IN VITRO SELECTION OF SALT TOLERANT SAPLINGS OF *MORUS ALBA* AND *MENTHA ARVENSIS* AND THEIR FIELD PERFORMANCE WITH BIOINOCULANTS

By

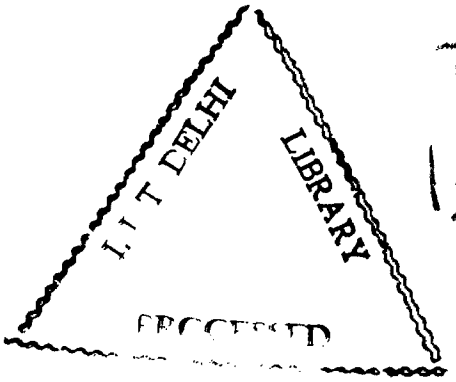
SUMAN KASHYAP

Centre for Rural Development and Technology

Submitted
In fulfilment of the requirements of the degree of Doctor of Philosophy
to the

Indian Institute of Technology, Delhi
May, 2002

saline soils; fungus



CERTIFICATE

This is to certify that the thesis entitled, "*In vitro* selection of salt tolerant saplings of *Morus alba* and *Mentha arvensis* and their field performance with bioinoculants" submitted by Mrs. Suman Kashyap has been prepared under our guidance with the rules and regulations of Indian Institute of Technology, Delhi, India. The research reports and results presented in this thesis have not been submitted for any degree or diploma in any other University or Institute.

Padma Vasudevan

(Prof. Padma Vasudevan)

Centre for Rural Development & Technology,
Indian Institute of Technology, Delhi
Hauz Khas, New Delhi – 110016
INDIA

Satyawati

(Dr. Satyawati Sharma)

Centre for Rural Development & Technology,
Indian Institute of Technology, Delhi
Hauz Khas, New Delhi – 110016
INDIA

TO
DEAR SIDDHARTH

ACKNOWLEGDEMENTS

I wish to express my heartfelt thanks and sincere gratitude to my supervisor, Dr.Satyawati Sharma for her invaluable guidance, constructive criticism, fruitful discussions and constant encouragement throughout the course of this work. Her painstaking efforts for providing lab facilities for tissue culture work are gratefully acknowledged.

I extend my deepest sense of obligation and warmest regards to my co-supervisor, Prof. Padma Vasudevan for her noble guidance and helpful suggestions.

I owe my indebtedness to Dr. M.V. Rajam (Department of Genetics, University of Delhi, Delhi) and Prof. Bisaria (DBEB, IIT, Delhi) for allowing me to work in their tissue culture laboratory during my initial stage of work. I am grateful to Prof. S.S Bhojwani (Department of Botany, University of Delhi, Delhi) and Dr. P.K. Chand(Department of Botany, Utkal University, Bhubaneswar) for their kind advice and for providing the reprints of their papers.

My sincere thanks are due to all colleagues and friends especially Anuja, Anshu, Vidya, Prabhat, Mohini, Neetu and Dhananjay for their helping and cooperative attitude. Thanks are also due to faculty and staff of the Centre for Rural Development & Technology for their help and encouragement.

I deem it my most pleasant duty to express my sincere appreciation to my husband, Mr. Dharmendra Kashyap for his continuous cooperation and support throughout the work. I am deeply obliged to my son, Siddharth for his love, affection and patience. This note would be incomplete if I fail to thank other family members especially to my father-in-law, Mr. Ranjit Kumar and mother-in-law, Mrs. Tara kashyap for providing me support in my research work.

I am deeply bestowed to my parents for their blessings and moral support.

All may not be mentioned here but none is forgotten.

(Suman Kashyap)

ABSTRACT

Salinity in soil is one of the major environmental stresses limiting plant growth and productivity. About 25 percent of the world's potentially arable land suffer from excessive salinity. There is an urgent need to exploit these saline soils to meet the increasing demands of expanding population for food and energy. In this context, the biological manipulation of plants by tissue culture is a promising, environment friendly and energy efficient approach for reclaiming and utilizing such type of land. The technique immensely reduces the labour and space requirements for evaluating and selecting large number of genotypes within a short span of time irrespective of the season. Further, the acclimatisation of *in vitro* selected salt resistant saplings with suitable bioinoculants like Arbuscular Mycorrhizal (AM) fungi and *Azotobacter* may enhance their survival percentage and enrich the biomass nutritionally.

Keeping the above in view, present study was focussed on *in vitro* selection of salt tolerant saplings of *Morus alba* (var. sujanpuri) and *Mentha arvensis* (var. piperascens) and their subsequent acclimatisation with native species of AM fungi and *Azotobacter*. Also, *in vivo* comparative studies were performed to select salt resistant saplings of both plant species in field conditions. Both *Morus alba* and *Mentha arvensis* are in great demand in rural areas due to their major use in sericulture and for extraction of mint oil respectively.

The dominating native AM fungi and *Azotobacter* species isolated from normal as well as saline soil by standard techniques were identified as *Glomus* and *Azotobacter chroococcum* respectively. The AM spores and number of *Azotobacter* cells in Micromodel (normal) soil were found to be more than in saline soil.

The salt resistant saplings of both plant species were screened and selected *in vitro* during different seasons of the year on MS medium with standardized phytohormone concentrations. Salt stress was induced by gradually applying NaCl (Sodium chloride) from 0.1% (w/v) onwards. Also, AM spore and root extracts of both plants species were tested *in*

vitro for inducing rhizogenesis. The selected saplings were acclimatized with/without bioinculants i.e, AM fungi (M), *Azotobacter* (A), AM fungi + *Azotobacter* (M+A) and control (C). Finally, some of the saplings were transplanted at wasteland (village Jamalpur, district Gurgaon) in Haryana.

Nodal explants with axillary buds of both plant species cultured on MS+6BAP (6 Benzyl Amino Purine, 2.5 mg/l for *Morus alba* and 2.0 mg/l for *Mentha arvensis)* were found to be the best for shoot multiplication *in vitro*. The addition of 0.3 mg/l of GA_3 to 6 BAP further enhanced shoot multiplication in case of *Morus alba.* Rooting was induced with 1.0 mg/l of IBA (Indole Butyric Acid) in *Morus alba* and 1.0 mg/l of IAA (Indole Acetic Acid) in *Mentha arvensis.* The culturing of explants during different seasons of year also influenced shoot multiplication in both plant species. The period between November to February for *Morus alba* and March to June for *Mentha arvensis* was found best for explant culturing with maximum shoot multiplication. On the basis of survival percentage, the salt resistant saplings were selected upto 0.4% (w/v) NaCl in *Morus alba* and 0.7% (w/v) NaCl in *Mentha arvensis* by gradually increasing salt concentration from 0.1% NaCl onwards. The AM spore extract and root extract of *Morus alba* were found better than IBA in inducing rhizogenesis while in *Mentha arvensis*, possibly due to presence of flavonoids and monoterpenes, these extracts did not exert better effects than IAA. The acclimatisation of selected saplings were found best on M+A due to positive synergistic effect of two microorganisms. The *in vitro* selected salt resistant saplings revealed more survivability (10-30% in *Morus alba*, 10-40% in *Mentha arvensis*) on wasteland transplantation than saplings developed without NaCl which resulted in 100% mortality.

In vivo studies were conducted for salt resistant saplings selection, by applying standardized auxin concentrations (15 ppm IBA in *Morus alba,* 10 ppm IAA in *Mentha arvensis*), NaCl regular watering [0.05% (w/v) in *Morus alba,* 0.08% (w/v) in *Mentha*

arvensis] and suitable bioinoculants. The M+A+auxin treatment resulted in maximum survival, saplings' growth and rhizosphere micoflora in both plant species. But these saplings survived very less (10%) on transplantation in Jamalpur wasteland.

Overall, the results indicate that the *in vitro* methods of selection of salt tolerant saplings were superior to *in vivo* methods due to the selection of large number of saplings in lesser time, tolerance to higher level of NaCl concentration and higher survival rate after transplantation to wasteland.

The salt tolerant characteristics observed in plants may either be due to some genetic changes or possible pre-existing variations in parental stock. It is further required to study the inheritance of the variants to propagate these on saline land.

CONTENTS