Thapar University

DECLARATION

I hereby declare that the work which is being presented in this thesis **"Evaluation and production of Bacosides from selected clones of *Bacopa monnieri* (L.) Wettst."** submitted by me for the award of the degree of *Doctor of Philosophy* in the Department of Biotechnology, Thapar University, Patiala, is true and original record of my own independent and original research work carried out under the supervisions of **Dr. Anil Kumar**, Associate Professor and **Dr. M. Sudhakara Reddy**, Professor, Department of Biotechnology, Thapar University, Patiala, India. The matter embodied in this thesis has not been submitted in part or full to any other university or institute for the award of any degree in India or Abroad.

(Mahima Bansal)

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Place Patiala

(Mahima Bansal)

I dedicate this thesis to

My parents for their constant support and unconditional love!!

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The following publications are the outcome of the present research work:

1. **Bansal M**, Kumar A, Reddy MS (2014). Diversity among wild accessions of *Bacopa monnieri* (L.) Wettst. and their morphogenetic potential. Acta Physiologiae Plantarum. 36: 1177-1186.

2. **Bansal M**, Kumar A, Reddy MS (2014). Influence of *Agrobacterium rhizogenes* strain on hairy roots induction and 'bacoside A' production from *Bacopa monnieri* (L.) Wettst. Acta Physiologiae Plantarum. DOI 10.1007/s11738-014-1650-5.

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Conference presentations

- **Bansal M**, Khilwani B, Kumar A, Reddy MS (2010). Biotechnological approaches for the production of bacosides from *Bacopa monnieri* (L.) Wettst., National Conference on emerging trends in biopharmaceuticals: relevance to human health, held at Thapar University, Patiala, 11-13 November 2010

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ABSTRACT

Plant derived natural products represent some of the most important pharmaceuticals available today. However, uncertainty regarding the commercial supply due to the limited availability of many plants in nature has resulted in a dramatic reduction in the use of natural products. Plant cell suspension culture capable of large scale industrial production of such pharmaceutically important molecules is the alternative which promises sustained and assured supply of these important molecules. Hairy roots induced following infection with *Agrobacterium rhizogenes* are capable of unlimited growth in culture in the absence of plant growth regulator and exhibit higher potential for the production of secondary metabolite production. The objectives of the present study were to select elite clones of *B. monnieri*, investigate the production of 'bacoside A' using cell suspension cultures and hairy root cultures and finally enhancing the production of 'bacoside A' of cell suspension cultures and hairy root cultures.

Fourteen accessions of *B. monnieri* (BM1- BM14) collected from different locations across India were maintained in nursery at Thapar University. Variation in the content of 'bacoside A' and biomass per plant in fourteen accessions of *B. monnieri* were studied during the different seasons of the year. Maximum biomass accumulation and 'bacoside A' contents were recorded in the samples processed in summer (June) in all the accessions and minimum biomass and 'bacoside A' content was recorded in winter (December). Amongst accessions, BM1 and BM7 recorded higher biomass accumulation, 'bacoside A' content, Relative growth rate (RGR) and Harvest Index (HI). These parameters showed minimum values in accession BM14. Molecular diversity was then investigated amongst these accessions using random amplified polymorphic DNA (RAPD) and Inter simple sequence repeats (ISSR). About, 35 % variations were detected in these populations based on combined data of RAPD and ISSR.

Clustering based on molecular marker data grouped these accessions into two major groups and placed accession BM14 as an out group. Maximum shoot organogenic potential was observed in accession BM6 and maximum rooting potential was observed in accessions BM1, BM2, BM7, BM10 and BM14. Based on *in vitro* morphogenetic response, 'bacoside A' content and growth, accession BM6 was selected for the studies of cell suspension and hairy root cultures. Cell suspension cultures established on MS medium supplemented with α-Napthaleneacetic acid (NAA) at 5.0 µM and Kinetin (KIN) at 1.15 µM showed maximum cell growth and levels of 'bacoside A'. Hairy roots induced by strain MTCC 2364 showed higher biomass accumulation and 'bacoside A' content by five-folds. Further, attempts were made to optimize growth and production of 'bacoside A' using cell suspension and hairy root cultures for commercial supply. Optimization of conditions for 'bacoside A' production was explored using conventional method (one-variable-at-a-time approach) and statistical method (Response surface methodology, RSM). Optimization of medium components using RSM leads to around two-fold increment in biomass accumulation and levels of 'bacoside A'. The current study uncovered several aspects of the strategies for the conservation of this medicinally important herb and increased the 'bacoside A' production using cell suspension and hairy root cultures. These have the potential of up-scaling in the bioreactors for the production of 'bacoside A'. Thus can be helpful to reduce the pressure on the wild populations.

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Abbreviations

Abbreviations	Word (s)
%	Percent
2,4-D	2,4-Dichlorophenoxy acetic acid
ºC	Degree Celsius
Abs	Absorbance
BA	6-Benzylaminopurine
bp	Base pair
cm	Centimeter
DNA	Deoxyribonucleic acid
dNTPs	deoxynucleotide Triphosphates
EDTA	Ethylene Diamine Tetraacetic Acid
g	Gram
h	Hour
IAA	indole-3-acetic acid
IBA	indole-3-butyric acid

ISSR	Inter-simple sequence repeat
kb	Kilobase
KIN	Kinetin (N6-furfuryladenine)
M	Molar
m	Meter
mg	Milligram
min	Minute
ml	Mililitre
mM	Milli molar
µM	Micro molar
MS	Murashige and Skoog medium 1962
NAA	1-Naphthaleneacetic acid
OD	Optical density
PCR	Polymerase chain reaction
PGRs	Plant growth regulator (s)
rpm	Rotation per minute
PCA	Principal component analysis
RAPD	Random amplified polymorphic DNA

s	Second
TAE	Tris-Acetate-EDTA
TBE	Tris-Borate-EDTA
TE	Tris-EDTA
Tris	Tris-(hydroxymethyl)- aminomethane
U	Unit
UV	Ultra Violet
V	Volt
v/v	volume by volume
w/v	weight by volume`
YMB	Yeast Mannitol Broth
µg	Microgram
µl	Microliter
µm	Micrometre
µmol	Micromole
µM	Micromolar