

# Studies on development of Saline Tolerance in plants

## TABLE OF CONTENTS

No.	Title		Page No.
<b>1</b>	<b>REVIEW OF LITERATURE</b>		
1.0	Introduction		1
1.1	Perception of salt stress		1
	1.1.1	Secondary Messengers involved in salt stress signaling	2
	1.1.2	Ion Homeostasis	3
	1.1.3	Sodium uptake	4
	1.1.4	Sodium Efflux	5
	1.1.5	Sodium compartmentation	5
1.2	Oxidative Stress Management		6
1.3	Synthesis of osmolytes		6
1.4	Halophytes		7
1.5	Glycophytes		7
1.6	Identifying determinants for salt tolerance		8
1.7	Mechanism of salt tolerance		9
1.8	Development of salt tolerance in plants		10
1.9	Increasing plant salt tolerance through genetic engineering		11
	1.9.1	Genes involved in Ion homeostasis	11
	1.9.2	Genes involved in Oxidative Stress Management	13
	1.9.3	Genes involved in Synthesis of osmolytes	13

	1.10	Synthesis of Glycine betaine	17
	1.11	Genetic engineering using the gene for choline oxidase from <i>Arthrobacter</i>	21
	1.12	Aim and scope of the study	23
	1.12.1	Work plan for present investigation	24
<b>2</b>	<b>TISSUE CULTURE STUDIES FOR DEVELOPMENT OF REGENERATION PROTOCOL FOR GROUNDNUT</b>		
	2.0	Introduction	25
	2.1	Literature studies	26
	2.2	Materials and Methods	27
	2.2.1	Standardization of surface sterilization treatment	27
	2.2.2	Culture Medium and growth conditions	29
	2.3	Results and Discussions	30
	2.3.1	Direct Organogenesis from young leaflets	30
	2.3.2	Direct Organogenesis from embryo axis	35
	2.3.3	Direct Organogenesis from Cotyledon explants	37
	2.3.4	Indirect organogenesis from mature leaves	41
<b>3</b>	<b>BIOCHEMICAL CHANGES SEEN IN GROUNDNUT DUE TO SALT STRESS</b>		
	3.0	Introduction	44
	3.1	Effect of salt stress on biochemical parameter: H <sub>2</sub> O <sub>2</sub> , catalase and paroxidase.	45
	3.2	Damages caused by ROS	46
	3.3	Methods and Material	52
	3.3.1	Effect of salt stress on seed germination and growth of ground nut <i>in vivo</i>	52
	3.3.2	Effect of salt stress on <i>in vitro</i> seed germination and growth of cultures	53

		3.3.3	Role of Hydrogen Peroxide in Plant	53
			3.3.3.1      H <sub>2</sub> O <sub>2</sub> Estimation	54
		3.3.4	Role of catalase in plants	55
			3.3.4.1      Catalase Estimation	56
		3.3.5	Role of Peroxidases in plants	56
			3.3.5.1      Peroxidase Estimation	57
	3.4		Result and Discussion	58
		3.4.1	Effect of salt stress on seed germination and growth of ground nut <i>in vivo</i>	58
		3.4.2	Effect of salt stress on <i>in vitro</i> seed germination and growth of cultures.	63
		3.4.3	Effect of salt stress on H <sub>2</sub> O <sub>2</sub> release	64
		3.4.4	Effect of salt stress on catalase release	65
		3.4.5	Effect of salt stress on Peroxidase release	66
<b>4</b>			<b>TRANSFORMATION OF AGROBACTERIUM</b>	
	4.0		Introduction	68
	4.1		Materials and Methods	69
		4.1.1	Cultures and Maintenance	69
		4.1.2	Media & Antibiotics	70
		4.1.3	Antibiotic Stocks	70
	4.2		Methods	70
		4.2.1	Plasmid Extraction by Alkaline Lysis Method	70
		4.2.2	Competent Cell Preparation	71

		4.2.3	Agarose gel electrophoresis	73
		4.2.4	Triparental Mating	73
		4.5.5	Ketolactose Test	75
	4.6		Results and discussion	75
		4.6.1	Transformation of pHS724 into <i>E.coli</i> DH5 $\alpha$	75
		4.6.2	Transformation of pHS724 into <i>Agrobacterium tumefaciens</i> LBA4404	78
		4.6.3	Confirmation of <i>A.tumefaciens</i> transformants by Ketolactose test	80
		4.6.4	Expression of Choline Oxidase (cox) in <i>Agrobacterium</i>	80
	4.7		Discussion	84
<b>5</b>			<b>TRANSFORMATION OF GROUNDNUT PLANTS FOR SALINE TOLERANCE</b>	
	5.1		Strategies of generating transgenic plants	86
		5.1.1	Electroporation	86
		5.1.2	Microprojectile Bombardment	86
		5.1.3	Microinjection	87
		5.1.4	<i>Agrobacterium tumefaciens</i> mediated transformation	87
			5.1.4.1 Ti Plasmid	88
			5.1.4.2 Cointegrative vector	91
			5.1.4.3 Binary vector	91
	5.2		Materials and Methods	92
		5.2.1	Plant Material, culture initiation and maintenance	92
		5.2.2	<i>Agrobacterium strains and plasmid vectors</i>	94
		5.2.3	Vector Map	94
		5.2.4	Co-cultivation	95
			5.2.4.1 Preparation of <i>Agrobacterium</i> suspension for co-cultivation	95

		5.2.4.2	Protocol used for Agrobacterium mediated transformation	96
		5.2.5	Selection of transformants, multiplication and growth conditions	99
		5.2.6	GUS analysis	100
		5.2.6.1	Preparation of GUS assay solution	100
	5.3	Results and Discussion		101
		5.3.1	Effect of Explant type on transformation	103
		5.3.2	Effect of Induction medium	105
		5.3.3	Effect of co-cultivation periods on transformation	105
		5.3.4	Effect of kanamycin concentration in selection	106
		5.3.5	Confirmation of transformants	108
		5.3.5.1	Phenotypic Expression	108
		5.3.5.2	GUS Expression	113
	<b>SUMMARY</b>			114
	<b>CONCLUSION</b>			117
	<b>REFERENCES</b>			118
	<b>PRESENTATION AND PUBLICATIONS</b>			138