acid
HEPES	N-(2-hydroxyethyl)piperzine-N'-(ethanesulfonic acid)
HI	Harvesting index
HIO_4	Periodic acid
HNO_3	Nitric acid
hv	Light
IAA	Indole 3 acetic acid
IBA	Indole butyric acid
IRGA	Infra-red gas analyser
ISH	*in situ* hybridization
ISTA	International Seed Testing Association
k	Flask constant
KCl	Potassium chloride
KH_2PO_4	Potassium dihydrophosphate
KI	Potassium iodide
KN	Kinetin
KNO_3	Potassium nitrate
L	Emissivity
L	Length
LA	Leaf area

LAD	Leaf area duration
LAI	Leaf area index
LAR	Leaf area ratio
LDH	Lactate dehydrogenase
LEA	Late embryogenesis proteins
LED	Light emitting diode
LS	Longitudinal section
LW	Leaf weight
LWR	Leaf weight ratio
M	Mass
m	Millie 10^{-3}
mRNA	Messenger RNA
M	Molar solution
MDH	Malate dehydorgenase
$MgCl_2$	Magnesium chloride
$MgSO_4$	Magnesium sulfate
MI	Magnification of intermediate lense
Mn	Manganese
$MnSO_4$	Manganese sulfate
MO	Magnification of objective lense
Mo	Molybdenum
MP	Magnification of projector lense
MPa	Mega Pascal
MS	Murashige & Skoog's medium
MT	Final magnification
MTS	membrane stability index
MW	Molecular weight
Na FeEDTA	Sodium, iron salt of EDTA
NA	Numerical aperture
Na_2CO_3	Sodium carbonate
Na_2HPO_4	Disodium hydrogen phosphate
Na_2MoO_4	Sodium molybadate

NAA	Naphthalene acetic acid
NaCl	Sodium chloride
NAD(P)	Oxidized form of nicotinamide adenine dinucleotide (3-phosphate)
NAD(P)H	Reduced form of nicotinamide adenine dinucleotide (3-phosphate)
NaH_2PO_4	Dihydrogen sodium phosphate
$NaHCO_3$	Sodium bicarbonate
NaOH	Sodium hydroxide
NAR	Net assimilation rate
NBT	Nitrobluetoludine
NH_4Cl	Ammonium chloride
NH_4NO_3	Ammonium nitrate
NH_4SO_4	Ammonium sulfate
NMA	Nadic methyl anhydride
NN	Nitsch & Nitsch's medium
OAA	Oxaloacetic acid
OsO_4	Osmium tetraoxide
PBS	Phosphate buffered saline
PC	Personal computer
p-CPA	p-chlorophenoxy acetic acid
PEA	Plant efficiency analyzer
PEG	Polyethylene glycol
PEP	Phosphoenol pyruvate
PGA	Phosphoglyceric acid
PGR	Plant growth regulator
pH	Negative log of active hydrogen concentration
Pi	Inorganic phosphate
PP	Pleated paper
PPi	Inorganic pyrophosphate
PS-I	Photosystem I
PS-II	Photosystem-II
PTA	Phosphotungestic acid

PVP	Polyvinylpyrrolidone (insoluble)
R-5-P	Ribose -5-phosphate
RC	Reaction centers
Rf	Relative to front or retardation factor
RGA	Relative growth rate
RH	Relative humidity
RNA	Ribo- or se nucleic acid
Ru-5-P	Ribulose-5-phospahe
RuBP	Ribulose-1,5-bisphosphate
RWC	Relative water content
S	In sand
SDS	Sodium dodecyl sulfate
SEM	Scanning electron microscope
SF	Stomatal frequency
SI	Stomatal index
SLA	Specific leaf area
T	Reaction temperature in ^{0}K
t	Time
TBA	Tertiary butyl alcohol
TCA	Trichloroacetic acid
TDZ	Thidiazuron
TEM	Transmission electron microscope
T_{max}	Area over fluorescence
TMV	Tobacco mosaic virus
TP	Top of paper
TR	Trapping flux
Tris	(Hydroxymethyl) aminoethane
TS	Top of sand
TS	Transverse section
TTC	2,3,5 Triphenyl tetrazolium chloride
TZ	Tetrazolium
ULR	Unit leaf rate

UV	Ultra violet region of light spectrum
V	Volume
v/v	Volume by volume
Vg	Gas volume
w	Weight
W	Water content
w/v	Weight by volume
WHC	Water holding capacity
WSD	Water saturation deficit
XET	Xyloglucan endotransglycosylase
$ZnSO_4$	Zinc sulfate
1,3-diPGA	1,3-diphosphoglyceric acid
2.4-D	2,4 dichlorophenoxy acetic acid
2-D	Double dimensional views
3-D	Three dimensional views
μ	micron 10^{-6}m
λ	lambda or wave length
π	pye
ψ	psi
ψ_p	Turgor potential
ψ_s	Solute potential
ψ_w	Water potential

CONTENTS

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ANNEXURE

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ALLELOPATHY AND PLANT PHYSIOLOGY

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1. INTRODUCTION

Allelopathy refers to any process involving secondary metabolites produced by plants, microorganism, viruses, fungi that influence the growth and development of Agricultural and Biological systems. It has been established that allelopathy offers great potential (a) to increase agricultural production crop, vegetable and ornamental plants, fruit and forest trees, (b) to decrease harmful effects of modern agricultural practices [multiple cropping, leaching losses from N fertilizers, indiscriminate use to pesticides (weedicides, fungicides, insecticides, nematicides), tolerant/resistant biotypes in pest] on soil health/productivity and on environment and (c) to maintain soil productivity and a pollution free environment for our future generations. It is likely that in the near future allelopathy will be used in crop production, crop protection, agroforestry and agrohorticultural practices in developed and developing countries. Allelopathy may become one of the strategic sciences to reduce the environmental pollution and to increase agricultural production in Sustainable Agriculture of the 21st Century. The Allelopathy provides basis to Sustainable Agriculture, hence, currently the Allelopathy research is being done in most Countries Worldwide and is now receiving more attention from agricultural and bioscientists.

Allelopathy is a new field of science, as the term *'allelopathy'* was coined by Prof. Hans Molisch, a German Plant Physiologist in 1937. Therefore, till now there is no Book on Methodology of Allelopathy Research. It is causing lot of problems to researchers working in underdeveloped/Third World Countries, in small towns without Library facilities. Therefore, to make available the standard methods for conducting allelopathy research independently, this multi-volume book has been planned. Since allelopathy is multidisciplinary area of research, hence, volumes have been planned for each discipline.

2. ALLELOPATHY

Plant physiology is closely associated with allelopathy, both in the synthesis of allelochemicals and influence of allelochemicals on various physiological processes in plants. Physiological processed effect the seed germination, seedling growth, development and senescence of plant organs and plant maturity. Besides, it is also related to the synthesis of allelochemicals in plants and their release into the environment. Various factors (a) radiation, (b) mineral deficiencies, (c) water stress, (d) temperature, (e) allelopathic agents, (f) age of plant organs, (g) genetics and (h) pathogens and predators affect the amount of allelochemicals produced in plants (Rice, 1984). All these factors influence various physiological processes, which affects the synthesis of allelochemicals. These allelochemicals after the release into the environment influences the plant growth and development through the changes of following physiological processes (Rice, 1984): (a) cell division and cell elongation, (b) phytohormone induced growth, (c) membrane permeability, (d) mineral uptake, (e) availability of soil phosphorus and potassium, (f) stomatal opening (g) photosynthesis, (h) respiration, (i) protein synthesis and changes in lipid and organic acid metabolism, (j) inhibition of porphyrin synthesis, (k) inhibition or stimulation of specific enzymes, (l) corking and clogging of xylem elements, stem conductance of water and internal water relations and (o) miscellaneous mechanisms.

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Section I.
SEED PHYSIOLOGY