

Ankita Varshney
Mohammad Anis

Trees: Propagation and Conservation

Biotechnological Approaches
for Propagation of a Multipurpose Tree,
Balanites aegyptiaca Del.

Trees: Propagation and Conservation

Ankita Varshney • Mohammad Anis

Trees: Propagation and Conservation

Biotechnological Approaches for
Propagation of a Multipurpose Tree,
Balanites aegyptiaca Del.

Dr. Ankita Varshney
Department of Botany
Plant Biotechnology Laboratory
Aligarh Muslim University
Aligarh
India

Prof. Mohammad Anis
Department of Botany
Plant Biotechnology Laboratory
Aligarh Muslim University
Aligarh
India

ISBN 978-81-322-1700-8 ISBN 978-81-322-1701-5 (eBook)
DOI 10.1007/978-81-322-1701-5
Springer New Delhi Dordrecht Heidelberg London New York

Library of Congress Control Number: 2013957489

© Springer India 2014

This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed. Exempted from this legal reservation are brief excerpts in connection with reviews or scholarly analysis or material supplied specifically for the purpose of being entered and executed on a computer system, for exclusive use by the purchaser of the work. Duplication of this publication or parts thereof is permitted only under the provisions of the Copyright Law of the Publisher's location, in its current version, and permission for use must always be obtained from Springer. Permissions for use may be obtained through RightsLink at the Copyright Clearance Center. Violations are liable to prosecution under the respective Copyright Law.

The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.

While the advice and information in this book are believed to be true and accurate at the date of publication, neither the authors nor the editors nor the publisher can accept any legal responsibility for any errors or omissions that may be made. The publisher makes no warranty, express or implied, with respect to the material contained herein.

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Preface

Micropropagation or plant tissue culture comprises a set of in vitro techniques, methods, and strategies that are part of the group of technologies called plant biotechnology. Plant tissue culture technology is playing an increasingly important role in basic and applied studies. Also, the application of tissue-culture technology, as a central tool or as an adjunct to other methods, including recombinant DNA techniques, is at the vanguard in plant modification and improvement for agriculture, horticulture and forestry.

Tree propagation in vitro has been a difficult proposition compared to other plants. The response of explants is primarily determined by genotype, physiological state of the tissue, and time of the year when the explants are collected and cultured. While most nurserymen have been introduced to the techniques and advantages of micropropagation, few have ventured to use it as a propagation tool. The applicability of micropropagation for woody trees has been demonstrated as feasible since all aspects of the technology have confirmed the fact that trees produced by this method look like and grow like their counterparts produced by traditional methods of cloning. The tree species in forest, plantation, and urban environments are important biological resources that play a major role in the economy and the ecology of terrestrial ecosystems, and they have aesthetic and spiritual value. Because of these many values of the tree species, preserving forest tree biodiversity through the use of biotechnological approaches should be an integral component in any forestry program in addition to large-scale ecologically sustainable forest management and preservation of the urban forest environment. Biotechnological tools are available for conserving tree species as well as genetic characterization that will be needed for deployment of germplasm through restoration activities. This book concentrates on the biotechnological tools available for multiplying, conserving, characterizing, evaluating, and enhancing the forest tree biodiversity, especially a multipurpose semi-arid tree *Balanites aegyptiaca* Del.

Micropropagation has gained the status of a multibillion dollar-industry being practiced in hundreds of biotechnology laboratories and nurseries throughout the world. As compared to annual herbaceous plants, tissue culture of trees is a difficult proposition mainly because of their being intractable to regenerate and slow growing and having the problems of dormancy, juvenility versus maturity, phenolics, and endogenous infection as well as great physiological variation in explants collected from fields besides less consistent efforts made at the global level. In vitro micropropagation has proved, in

the recent past, a means for supply of planting material for forestry. Different basal media, plant growth regulators, media additives, and carbohydrate sources are being used to manipulate culture conditions in vitro for propagation of forest trees. Micropropagation of forest trees in vitro is not only a means for mass scale propagation of superior clones of tree species but it can also be used for developing transgenic plants and conservation of germplasm through cryopreservation.

We anticipate that the conclusion of this book will be an increased awareness of the fact that there is still a great need for strategic research in applied sciences like plant biotechnology. The contents of the book also provide an indication of some of the information in which plant biotechnology (in vitro culture of trees) is likely to go in the coming years. At the very least, we feel that it should provide a source of background information and references to both students and researchers alike who wish to initiate or broaden their interests in the field.

It is noticeable that many of the chapters described in this book tend to be complimentary to each other, particularly where a combination of techniques may be required to achieve an ideal objective. This reinforces a vision held by us that plant biotechnology can rightly be considered a novel and key area of research involving both the applied and basic aspects of plant sciences.

September 2013

Dr. Ankita Varshney
Prof. Mohammad Anis

Acknowledgments

The research support and assistance rendered by the Department of Science and Technology and the University Grant Commission, Govt. of India, New Delhi, in the form of DST-FIST (2011–2016) and UGC-DRS-I (2009–2014) programs is duly acknowledged. The award of Young Scientist under DST-FAST TRACK scheme to Ankita Varshney is also gratefully acknowledged. We would also like to acknowledge the help rendered by Dr. Anushi Arjument Jahan, in preparing the layout of the book. We would also like to place on record our sincere thanks to other colleagues in Plant Biotechnology Laboratory, Department of Botany, Aligarh Muslim University, Aligarh, for their support and cooperation. We would also like to thank Springer for providing us the opportunity to publish this book.

Contents

1	Introduction	1
1.1	Global and National Scenarios	1
1.2	In Vitro Approaches	3
1.3	Constraints with Tree Tissue Culture	4
1.4	Plant Tissue Culture for Trees	4
1.5	Taxonomy	5
1.5.1	Scientific Classification	5
1.5.2	Binomial name	5
1.5.3	Common names	5
1.5.4	Habitat	5
1.5.5	Morphological Description	5
1.5.6	Active Constituents.....	6
1.5.7	Medicinal Properties and Uses	7
1.5.8	Other Uses	7
1.5.9	Conventional Propagation Methods and its Limitations	7
	References	8
2	Review of Literature	11
2.1	Introduction.....	11
2.2	Micropropagation	13
2.3	Various Approaches for Micropropagation	14
2.3.1	The Propagation of Plants from Axillary Buds or Shoots.....	14
2.3.2	Propagation by Adventitious Shoot Organogenesis	15
2.4	Factors Affecting In Vitro Shoot Regeneration and Growth of Plants.....	17
2.4.1	Explant Type.....	17
2.4.2	Plant Growth Regulators.....	18
2.4.3	Medium pH Levels.....	21
2.4.4	Basal Media.....	22
2.4.5	Carbohydrate Source.....	24
2.4.6	Subculture Passages	25
2.5	Rooting of In Vitro-Regenerated Shoots	26
2.6	Acclimatization and Hardening of Plantlets	27
2.7	Advancement in Plant Tissue Culture: Synthetic Seed Technology	28

2.8	Clonal Fidelity of Micropropagated Plants	29
2.8.1	PCR-Based DNA Markers	30
2.9	Antioxidant Enzymes	31
	References	33
3	Materials and Methods	49
3.1	Plant Materials and Explant Source	49
3.2	Surface Disinfection of Seeds and Explants	49
3.3	Establishment of Aseptic Seedlings and Preparation of Explants	49
3.4	Culture Media	50
3.4.1	Composition of Basal Media	50
3.4.2	Preparation of Stock Solutions	50
3.5	Plant Growth Regulators	51
3.6	Adjustment of pH, Gelling of Medium, Carbon Source and Sterilization	52
3.7	Sterilization of Glassware and Instruments	52
3.8	Sterilization of Laminar AirFlow Hood	52
3.9	Inoculation and Culture Conditions	52
3.10	Rooting	52
3.11	Acclimatization and Hardening of Plantlets	52
3.12	Synthetic Seed	53
3.12.1	Explant Source	53
3.12.2	Encapsulation Matrix	53
3.13	Physiological and Biochemical Studies of in vitro Regenerated Plants During Acclimatization	53
3.13.1	Chlorophyll and Carotenoid Estimation	53
3.14	Assessment of Antioxidant Enzyme Activities	54
3.14.1	Superoxide Dismutase	54
3.14.2	Catalase	55
3.14.3	Ascorbate Peroxidase	56
3.14.4	Glutathione Reductase	56
3.14.5	Soluble Protein	57
3.15	Anatomical Studies	58
3.15.1	Fixation and Storage	58
3.15.2	Embedding, Sectioning and Staining	58
3.16	Chemicals and Glassware Used	58
3.17	Statistical Analysis	58
3.18	Genomic DNA Isolation and Purification	58
3.18.1	Preparation of Stock Solutions Required for DNA Extraction	58
3.18.2	Extraction and Purification Protocol	59
3.18.3	Quantitative and Qualitative Assessment of Genomic DNA	59
3.19	PCR Amplification of DNA Using ISSR Primers	60
3.19.1	Polymerase Chain Reaction Amplification	60
3.19.2	ISSR-PCR with Genomic DNA	61

3.19.3	Analysis of PCR Products by Agarose Gel Electrophoresis	61
3.19.4	Data Scoring and Analysis	61
References	61
4	Results	63
4.1	Direct Shoot Regeneration.....	63
4.1.1	Establishment of Aseptic Seedling.....	63
4.1.2	Regeneration from CN Explant Excised from 15-day-old Aseptic Seedlings.....	63
4.1.3	Regeneration from Nodal Explant Excised from 4-week old Aseptic Seedlings	68
4.1.4	Regeneration from Root Explants Excised from 4-week-old Aseptic Seedlings	75
4.1.5	Induction of Multiple Shoots from Intact Seedlings ...	80
4.1.6	Regeneration from Mature Nodal Explants Excised from 10-year-old Candidate Plus Tree	83
4.1.7	Rooting of Microshoots	88
4.1.8	Acclimatization	91
4.1.9	Synthetic Seeds.....	91
4.1.10	Rooting in Synthetic Seeds and Establishment of Plants in Soil	92
4.2	Assessment of Physiological and Biochemical Parameters...	93
4.2.1	Photosynthetic Pigments	93
4.2.2	Antioxidant Enzyme Activities	93
4.3	Clonal Fidelity in TC-Raised Plantlets Derived from Mature Nodal Explants	93
5	Discussion	101
5.1	Effect of Plant Growth Regulators on Different Explants ...	101
5.2	Effect of Different Media, Sucrose Concentrations and pH Levels	104
5.3	Rooting and Acclimatization	104
5.4	Synthetic Seeds	106
5.5	Antioxidant Enzymes Activities	107
5.6	Assessment of Genetic Fidelity	108
References	108
6	Summary and Conclusions	115
6.1	Summary	115
6.2	Conclusions	116

About the Authors

Ankita Varshney obtained her Ph.D. degree in Botany (Plant Biotechnology) in 2012 from the Aligarh Muslim University, Aligarh. Currently, she is working as a Young Scientist under DST-FAST TRACK scheme. Earlier, she worked as Research Associate of CSIR, New Delhi. She has published 10 research papers in journals of national and international repute. Her research focuses on in vitro regeneration, biochemical and molecular characterization of medicinal plants.

Mohammad Anis is a Professor and a Former Chairman in the Department of Botany at the Aligarh Muslim University, Aligarh. He has 26 years of teaching and research experience in cytogenetics, plant tissue culture and morphogenesis. He has published more than 150 research papers in journals of national and international repute. His current research focuses on morphogenesis and in vitro propagation of medicinal and woody plant species. He has been a Programme Coordinator of DST-FIST, DBT-HRD and UGC-DRS-I projects in the department. He has been conferred with “Vigyan Ratan Samman-2010” by the U.P. Council of Science and Technology.

Abbreviations

2-iP	2-isopentenyladenine
APX	Ascorbate peroxidase
B ₅	Gamborg's medium
%	Percent
°C	Degree celsius
2,4-D	2,4-Dichlorophenoxyacetic acid
BA	6-Benzyladenine
BSA	Bovine serum albumin
CAT	Catalase
Chl	Chlorophyll
cm	Centimeter
CN	Cotyledonary node
CTAB	Cetyl-trimethylammonium bromide
d	Days
dATP	Dinucleotide adenine triphosphate
dCTP	Dinucleotide cytosine triphosphate
DDW	Double distilled water
dGTP	Dinucleotide guanine triphosphate
DNA	Deoxyribonucleic acid
dNTPs	Dinucleotide triphosphates
dTTP	Dinucleotide thiamine triphosphate
ε	Extinction coefficient
EDTA	Ethylene diamine tetraacetic acid
EU	Enzyme units
FAA	Formalin acetic acid
FW	Fresh weight
g	Gram
GR	Glutathione reductase
GSH	Reduced glutathione
GSSG	Oxidized glutathione
h	Hour
H ₂ O ₂	Hydrogen peroxide
HCl	Hydrochloric acid
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
ISSR	Inter-sequence simple repeats
Kn	Kinetin (6-furfurylaminopurine)

L ₂	Phillips and Collin's medium
M	Molarity
Mg	Miligram
mg/l	Miligram per liter
MgCl ₂	Magnesium chloride
min	Minute
ml	Milliliter
mm	Millimeter
mM	Milimolar
MS	Murashige and Skoog medium
N	Normality
NAA	α-naphthalene acetic acid
NaCl	Sodium chloride
NADPH	Nicotinamide adenine dinucleotide phosphate
NaOH	Sodium hydroxide
NBT	Nitroblue tetrazolium
nm	Nanometer
OD	Optical density
PCR	Polymerase chain reaction
PGRs	Plant growth regulators
PPFD	Photosynthetic photon flux density
ppm	Parts per million
PVP	Polyvinyl pyrrolidone
RAPD	Random amplified polymorphism DNA
RH	Relative humidity
RNA	Ribonucleic acid
ROS	Reactive oxygen species
rpm	Rotation per minute
sec	Second
SOD	Superoxide dismutase
TBE	Tris-borate EDTA
TCA	Trichloroacetic acid
TDZ	Thidiazuron
TE	Tris-HCl EDTA
U	Unit
UBC	University of British Columbia
UV	Ultraviolet
V	Volt
v/v	Volume by volume
W	Watt
w/v	Weight by volume
μg	Microgram
μM	Micromolar