# TABLE OF CONTENTS

ACKNOWLEDGEMENTS
LIST OF FIGURES
LIST OF TABLES
ABBREVIATIONS
ABSTRACT

## CHAPTERS

I. **GENERAL INTRODUCTION** ........................................................... 1
II. **DEVELOPMENT AND COLLECTION OF DROUGHT STRESS INDUCED COTTON BOLL SAMPLES FROM FIELD GROWN PLANTS AND MICROARRAY BASED TRANSCRIPTOME ANALYSIS** .......................................................... 15

### INTRODUCTION
.......................................................... 15

### MATERIALS AND METHODS
.......................................................... 20

- Plant Materials, Drought Treatment, Collection of Cotton Boll Samples .................................................................................................................. 20
- Total RNA Extraction .............................................................................................................. 21
- Microarray Analysis .............................................................................................................. 21
- Microarray Data Analyses .................................................................................................... 22
- Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) Analysis ................................................................. 23

### RESULTS
.......................................................... 24

- Effect of Drought Stress on Plant Growth and Fibre Development ........................................ 24
- Analysis of Total RNA, Labeled cRNA and Fragmented Labeled cRNA ................................................................. 24
- Microarray Data Analysis .................................................................................................... 25
- Differentially Expressed Transcripts during Fibre Development Stages ................................................................. 25
- Cluster Analysis of Differentially Expressed Transcripts ....................................................................................... 27
- Functional Classification of Differentially Expressed Transcripts ....................................................... 27
Drought Responsive Transcripts Related to Protein Kinases and Phosphatases and Phytohormones Biosynthesis and Signal Transduction Pathways ................................................................. 28
Drought Responsive Transcription Factors ........................................... 29
Biotic and Abiotic Stress Responsive Transcripts .................................. 30
Cell Wall Modification, Cell Division and Growth Related Genes ....... 32
Metabolic Pathways Regulated by Drought Stress ................................. 33
Quantitative RT-PCR Based Expression Analysis ................................. 34

DISCUSSION ................................................................................................. 34
Cellular Communication and Signal Transduction Related Genes ...... 35
Transcription Factors (TFs) .............................................................. 38
Heat Shock Proteins (HSPs) .......................................................... 39
Transporters ...................................................................................... 40
Detoxification ..................................................................................... 42
Other Biotic and Abiotic Responsive Genes ........................................ 42
Carbohydrate and Fatty Acid Metabolism Related Genes ................. 43
Cell Wall Modification, Cell Division and Growth Related Genes ...... 45
Other Metabolic Pathway Regulated Under Drought Stress ............. 49

III. ISOLATION AND FUNCTIONAL CHARACTERIZATION OF THE DROUGHT TOLERANCE CANDIDATE GENE(S) IN TRANSGENIC TOBACCO ................................................................. 50

INTRODUCTION ............................................................................................ 50
MATERIALS AND METHODS .................................................................. 55
Plant Materials, Growth Conditions, and Stress Treatments .............. 55
Total RNA and RT-PCR Analysis .......................................................... 57
Full-Length cDNA cloning of GhCCL and Construction of Phylogenetic Tree............................................................... 58
Expression Analysis of GhDRIN1 .......................................................... 58
Cloning the Full Length GhDRIN1 and Sequence Analysis .............. 59
Amplification of the Genomic and Upstream Sequence of GhCCL ...... 60
Isolation and Analysis of the GhDRIN1 Promoter ............................... 60
Identification of *GhCCL* and *GhDRIN1* Copy Number in Cotton .......... 60
Subcellular Localization of *GhCCL-mGFP* and *GhDRIN1-mGFP* Fusion Proteins and Agroinfiltration Study ......................................................... 61
Construction of Plant Transformation Vector and Tobacco Transformation .................................................................................................................... 62
Molecular Analysis of Transgenic Plants ................................................. 63
PCR, Southern, and Northern Blot Analysis ............................................. 63
Analysis of Transgenic Tobacco Plants for Abiotic Stress Tolerance .... 65
Leaf Disc Assay .................................................................................... 65
Seed Germination Assay under Stress .................................................... 65
Long-Term Growth Performance of Transgenic Plants under Stress ..... 66
Insect Bioassay of *GhDRIN1* Transgenic Tobacco .............................. 66
Water-Deficit Stress Tolerance of Transgenic Tobacco ......................... 66
Statistical Analyses .............................................................................. 67

**RESULTS** ............................................................................................. 67

Isolation, Molecular Cloning, and Structural Organization of *GhCCL* and *GhDRIN1* .................................................................................. 67
Construction of Binary Vectors for Plant Transformation ..................... 68
*GhCCL* and *GhDRIN1* Promoter Analysis ............................................ 69
Identification of Introns and Copy Number Determination of *GhCCL* and *GhDRIN1* .................................................................................. 71
Sequence Analysis of *GhCCL* ................................................................. 72
Expression Profiles of *GhCCL* and *GhDRIN1* in Cotton Leaves and Boll Developmental Stages ................................................................. 73
Expression Profiles of *GhCCL* in Response to Diverse Abiotic Stresses in Cotton Seedlings ................................................................. 73
*In Silico* Prediction and Subcellular Localization of GhCCL and GhDRIN1 .............................................................................................. 75
Abiotic and Biotic Stress Tolerance Study of Transgenic Tobacco ......... 76
Overexpression of *GhCCL* and *GhDRIN1* in Transgenic Plants Shows no Negative Effect on Plant Growth ................................................. 76
Leaf Disc, Seed Germination Assay and Long term growth performance of *GhCCL* Transgens for Dehydration, Osmotic, and Salt Stresses ................................................................................ 77
<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1.1</td>
<td>Distribution of world cotton fibre production</td>
</tr>
<tr>
<td>Figure 1.2</td>
<td>Cotton fibre initiation and elongation stages</td>
</tr>
<tr>
<td>Figure 1.3</td>
<td>Electron microscope view of cotton fibre development</td>
</tr>
<tr>
<td>Figure 1.4</td>
<td>Chemical structure of cotton</td>
</tr>
<tr>
<td>Figure 1.5</td>
<td>Phylogenetic framework and genome size variation in cotton</td>
</tr>
<tr>
<td>Figure 2.1</td>
<td>Cotton plants grown in normal and drought-induced field condition under rainout shelter</td>
</tr>
<tr>
<td>Figure 2.2</td>
<td>Representative Agarose gel electrophoresis analysis of total RNA, Full length biotin labeled complementary RNA (cRNA) and Fragmented cRNA</td>
</tr>
<tr>
<td>Figure 2.3</td>
<td>Representative figures showing the analysis of total RNA, Full length Biotin labeled cRNA and Fragmented cRNA by Agilent Bioanalyzer 2100 system using RNA Nano chips</td>
</tr>
<tr>
<td>Figure 2.4</td>
<td>Overview of the GeneChip® 3’ IVT Express Kit Labeling Assay</td>
</tr>
<tr>
<td>Figure 2.5</td>
<td>Differentially expressed transcripts under drought stress during fibre development stages (0, 5, 10 and 20 dpa)</td>
</tr>
<tr>
<td>Figure 2.6</td>
<td>Hierarchical cluster analysis of differentially expressed transcripts (fold change ≥3) under drought stress as compared to their respective control samples during fibre development stages (0, 5, 10 and 20 dpa)</td>
</tr>
<tr>
<td>Figure 2.7</td>
<td>Functional classification of differentially expressed transcripts under drought stress during fibre development stages (0, 5, 10 and 20 dpa)</td>
</tr>
<tr>
<td>Figure 2.8</td>
<td>Overview of differentially up-regulated transcripts under drought stress during fibre development in <em>G. hirsutum</em></td>
</tr>
<tr>
<td>Figure 2.9</td>
<td>Overview of differentially down-regulated transcripts under drought stress during fibre development in <em>G. hirsutum</em></td>
</tr>
</tbody>
</table>
Figure 2.10 - Down-regulated transcripts (downwards arrow) under drought stress at various stages (in parenthesis) involved in phenylpropanoid and flavonoid biosynthesis pathway

Figure 2.11 - Number of differentially expressed transcripts under drought stress as compared to their respective control samples related to phytohormones induced genes during fiber development stages (0, 5, 10 and 20 dpa)

Figure 2.12 - Number of differentially expressed transcripts enriched in each transcription factor family under drought stress

Figure 2.13 - Number of differentially expressed transcripts related to cell wall Modifying enzymes and structural proteins

Figure 2.14 - Validation of expression patterns of selected differentially expressed transcription factors from 0, 5, 10 and 20 dpa using qRT-PCR

Figure 3.1 - Overview of 5’ Rapid Amplification cDNA Ends (5’-RACE)

Figure 3.2 - Overview of Genome Walker protocol

Figure 3.3 - EST sequence and 5’ RACE PCR analysis of GhCCL transcript

Figure 3.4 - RT-PCR analysis of GhCCL transcript and sequence and PCR analysis of GhDRIN1 transcript

Figure 3.5 - Phylogenetic tree analysis, genomic PCR amplification and circular map analysis of GhCCL gene

Figure 3.6 - Identification, amplification and cloning of rd29A promoter from Arabidopsis thaliana

Figure 3.7 - Construction and confirmation analysis of binary vector pBI121-P- rd29A::GUS

Figure 3.8 - Circular and linear map of binary vectors pBI121-CaMV35S::GhCCL and pBI121-Atrd29A::GhCCL

Figure 3.9 - RT-PCR analysis of GhDRIN1 transcript and construction binary vector pBI121-CaMV35S::GhDRIN1

Figure 3.10 - Colony PCR analysis of A. tumefaciens strain harboring the binary vectors
Figure 3.11 - Genome walking analysis of GhCCL and GhDRIN1 5’ upstream sequence
Figure 3.12 - Amplification, organization, copy number and conserved domain of GhCCL gene
Figure 3.13 - Restriction digestion and Southern blot analysis of cotton to identify the copy number of GhDRIN1 gene
Figure 3.14 - Phylogenetic tree and expression profiles analysis of GhCCL transcript
Figure 3.15 - RT-PCR analysis of GhDRIN1 transcript in stress induced cotton samples
Figure 3.16 - Expression analysis of GhDRIN1 transcript in stress induced cotton samples
Figure 3.17 - Map of pCAMBIA1302-CaMV35S::mGFP binary vector and confirmation analysis
Figure 3.18 - PCR amplification of GhCCL cDNA fragment, construction of binary vector pCAMBIA1302-GhCCL::mGFP and colony PCR confirmation of A. tumefaciens strain
Figure 3.19 - PCR amplification of GhDRIN1 cDNA fragment, construction of binary vector pCAMBIA1302-GhDRIN1::mGFP and colony PCR confirmation of A. tumefaciens strain
Figure 3.20 - Subcellular localization study of the GhCCL and GhDRIN1 protein in tobacco leaf tissue
Figure 3.21 - Agrobacterium-mediated genetic transformation of tobacco (Nicotiana tabacum var. Petit Havana) using leaf disc method
Figure 3.22 - PCR, Southern and Northern blot analysis of GhCCL transgenic tobacco plants
Figure 3.23 - PCR, Southern and Northern blot analysis of GhDRIN1 transgenic Tobacco plants
Figure 3.24 - Leaf disc senescence assay of WT and T₀ GhCCL Transgenic plants
Figure 3.25 - Performance and percentage (%) of WT and T₁ GhCCL transgenic seed germination on amended MS media
Figure 3.26 - Long-term growth performance and fresh weight of WT and GhCCL transgenic tobacco plants under amended MS media medium

Figure 3.27 - Effect of long term stress and insect bioassay of WT (Wild-type) and T1 GhDRIN1 transgenic tobacco

Figure 3.28 - Evaluation of water-deficit stress tolerance, relative water content, and total chlorophyll content analysis in WT and GhCCL transgenic plants under glass house conditions

Figure 3.29 - Representative photos for water-deficit stress analysis of WT and GhDRIN1 transgenic tobacco lines
**LIST OF TABLES**

| Table 2.1  | Parameters observed in cotton plant growth and fibre development in response to drought stress under field conditions |
| Table 2.2  | List of transcription factor specific primers used for qRT-PCR study |
| Table 2.3  | List of primers for differentially expressed transcripts at 0 dpa stage to validate microarray data using qRT-PCR |
| Table 2.4  | List of primers for differentially expressed transcripts at 5 dpa stage to validate microarray data using qRT-PCR |
| Table 2.5  | List of primers for differentially expressed transcripts at 10 dpa stage to validate microarray data using qRT-PCR |
| Table 2.6  | List of primers for differentially expressed transcripts at 20 dpa stage to validate microarray data using qRT-PCR |
| Table 2.7  | List of primers for differentially expressed transcripts at leaf stage to validate microarray data using qRT-PCR |
| Table 2.8  | List of commonly up-regulated transcripts among fibre elongation (5 and 10 dpa) and secondary cell wall synthesis (20 dpa) stage under drought stress |
| Table 2.9  | List of commonly down-regulated transcripts among fibre elongation (5 and 10 dpa) and secondary cell wall synthesis (20 dpa) stage under drought stress |
| Table 2.10 | Differentially expressed transcripts (DETs) among fiber elongation (5 and 10 dpa) and secondary cell wall synthesis (20 dpa) stage present in different functional groups |
| Table 2.11 | Differentially expressed transcripts (DETs) encoding protein kinases at various stages under drought stress |
| Table 2.12 | Differentially expressed transcripts at various stages under drought stress related to phytohormone biosynthesis and signal transduction pathways |
Table 2.13 - Differentially expressed transcripts (DETs) at various stages under drought stress related to transporters, heat shock proteins, detoxification, dehydration and biotic stress response

Table 2.14 - Statistically enriched metabolic pathways identified using KOBAS database in differentially expressed transcripts at various stages under drought stress

Table 3.1 - List of primers used for GhCCL (CCR-like) and GhDRIN1 gene characterization study

Table 3.2 - Prediction of the sub-cellular localization of GhCCL protein

Table 3.3 - Prediction for sub-cellular localization of GhDRIN1 protein

Table 3.4 - Putative cis-elements in the GhCCL promoter

Table 3.5 - Putative cis-acting elements in the promoter sequence of cotton GhDRIN1.

Table 3.6 - Leaf disc senescence assays of wild-type and T₁ transgenic tobacco in distilled water supplemented with 10% PEG-6000, 200 mM each mannitol and NaCl

Table 3.7 - Seed germination data of wild-type and T₁ transgenic tobacco in MS agar medium supplemented with 10% PEG-6000, 200 mM each mannitol and NaCl

Table 3.8 - Wild-type and T₁ GhCCL transgenic tobacco stem portion was cultured on MS medium supplemented with 10% PEG-6000, 200 mM each mannitol and NaCl

Table 3.9 - Percentage of fresh weight of WT (Wild-type) and GhDRIN1 T₁ transgenics under long term stress amended MS medium supplemented with 200 mM each of NaCl, mannitol and 10 % PEG

Table 3.10 - Relative water content analysis of wild-type and T₁ GhCCL transgenic tobacco plants under glass house condition upon before and after 15 days water withholding

Table 3.11 - Total chlorophyll content analysis of wild-type and T₁ GhCCL transgenic tobacco plants under glass house condition upon before and after 15 days water withholding
Table 3.12 - Analysis of relative water content for WT and $T_1$ *GhDRIN1* transgenic tobacco plants under transgenic glass house condition for 0 days and 15 days after water withholding.

Table 3.13 - Analysis of total chlorophyll content in WT and $T_1$ *GhDRIN1* transgenic tobacco plants under transgenic greenhouse condition for 0 and 15 days after water withholding.