

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

By

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Submitted

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



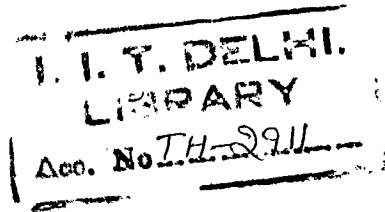
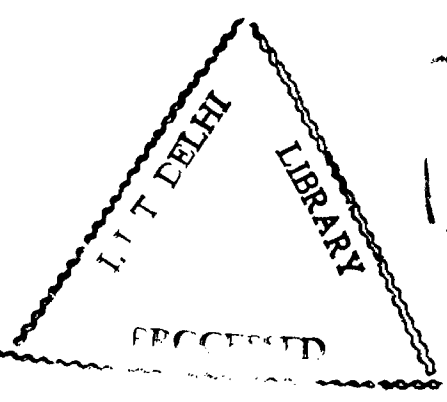
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## CERTIFICATE

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.

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**TO  
DEAR SIDDHARTH**

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(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*

*in vitro* for inducing rhizogenesis. The selected saplings were acclimatized with/without bioinoculants 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<sub>3</sub> 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

LIST OF FIGURES .....	1
LIST OF TABLES .....	2
LIST OF PLATES .....	3
ABBREVIATIONS .....	4
CHAPTER – I (INTRODUCTION) .....	5
1.1. Scope of the present work .....	13
1.2. Objectives .....	14
CHAPTER – II (REVIEW OF LITERATURE) .....	16
2.1. Land degradation and soil salinity .....	16
2.2. Tissue culture and production of salt tolerant saplings .....	20
2.3. Tissue culture and Bioinoculants .....	26
2.3.1 AM fungi and soil salinity .....	26
2.3.2 Mycorrhization of Tissue Cultured Plantlets .....	28
2.3.3 <i>Azotobacter</i> and soil salinity .....	31
2.4 <i>Morus</i> (mulberry) and micropropagation .....	33
2.5 <i>Mentha</i> (mint) and micropropagation .....	36
CHAPTER – III (EXPERIMENTAL WORK) .....	39
3.1. Culturing of native AM fungi and <i>Azotobacter</i> species .....	39
3.1.1. Culturing of native AM fungi and production of inocula .....	39
3.1.1a. Screening, isolation and identification of AM fungi .....	39
3.1.1b. Inoculation and multiplication of AM fungi .....	40
3.1.1c. Production of AM inocula .....	41
3.1.2. Culturing of <i>Azotobacter</i> species .....	42
3.2. <i>In vitro</i> studies .....	44
3.2.1. Explant collection and sterilization .....	44
3.2.2. Standardization of phytohormones for culture .....	45
3.2.2a. Culture medium for shoot multiplication .....	45
3.2.2b. Culture medium for root multiplication .....	46
3.2.3. Screening and selection of salt tolerant saplings .....	47
3.2.3a. Shoot multiplication .....	47
3.2.3b. Root multiplication .....	47
3.2.4. AM spore and root extracts for rhizogenesis .....	48
3.2.4a. Plantation of <i>Morus alba</i> and <i>Mentha arvensis</i> with/without AM inocula .....	48
3.2.4b. Preparation of AM root extracts .....	49
3.2.4c. Preparation of AM spore extracts .....	50
3.2.5. Acclimatization and transfer of tissue cultured saplings to the field level .....	50
3.3. <i>In vivo</i> studies .....	53
3.3.1. Standardization of auxin concentration .....	53
3.3.2. Standardization of NaCl (Sodium chloride) concentration .....	54
3.3.3. Screening and selection of salt tolerant saplings .....	55
3.4. Statistical Analysis .....	57
CHAPTER – IV (RESULT & DISCUSSION) .....	58
4.1. Culturing and production of inocula of native AM fungi and <i>Azotobacter</i> species .....	58
4.1.1. Culturing of AM fungi .....	58
4.1.2. Culturing of <i>Azotobacter</i> .....	68
4.2. <i>In vitro</i> studies .....	69
4.2.1. Selection of explants .....	70
4.2.1a. Internodal explants .....	71

4.2.1b.	Nodal explants without axillary buds .....	71
4.2.1c.	Apical shoot buds .....	72
4.2.1d.	Nodal explants with axillary buds .....	72
4.2.2.	Standardization of phytohormones .....	77
4.2.2.1.	Phytohormones for shoot multiplication .....	77
4.2.2.1a.	Shoot multiplication in internodal explant .....	78
4.2.2.1b.	Shoot multiplication in nodal explants (without axillary buds) ....	79
4.2.2.1c.	Shoot multiplication in apical shoot buds .....	80
4.2.2.1d.	Shoot multiplication in nodal explants with axillary buds .....	81
4.2.2.2.	Phytohormones for root multiplication .....	84
4.2.2.2a.	Phytohormones for root multiplication in <i>Morus alba</i> .....	91
4.2.2.2b.	Phytohormones for root multiplication in <i>Mentha arvensis</i> .....	92
4.2.3.	Screening and selection of salt tolerant saplings .....	95
4.2.3.1.	Effect of seasons in selection of explants .....	96
4.2.3.2.	Effect of NaCl on shoot multiplication .....	96
4.2.3.2a.	Effect of NaCl on shoot multiplication in <i>Morus alba</i> in .....	98
	different seasons	
4.2.3.2b.	Effect of NaCl on shoot multiplication in <i>Mentha arvensis</i> in ...	103
	different seasons	
4.2.3.3.	Effect of NaCl on root multiplication .....	108
4.2.3.3a.	Effect of NaCl on root multiplication in <i>Morus alba</i> .....	108
4.2.3.3b.	Effect of NaCl on root multiplication in <i>Mentha arvensis</i> .....	116
4.2.3.4.	Effect of AM root and spore extracts on rhizogenesis in <i>Morus alba</i> .....	125
4.2.3.4a.	Effect of AM root and spore extracts on rhizogenesis in .....	125
	<i>Morus alba</i>	
4.2.3.4b.	Effect of AM root and spore extracts on rhizogenesis in .....	132
	<i>Mentha arvensis</i>	
4.2.4.	Acclimatization/Hardening of <i>in vitro</i> selected salt tolerant saplings .....	133
4.2.4a.	Effect of different treatments on growth of acclimatized saplings of .....	134
	<i>Morus alba</i>	
4.2.4b.	Effect of different treatments on growth of acclimatized saplings of .....	137
	<i>Mentha arvensis</i>	
4.2.4c.	Effect of different treatments on microbial number in pot soil of .....	141
	<i>Morus alba</i>	
4.2.4d.	Effect of different treatments on microbial number in pot soil of .....	143
	<i>Mentha arvensis</i>	
4.2.5.	Transplantation of salt tolerant saplings in field .....	146
4.2.5a.	Transplantation of salt tolerant saplings of <i>Morus alba</i> .....	146
4.2.5b.	Transplantation of salt tolerant saplings of <i>Mentha arvensis</i> .....	147
4.3.	<i>In vivo</i> studies .....	154
4.3.1.	Standardization and selection of auxin concentration with bioinoculants .....	154
4.3.1a.	Standardization of IBA concentration in <i>Morus alba</i> .....	155
4.3.1b.	Standardization and selection of IAA concentration in <i>Mentha arvensis</i> ....	158
4.3.2.	Standardization of NaCl concentration.....	163
4.3.2a.	Standardization of NaCl concentration in <i>Morus alba</i> .....	163
4.3.2b.	Standardization of NaCl concentration in <i>Mentha arvensis</i> .....	165
4.3.3.	Screening and selection of salt tolerant saplings .....	169
4.3.3a.	Effect of different treatments on growth of <i>Morus alba</i> .....	169
4.3.3b.	Effect of different treatments on growth of <i>Mentha arvensis</i> .....	177
4.3.3c.	Effect of different treatments on microbial number in <i>Morus alba</i> .....	182
4.3.3d.	Effect of different treatments on microbial number in <i>Mentha arvensis</i> ....	183
4.3.4.	Transplantation of selected saplings in field conditions .....	184
4.3.4a.	Transplantation of salt tolerant saplings of <i>Morus alba</i> .....	184
4.3.4b.	Transplantation of salt tolerant saplings of <i>Mentha arvensis</i> .....	185
	CHAPTER – V (SUMMARY AND CONCLUSIONS) .....	189
	REFERENCES .....	197
	APPENDIX .....	217
	CURRICULUM VITAE .....	220