Characterization of Cotton *GhMATE* and *GhALMT1* Systems to Enhance the Acid Soil Tolerance of Cotton Plants (*Gossypium hirsutum* L.)

Thesis submitted to the Presidency University for the award of the degree of

Doctor of Philosophy in Science

by

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This thesis is

Dedicated to

My beloved parents

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CERTIFICATE

This is to certify that the thesis entitled "**Characterization of Cotton *GhMATE* and *GhALMT1* Systems to Enhance the Acid Soil Tolerance of Cotton Plants (*Gossypium hirsutum* L.)**" submitted to Presidency University, Kolkata, for the award of the Degree of Doctor of Philosophy in Science is an authentic record of original work carried out by Atreyee Kundu (*R-14RS18210022*), under my supervision and guidance at Department of Life Sciences, Presidency University, Kolkata, India.

I further certify that no part of this thesis has previously formed the basis for the award of the candidate of any Degree, Diploma, Assistantship, Fellowship or other similar titles of this or any other University or Society.

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DECLARATION

I hereby declare that this work has been carried out by me under the guidance of Dr.M.Ganesan, Assistant Professor, Department of Life Sciences, Presidency University, Kolkata, West Bengal, India, and this work has not been submitted for any degree either in part or in full to any other University.

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PREFACE

Soil acidity is one of the most significant limiting factors to the crop yield. The ionic form of Aluminum (Al^{3+}) is one of the dangerous toxins under acid soil condition. Approximately 30 - 40% of world cultivable land is categorized under soil acidity. Under Al stress conditions, plant loses its strength, fails to uptake nutrient, following the prohibition of root development which leads to decrease in shoot and root biomass. During last two decades, significant studies have been made at molecular level to know about the acid soil stress tolerance mechanisms which are controlled by several genes, transcription factors and transporters. A principal functional tool of plant Al^{3+} tolerance encompasses Al^{3+}-induced stimulation of membrane transporters, which accelerates the organic acid release from the root region of plant (Kochian et al. 2005). Furthermore, the toxic Al^{3+} and the released organic acids form stable and nontoxic complexes in the rhizosphere. Meantime, several genes were isolated and characterized from the number of plant species to understand the acid soil tolerance mechanisms. Recent reports proved that Al-activated *AtALMT1*-mediated malate efflux from the apical part of the root and Al-activated *AtMATE*-mediated citrate efflux from the mature part of the roots are effectively involved in acid soil stress tolerance mechanisms. Therefore, the genes responsible for malate (ALMT1) and citrate (MATE) release are cloned and characterized from economically important crop plants (Sasaki et al. 2004; Magalhaes et al. 2007; Furukawa et al. 2007; Tiwari *et al*. 2014; Chen *et al*. 2018; Liu *et al*. 2016; Lu *et al*. 2018; Min *et al*. 2019; He *et al*. 2019). Based on the above principles and mechanisms, in this present work, the citrate (*GhMATE1*) and malate (*GhALMT1*) transporter has been cloned and characterized in Cotton.

Like most of crop plants, Cotton plants are also sensitive to soil acidity and Al^{3+} stress, under hydroponics conditions; the Cotton seedlings exhibited weak phenotypes to low pH and Al^{3+} stress conditions by exhibiting retarded primary and secondary root growths and small cotyledons. Meanwhile, preliminary experiments proved that the acid soil condition (both low pH and high Al^{3+} stress), cause negative impact on Cotton root growth and whole plant development. With the above importance, this study is executed in the following chapter wise methodology.

Chapter 1

This chapter includes general introduction about soil acidity, Al stress tolerance mechanisms in plants, role of organic acids, Aluminum exclusion mechanisms, genetic engineering in plants to combat soil acidity and importance of Cotton. The aims and objectives of the present work are also envisaged in this chapter.

Chapter 2

This chapter deals with characterization of Cotton plants under low proton stress and Al stress which includes variations in root and shoot growth variation, cloning, sequence analysis and expression profile studies of *GhALMT1* and *GhMATE1*.

Chapter 3

This chapter reports about development methodology of pBI121:35S:RNAi-*GhMATE1* vector and *Agrobacterium*-mediated pBI121:35S:RNAi-*GhMATE1* gene transfer procedure by using embryo apex explant in Cotton. It also includes, development of transgenic Arabidopsis plants by using floral dip method with pBI121:35S:*GhMATE1* vector. The details of Al and proton toxicity responses of the above two transgenics (*GhMATE1-RNAi* and *GhMATE1*-overexpression in Arabidopsis plants) will be presented in this chapter.

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LIST OF ABBREVIATIONS

Al	Aluminum
Ara	Arabidopsis
$AlCl_3$	Aluminum chloride
BAP	Benzyl amino purine
B5	Gamborg's medium
C	Control
C2-H2	Cystine-histidine
CS	Citrate synthase
CDS	Coding sequence
cDNA	Complementary DNA
$CaCl_2$	Calcium chloride
Cef	Cefotaxime
Car	Carbenicillin
°C	Centigrade
cm	Centimeter
CTAB	Cetyl trimethy ammonium bromide
cv	Cultivar
d	Days
DNA	Deoxyribo nucleic acid
dNTP	Deoxy-nucleotide triphosphate
dT	Deoxythymine
DMRT	Duncan's multiple range test
2,4-D	2,4-Dichloro phenoxy acetic acid
EDTA	Ethylene diamine tetra acetate

g/L	Gram per litre
GA_3	Gibberellic acid
HCl	Hydrochloric acid
$HgCl_2$	Mercuric chloride
hr	Hours
IAA	Indole acetic acid
IBA	Indole-3-Butyric acid
Kb	Kilo base pair(s)
DR	Down-regulated
kD	Kilo Dalton
Kan	Kanamycin
KIO_3	Potassium iodate
L	Litre
LB	Luria broth
lb	Pound
mRNA	Messenger RNA
µM	Micro molar
µm	Micro meter
mg	Milligram
mm	Millimeter
mM	Millimolar
mL	Milliliter
min	Minutes
M	Molar
MS	Murashige and Skoog's Medium
MES	2-(N-morpholino) ethanesulfonic acid

NADH	Nicotinamide adenine dinucleotide
NAA	Naphthalene acetic acid
NaOH	Sodium hydroxide
OD	Optical density
PCR	Polymerase chain reaction
%	Percentage
PGRs	Plant growth regulators
Pic	Picloram
psi	Pound per square inch
pL	Pico litre
RNA	Ribo nucleic acid
RACE	Rapid Amplification of cDNA Ends
RT	Real time
RH	Relative humidity
RNase	Ribonuclease
Rif	Rifampicin
RNA-i	RNA-interference
rpm	Rotation per minute
SOC	Super Optimal broth with Catabolite repression
SPSS	Statistical Package for the Social Sciences
sec	Second(s)
SD	Standard deviation
SDS	Sodium dodecyl sulfate
SE	Standard error
TAE	Tris acetate – EDTA
TE	Tris-EDTA

T-DNA	Transferred DNA
Ti	Tumor - inducing
Tris	Tris (hydroxymethyl)-aminomethane
μg	Microgram
μL	Microliter
v/v	Volume/volume
VC	Vector control
WT	Wild type
w/v	Weight / volume