DOCTOR OF PHILOSOPHY

Salt tolerance in cotton

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Abstract

Cotton is the world's most important fibre crop. It is also the second oil seed crop of the world (Khan *et al.*, 1999). It is a major foreign exchange earner in Pakistan, where it is cultivated mostly in Sindh and Punjab provinces. Cotton is a halophytic crop, which is generally grown in mildly saline soil (Ashraf, 2002). However, its yield and quality is reduced at high salt levels. Some 6.67 million hectare in Pakistan is seriously affected (Alam *et al.*, 2000). Introduction of salt tolerant cotton varieties will play a key role in increasing the agriculture productivity of cotton under saline condition. The object of this project was the identification of genetic markers associated with the salt tolerance of cotton. 40 genotypes were tested with salt treatments. Sixteen genotypes were tested with (150 followed by 250 mM) and the response of nutrition; photosynthesis and basic geometry were measured. The plant height, nodes per plants, fresh and dry weight, chlorophyll content, transpiration rate, stomatal conductance and CO_2 uptake of cotton plants were reduced significantly by NaCl. Na^+ uptake in all young and old plants parts were increased significantly by salt (150 followed by 250 mM) treatments as compared to control treatments. K^+ and Ca^{++} uptake in some young and old plants parts were increased significantly and in some of plant parts decreased by salt (150 followed by 250 mM) treatments as compared to control treatments. Genotypic response was significantly different in most of genotypes by salt. A rudimentary mapping population was developed for breeding and genetics. But no phenotypic and genenotypic variation were observed between genotypes.

Meanwhile, microsatellite DNA sequences (SSR; potential genetic markers) were sought. Seventeen pairs of primers were used on the three selected lines and on 28 different cotton genotypes. The number of alleles per primer with a range of 1 to 5 with in average of 2.5 SSR fragment per primer were observed. The polymorphism information content (PIC) values with a range of 0.00 to 70. The genetic similarity of SSR between genotypes with a range of 0.72 to 0.95 in *G. hirsutum* was higher than of *G. stocksii,* S6 x DNH 40 and *G. stocksii x G. hirsutum* (0.14).

Dedication

This thesis is dedicated to my parents, who are no longer with us, and for my sister, whose support, patience and love have encouraged me throughout my study.

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Table of content

Declaration i

Abstract ii

Dedication iii

Acknowledgements iv

Table of contents v

List of figures xiii

List of tables xxi

Abbreviations xxii

Chapter 1 1

Introduction 1

1.1. History of cotton 1

1.2. Importance of cotton in Pakistan 1

1.3. Growth and development of cotton 2

1.4. Botany and Genetics of cotton 3

1.4.1. Ploidy 3

1.4.2. Genome 4

1.4.3. Classical genetics 6

1.5. Salinity in Agriculture 6

1.5.1. Salt tolerance 7

1.5.1. Salt tolerance in cotton 8

1.5.2. Effect of salinity in whole plant 8

1.5.3. Effect of salinity in Photosynthesis 9

1.5.4. Effect of salinity on cotton yield 9

1.5.5. Mechanisms of plant salt stress tolerance 10

1.6. Application of Molecular techniques in cotton 10

1.6.1 Evolutionary studies 12

1.6.2. Marker assisted selection (MAS) 14

1.6.3. Simple sequence repeats (SSR) 15

1.7. Aims and objectives 16

Chapter 2 17

Materials and methods 17

2.1. Plant materials 17

2.2. Temperature and light 17

2.3. Flood bench system 19

2.3.1. 150 mM NaCl Treatment 21

2.3.2. 150/250 mM Treatment 22

2.3.3. Constant $CaCl_2$ Treatment 23

2.4. Physiological parameters 23

2.4.1. Plant height (mm) 23

2.4.2. Nodes per plant.....23

2.4.3. Fresh weight of plant shoots (g) 23

2.4.4. Dry weight of plant shoots (g) 23

2.5. Sap extraction 24

2.6. Determination of Sodium (Na^+), Potassium (K^+) and Calcium (Ca^{++}).....25

2.7. Photosynthesis and Transpiration Rate measurement.....25

2.8. Chlorophyll content (measured in vivo) 26

2.9. DNA extraction and Quantification 26

2.9.1. DNeasy plant mini kits (Qiagen, UK) and Agarose gel electrophoresis.....26

2.9.2. Qiagen Puregene Core kit A and Spectrophotometer 27

2.10. PCR (Polymerase chain reaction) amplification 27

2.10.1. Programme 1 (Turner, 2003) 27

2.10.2. Programme 2 27

2.10.3. Programme 3 (Touchdown programme 1; adopted from Cho *et al*, 2000).....28

2.11. Gel electrophoresis 28

2.12. Scoring of SSR fragments through capillary electrophoresis. 28

2.12.1. Binary data matrix.....29

2.13. Ranking data.....29

2.14. Experiment layout and Statistical analysis.....29

Chapter three 30

To study the effects of salt on physiological traits of different cotton varieties.....30

Introduction.....30

3.1.1. The effect of 150 Mm NaCl on cotton varieties 32

3.1.2. Material and methods.....32

3.1.3. Results.....33

3.1.3.1. Plant height, fresh weight, dry weight and Na^+ in leaf, petiole and stem of cotton genotypes.....33

3.1.3.2. K^+ concentration and K^+/Na^+ ratio in leaf, petiole and stem of cotton genotypes.....35

3.1.4. Principal component analysis of phenotypes.....39

3.2.1. Effect of two different treatments of salt (150 - 250 mM NaCl) on cotton.....42

3.2.2. Material and methods 42

3.2.3. Results.....43

3.2.3.1. Plant height 43

3.2.3.2. Nodes per plants 45

3.2.3.3. Fresh weight 47

3.2.3.4. Dry weight 49

3.2.3.5. Na^+ concentration in young leaf.....51

3.2.3.6. K^+ concentration in young leaf.....53

3.2.3.7. K^+/Na^+ ratio in young leaf.....55

3.2.3.8. Na^+ concentration in young petiole.....57

3.2.3.9. K^+ concentration in young petiole 59

3.2.3.10. K^+/Na^+ ratio in young petiole 61

3.2.3.11. Na^+ concentration in young stem.....63

3.2.3.12. K^+ concentration in young stem 65

3.2.3.13. K^+/Na^+ ratio in young stem 67

3.2.3.14. Na^+ concentration in old leaf.....68

3.2.3.15. K^+ concentration in old leaf.....70

3.2.3.16. K^+/Na^+ ratio in old leaf.....72

3.2.3.17. Na^+ concentration in old petiole.....74

3.2.3.18. K^+ concentration in old petiole 76

3.2.3.19. K^+/Na^+ ratio in old petiole 77

3.2.3.20. Na^+ concentration in old stem.....79

3.2.3.21. K^+ concentration in old stem 80

3.2.3.22. K^+/Na^+ ratio in old stem 82

3.2.5. Principal component analysis of phenotypes.....83

3.3.1. The effect of 150 Mm NaCl on cotton genotypes with constant $CaCl_2$. 86

3.3.2. Material and methods.....86

3.3.3. Results.....88

3.3.3.1. Plant height, nodes per plant, fresh weight and Dry weight.....88

3.3.3.2. Chlorophyll content, transpiration rate, stomatal conductance and CO_2 uptake.....90

3.3.3.3. Na^+ concentration in young and old leaves, petiole and stem.....92

3.3.3.4. K^+ concentration in young and old leaves, petiole and stem.....94

3.3.3.5. K^+/Na^+ ratio in young and old leaves, petiole and stem.....97

3.3.3.6. Ca^{++} concentration in young and old leaves, petiole and stem.....99

3.3.5. Principal component analysis of phenotypes.....108

3.4.1. A comparison of the response of three varieties of cotton stressed by direct addition of salt with responses obtained using the flood-bench system.....110

3.4.2. Material and methods 110

3.4.3. Results.....112

3.4.3.1. Plant height 112

3.4.3.2. Nodes per plants 113

3.4.3.3. Fresh weight 114

3.4.3.4. Dry weight 115

3.4.3.5. Na^+ concentration in young leaf.....116

3.4.3.6. K^+ concentration in young leaf.....117

3.4.3.7. K^+/Na^+ ratio in young leaf.....118

3.4.3.8. Na^+ concentration in young petiole.....119

3.4.3.9. K^+ concentration in young petiole 120

3.4.3.10. K^+/Na^+ ratio in young petiole 121

3.4.3.11. Na^+ concentration in young stem.....122

3.4.3.12. K^+ concentration in young stem 123

3.4.3.13. K^+/Na^+ ratio in young stem 124

3.4.3.14. Na^+ concentration in old leaf.....125

3.4.3.15. K^+ concentration in old leaf.....126

3.4.3.16. K^+/Na^+ ratio in old leaf.....127

3.4.3.17. Na^+ concentration in old petiole.....128

3.4.3.18. K^+ concentration in old petiole 129

3.4.3.19. K^+/Na^+ ratio in old petiole 130

3.4.3.20. Na^+ concentration in old stem.....131

3.4.3.21. K^+ concentration in old stem 132

3.4.3.22. K^+/Na^+ ratio in old stem 133

3.5. Discussion.....134

3.6. Conclusion.....140

Chapter four 143

Molecular marker diversity in cotton genotypes.....143

4.1. Introduction.....143

4.2. Material and methods 144

4.2.1. Polymorphic information content (PIC).....146

4.2.1.1. Percentage of polymorphic loci (P).....147

4.2.1.2. Average number of alleles per locus (A).....147

4.2.1.3. Average number of alleles per polymorphic locus (AP).....147

4.3. Results.....148

4.3.1. Fragment analysis using agarose gel electrophoresis.....148

4.3.1.1. BNL 1434.....149

4.3.1.2. BNL 1878.....152

4.3.2. Fragment analysis using CEQ 8000.....154

4.4. Discussion.....158

4.4.1. Diversity analysis 158

4.4.2. Diversity observed in clustering analysis.....159

4.5. Conclusion.....159

Chapter five.....160

Developing a mapping populations for cotton.....160

5.1 Introduction.....160

5.2 Material and methods.....162

5.3. Results.....165

5.3.1. The effect of salt on plant height, nodes per plant, fresh weight, dry weight and chlorophyll content.....165

5.3.2. The effect of salt on Na^+ concentration in young and old leaves, petiole and stem.....166

5.3.3. The effect of salt on K^+ concentration in young and old leaves, petiole and stem.....169

5.3.4. The effect of salt on Na^+/K^+ ratio in young and old leaves, petiole and stem.....171

5.3.5. The effect of salt on Ca^{++} concentration in young and old leaves, petiole and stem.....173

5.5. Principal component analysis of phenotypes.....182

5.3.33 Fragment analysis using agarose gel electrophoresis.....184

5.4 Discussion.....186

5.4.1 Effect of salinity on Physiological characters.....186

5.4.2 Molecular diversity..... 188

5.5 Conclusion.....188

Chapter six.....190

General Discussion, conclusions and Recommendations.....190

6.1. General discussion.....190

6.1.1. Shoot growth, chlorophyll content and photosynthesis under NaCl.....192

6.1.2. Na^+ uptake by all young and old parts of plants under NaCl.....193

6.1.3. K^+ uptake by all young and old parts of plants under NaCl.....194

6.1.4. K^+/Na^+ ratio of all young and old parts of plants under NaCl.....195

6.1.5. Ca^{++} uptake by all young and old parts of plants (Constant $CaCl_2$) under (150 mM) NaCl.....195

6.1.6. Molecular diversity.....196

6.1.7. Mapping population.....197

6.2. Conclusions.....198

6.3. Recommendations.....199

References.....200

Appendix.....215

List of Figures

Figure 2.1. . Flood bench system for assessment of phenotypic responses of control and salt treatments.....20

Figure 2.2. Flood bench system for assessment of phenotypic responses of control and salt treatments. (Pen-y-Ffridd Research Station, Bangor University, Bangor UK).....21

Figure 2.3. Diagram for Sap extraction, shows in step 1. Collect the sample in the tube, 2. Hole on the top on the same tube, 3. Hole on the bottom on the same tube, 4. cut the lid of the emty tube, 5, Place the samle tube in the empty tube and centrifuge, 6. leaf sap after centrifuge.....24

Figure 3.1.4.1 Scatter plot of genotypes based on the principal component analysis of physiological traits.....41

Figure 3.1.4.2 Dendrogram of cotton varieties based on principal component analysis generated by significant physiological traits.....41

Figure 3.2.3.1 Effect of 150 followed by 250 mM NaCl on plant shoots height of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd. DAT= days after treatments.....44

Figure 3.2.3.2 Effect of 150 followed by 250 mM NaCl on nodes per plant of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd. DAT= days after treatments.....46

Figure 3.2.3.3 Effect of 150 followed by 250 mM NaCl on fresh weight of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd. DAT= days after treatments.....48

Figure 3.2.3.4 Effect of 150 followed by 250 mM NaCl on dry weight of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd. DAT= days after treatments.....50

Figure 3.2.3.5 Effect of 150 followed by 250 mM NaCl on Na^+ concentration in young leaf of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd. DAT= days after treatments.....52

Figure 3.2.3.6 Effect of 150 followed by 250 mM NaCl on K^+ concentration in young leaf of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd. DAT= days after treatments.....54

Figure 3.2.3.7 Effect of 150 followed by 250 mM NaCl on K^+/Na^+ ratio in young leaf of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd. DAT= days after treatments.....56

Figure 3.2.3.8 Effect of 150 followed by 250 mM NaCl on Na^+ concentration in young petiole of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd. DAT= days after treatments.....58

Figure 3.2.3.9 Effect of 150 followed by 250 mM NaCl on K^+ concentration in young petiole of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd. DAT= days after treatments.....60

Figure 3.2.3.10 Effect of 150 followed by 250 mM NaCl on K^+/Na^+ ratio in young petiole of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd. DAT= days after treatments.....62

Figure 3.2.3.11 Effect of 150 followed by 250 mM NaCl on Na^+ concentration in young stem of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd. DAT= days after treatments.....64

Figure 3.2.3.12 Effect of 150 followed by 250 mM NaCl on K^+ concentration in young stem of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd. DAT= days afer treatments.....66

Figure 3.2.3.13 Effect of 150 followed by 250 mM NaCl on K^+/Na^+ ratio in young stem of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd. DAT= days after treatments.....67

Figure 3.2.3.14 Effect of 150 followed by 250 mM NaCl on Na^+ concentration in old leaf of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd. DAT= days after treatments.....69

Figure 3.2.3.15 Effect of 150 followed by 250 mM NaCl on K^+ concentration in old leaf of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd. DAT= days after treatments.....71

Figure 3.2.3.16 Effect of 150 followed by 250 mM NaCl on K^+/Na^+ ratio in old leaf of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd. DAT= days after treatments.....73

Figure 3.2.3.17 Effect of 150 followed by 250 mM NaCl on N^+ concentration in old petiole of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd. DAT= days after treatments.....75

Figure 3.2.3.18 Effect of 150 followed by 250 mM NaCl on K^+ concentration in old petiole of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd. DAT= days after treatments.....76

Figure 3.2.3.19 Effect of 150 followed by 250 mM NaCl on K^+/Na^+ ratio in old petiole of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd. DAT= days after treatments.....78

Figure 3.2.3.20 Effect of 150 followed by 250 mM NaCl on Na^+ concentration in old stem of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd. DAT= days after treatments.....79

Figure 3.2.3.21 . Effect of 150 followed by 250 mM NaCl on K^+ concentration in old stem of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd. DAT= days after treatments.....81

Figure 3.2.3.22 Effect of 150 followed by 250 mM NaCl on K^+/Na^+ ratio in old stem of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd. DAT= days after treatments.....82

Figure 3.2.5.1 Scatter plot of genotypes based on the principal component analysis of physiological traits.....85

Figure 3.2.5.2 Dendrogram of cotton varieties based on principal component analysis generated by significant physiological traits.....85

Figure 3.3.1. Reduced plant growth of Qalandari and CIM 1100 under 150mM NaCl ((constant $CaCl_2$ treatment).....87

Fig.3.3.4.1. Scatter plot of sixteen cotton varieties based on principal component analysis generated by significant physiological traits.....109

Fig.3.3.4.2. Dendrogram of sixteen cotton varieties based on principal component analysis generated by significant physiological traits.....109

Fig.3.4.1 Cotton genotypes with control and salt treatment.....111

Fig.3.4.3.1. Effect of 150 mM NaCl with flood benches (WF) and 150 mM NaCl with out flood benches (WOF) on plant shoots height of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd (see in Appendix Table 15).....112

Fig.3.4.3.2. Effect of 150 mM NaCl with flood benches (WF) and 150 mM NaCl with out flood benches (WOF) on nodes per plant of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd (see in Appendix Table 15).....113

Fig.3.4.3.3. Effect of 150 mM NaCl with flood benches (WF) and 150 mM NaCl with out flood benches (WOF) on fresh weight of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd (see in Appendix Table 15).....114

Fig.3.4.3.4. Effect of 150 mM NaCl with flood benches (WF) and 150 mM NaCl with out flood benches (WOF) on dry weight of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd (see in Appendix Table 15).....115

Fig.3.4.3.5. Effect of 150 mM NaCl with flood benches (WF) and 150 mM NaCl with out flood benches (WOF) on Na^+ concentration in young leaf of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd (see in Appendix Table 16).....116

Fig.3.4.3.6. Effect of 150 mM NaCl with flood benches (WF) and 150 mM NaCl with out flood benches (WOF) on K^+ concentration in young leaf of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd (see in Appendix Table 17).....117

Fig.3.4.3.7. Effect of 150 mM NaCl with flood benches (WF) and 150 mM NaCl with out flood benches (WOF) on K^+/Na^+ ratio in young leaf of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd (see in Appendix Table 18).....118

Fig.3.4.3.8. Effect of 150 mM NaCl with flood benches (WF) and 150 mM NaCl with out flood benches (WOF) on Na^+ concentration in young petiole of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd (see in Appendix Table 16).....119

Fig.3.4.3.9. Effect of 150 mM NaCl with flood benches (WF) and 150 mM NaCl with out flood benches (WOF) on K^+ concentration in young petiole of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd (see in Appendix Table 17).....120

Fig.3.4.3.10. Effect of 150 mM NaCl with flood benches (WF) and 150 mM NaCl with out flood benches (WOF) on K^+/Na^+ ratio in young petiole of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd (see in Appendix Table 18).....121

Fig.3.4.3.11. Effect of 150 mM NaCl with flood benches (WF) and 150 mM NaCl with out flood benches (WOF) on Na^+ concentration in young stem of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd (see in Appendix Table 16).....122

Fig.3.4.3.12. Effect of 150 mM NaCl with flood benches (WF) and 150 mM NaCl with out flood benches (WOF) on K^+ concentration in young stem of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd (see in Appendix Table 17).....123

Fig.3.4.3.13. Effect of 150 mM NaCl with flood benches (WF) and 150 mM NaCl with out flood benches (WOF) on K^+/Na^+ ratio in young stem of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd (see in Appendix Table 18).....124

Fig.3.4.3.14. Effect of 150 NaCl with flood benches (WF) and 150 mM NaCl with out flood benches (WOF) on Na^+ concentration in old leaf of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd (see in Appendix Table 16).....125

Fig.3.4.3.15. Effect of 150 mM NaCl with flood benches (WF) and 150 mM NaCl with out flood benches (WOF) on K^+ concentration in old leaf of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd (see in Appendix Table 17).....126

Fig.3.4.3.16. Effect of 150 mM NaCl with flood benches (WF) and 150 mM NaCl with out flood benches (WOF) on K^+/Na^+ ratio in old leaf of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd (see in Appendix Table 18).....127

Fig.3.4.3.17. Effect of 150 mM NaCl with flood benches (WF) and 150 mM NaCl with out flood benches (WOF) on N^+ concentration in old petiole of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd (see in Appendix Table 16).....128

Fig.3.4.3.18. Effect of 150 mM NaCl with flood benches (WF) and 150 mM NaCl with out flood benches (WOF) on K^+ concentration in old petiole of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd (see in Appendix Table 17).....129

Fig.3.4.3.19. Effect of 150 mM NaCl with flood benches (WF) and 150 mM NaCl with out flood benches (WOF) on K^+/Na^+ ratio in old petiole of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd (see in Appendix Table 18).....130

Fig.3.4.3.20. Effect of 150 mM NaCl with flood benches (WF) and 150 mM NaCl with out flood benches (WOF) on Na^+ concentration in old stem of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd. (see in Appendix Table 16).....131

Fig.3.4.3.21. Effect of 150 mM NaCl with flood benches (WF) and 150 mM NaCl with out flood benches (WOF) on K^+ concentration in old stem of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd (see in Appendix Table 17).....132

Fig.3.4.3.22. Effect of 150 mM NaCl with flood benches (WF) and 150 mM NaCl with out flood benches (WOF) on K^+/Na^+ ratio in old stem of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd (see in Appendix Table 18).....133

Figure. 4.3.1.1.a Microsatellite DNA profiles of 43 cotton genotypes amplified using by primer pair BNL 1434. The amplified microsatellite DNA was separated in 3% agarose gel and detected by ethidium bromide. Hyper ladder was used. The names of genotypes used in this gel are given in Table. 4.2.....150

Figure. 4.3.1b. Microsatellite DNA profiles of 8 cotton genotypes amplified using by primer pair BNL 1434. The amplified microsatellite DNA was separated in 3% agarose gel and detected by ethidium bromide. Hyper ladder was used. The names of genotypes used in this gel are given in Table. 4.2.....151

Figure. 4.3.1c. Microsatellite DNA profile of S6 x DNH 40 (track 23) genotype detected by primer pair BNL 1434. Red colour peaks shows the size standard using size fragments (PA 400).....152

Figure. 4.3.1d. Microsatellite DNA profile of DNH 40 (track 22) genotype detected by primer pair BNL 1434. Red colour peaks shows the size standard using size fragments (PA 400).....152

Figure. 4.3.1.2a. Microsatellite DNA profiles of 43 cotton genotypes amplified using by primer pair BNL 1878. The amplified microsatellite DNA were separated in 3% agarose gel and detected by ethidium bromide. The names of genotypes used in this gel are given in Table. 4.2.....153

Figure. 4.3.1.2b. Microsatellite DNA profile of *G.stocksii x G. hirsutum* (track 26) genotype detected by primer pair BNL 1878. Red colour peaks shows the size standard using size fragments (PA 400).....154

Figure. 4.3.1.2c. Microsatellite DNA profile of MNH 147 (track 19) genotype detected by primer pair BNL 1878. Red colour peaks shows the size standard using size fragments (PA 400).....154

Fig. 4.3.3.1. Dendrogram showing how the twenty eight cotton genotypes clustered on the basis of SSR.....157.

Figure. 4.3.3.2. Groups of genotypes on the bases of their pedigree.....158

Figure. 5.2.1. Cotton genotypes grown for backcross generation in Pen-y-Ffridd Research Station, Bangor University, Bangor, UK.....164

Figure. 5.2.2. One example of growth reduction in backcross generation after salt treatment in green house (Memorial building), Bangor University, Bangor, UK.....165

Figure. 5.3.31. Scatter plot of three cotton varieties with their BC1 based on principle component analysis generated by significant physiological traits bases.....184

Figure. 5.3.32. Dendrogram of three cotton varieties with their BC1 based on principle component analysis generated by significant physiological traits bases.....184

Fig.5.3.33. Genetic map constructed using 43 cotton varieties with 17 SSR primers. Eleven of these on 10 chromosomes were confirmed by different literature. (Nguyen *et al.*, 2004, Song *et al.,* 2005 and Wang *et al.*, 2006). The location of six primers are unknown.....186

List of Tables

Table 1.1. Cultivated wild species and sub species of genus *Gossypium* diploid (2n = 2x= 26). (Fryxell *et al* 1992, Khan *et al* 1999).....5

Table1.2. Allotetraploids (2n =4x = 52) (Fryxell *et al* 1992, Khan *et al* 1999).....5

Table 1.3. Reported linkage maps for tetraploid cotton.....13

Table.2.1. List of varieties used in this study.....18

Table 2.1. The list of nutrient concentrations of 1.045 g/l Phostrogen.....22

Table 2.2: Micronutrients (Hoagland and Arnon, 1950).....23

Table 3.1.3.1. Means and standard deviation of plant height, fresh weight, dry weight and Na^+ in leaf, petiole and stem of cotton genotypes.....35

Table 3.1.3.2. Means and standard deviation of K^+ and K^+/Na^+ in leaf, petiole and stem of cotton genotypes.....37

Table 3.1.3.3. Mean square, degree of freedom and P values of cotton genotypes treated with 150mM NaCl.....38

Table 3.1.3.4. Correlation of all physiological traits.....38

Table 3.3.1. Means and standard deviation of plant height, fresh weight and dry weight of cotton genotypes.....89

Table. 3.3.2. Means and standard deviation of chlorophyll content, transpiration rate, stomatal conductance and CO_2 uptake of cotton genotypes.....92

Table. 3.3.3. Means and standard deviation of Na^+ concentration of all young and old plant tissues of cotton genotypes.....102

Table. 3.3.4. Means and standard deviation of K^+ concentration of all young and old plant tissues of cotton genotypes.....102

Table. 3.3.5. Means and standard deviation of K^+/Na^+ ratio of all young and old plant tissues of cotton genotypes.....103

Table. 3.3.6. Means and standard deviation of Ca^{++} concentration of all young and old plant tissues of cotton genotypes.....103

Table.3.3.7. Correlation of plant height, nodes per plant, fresh and dry weight, chlorophyll content, transpiration rate, CO2 uptake and Na^+ concentration in young and old leaf, petiole and stem.....104

Table 3.3.8 Correlation of plant K^+ concentration and K^+/Na^+ ratio in young and old leaf, petiole and stem.....105

Table Correlation of plant Ca^{++} concentration in young and old leaf, petiole and stem.....106

Table 3.3.10. Mean square, degree of freedom and P value of cotton genotypes.....107

Table 3.5.1. Ranking of growth parameters, chylorophll content, photosynthesis, Na^+, K^+, K^+/Na^+ and Ca^{++} in 150 mM stress.....142

Table 3.5.2. Ranking of growth parameters, Na^+, K^+ and K^+/Na^+ in 250 mM stress.....143

Table 4.1. Microsatellite (SSR) primers, their sequence (R & F), motif, frequency and size.....146

Table. 4.2. Track numbers of cotton genotypes used in gel photographs.....147

Table 4.3. Number of alleles of each SSR locus in different cotton genotypes.....149

Table 4.4. Number of alleles of each SSR locus in different cotton genotypes.....156

Table 5.3.1. Means and standard deviation of plant height, fresh weight, dry weight and chlorophyll content of cotton genotypes.....177

Table 5.3.2. Means and standard deviation of Na^+ concentration in young and old plant tissues of cotton genotypes.....177

Table 5.3.3. Means and standard deviation of K^+ concentration in young and old plant tissues of cotton genotypes.....178

Table 5.3.4. Means and standard deviation of K^+/Na^+ concentration in young and old plant tissues of cotton genotypes.....178

Table 5.3.5. Means and standard deviation of Ca^{++} concentration in young and old plant tissues of cotton genotypes.....179

Table 5.3.6. Mean square, degree of freedom and P values of different traits of cotton genotypes.....180

Table 5.3.7. Correlation of plant height, nodes per plant, fresh and dry weight, chlorophyll content, Na^+ and K^+ concentration in young and old leaf, petiole and stem.....181

Table 5.3.8. Correlation of K^+ and Ca^{++} concentration and K^+/Na^+ ratio in young and old leaf, petiole and stem.....182

Table 5.2. Number of alleles of each SSR locus in different cotton genotypes.....185

Table 5.3. Cotton genotypes and the track number identifies used in the illustrators of gels predicted in this Table.....185

Table 5.4.1 Ranking of growth parameters, chlorophyll content, photosynthesis, Na^+, K^+, K^+/Na^+ and Ca^{++} in 150 mM stress.....190

Abbreviations

AFLP = Amplified Fragment Length Polymorphism

ANOVA = Analysis of Variance

BAC = Becterial Artificial Chromosomes

BC = Back cross

BNL = Brookhaven National Laboratory

CCRI = Central Cotton Research Institute

CRS = Cotton Research Station

CRI = Cotton Research Institute

cM = Centimorgan

CMD = Cotton Marker Database

DAT = Days After Treatment

DNA = Deoxyribonucleic acid

EC = Electrical Conductivity

EST = Expressed Sequence Tag

Gh = *G. hirsutum*

IRGA = Infra Red Gas Analyzer

MAS = Marker Assisted Selection

mM = milli molar

MUGHES = Mississippi *G. hirsutum* EST SSR.

NAU = Nanjing Agriculture University

NIAB = Nuclear Institute for Agriculture & Biology

PARF = Polymorphic Amplified Restriction Fragment

PCA = Principle Component Analysis

PCR = Polymerase Chain Reaction

PIC = Polymorphism Information Content

RAPD = Random Amplified Polymorphic DNA

RFLP = Restriction Fragment Length Polymorphism

SFR = Supper Fine Resolution

SPAD = Soil and Plant Analyzer Development

SRAPS = Sequence related Amplified Polymorphism

SSR = Simple Sequence Repeats

TRAP = Target Region Amplified Polymorphism

UPGUMA = Unweighted Paired Group Method using Arithmetic average.

QTL = Quantitative Trait Loci

Chapter 1

Introduction

1.1. History of cotton.

Cotton (*Gossypium hirsutum* L.) is one of the oldest fibre crops, and is an important cash crop around the world (Asif *et al*., 2008). The genus *Gossypium* is grown in tropical and sub tropical region of the world. It is grown commercially in about 60 developing as well as developed countries (Rafiq, 2006). The genus has many wild and cultivated species, but only four species of cotton are grown commercially. The species *G. arboreum* (Desi) is native to the Pakistan and India region. There is evidence of its cultivation as long ago as 3000-4000 BC by the Mohanjo Daro, Mehrab garh and Harappa people (Venkatesh *et al*, 2009). *G. barbadense*, a species with an extra long- staple (Adawy, 2007), originated in Western South America, where cotton dating to 2000 BC has been found. *G.hirsutum* (upland cotton) is the most widely planted species. It represents about 90 % of all production (Adawy *et al*, 2006) and has long staple and fine cotton. The cultivation of this species began about 3500 BC in Mexico (Fryxell, 1992). Desi was cultivated in Pakistan before the introduction of upland cotton (*G. hirsutum*). Now in Pakistan 98 % of cultivated cotton is upland (Rahman *et al*, 2002).

1.2. Importance of cotton in Pakistan.

Cotton (as *G. hirsutum L.*) is an important cash crop of Pakistan. It is known as a white gold crop, because the cotton crop is grown for its fibre that is used in the manufacture of clothing, many industrial and other products. Cotton lint varies between different varieties and is used as a raw material for the textile industries. Long-staple cotton can be better for clothing purposes. Cotton seed is also an important source of oil which is used mainly for human consumption (Sekhar and Bhaskara, 2011). Cottonseed cake is used as a livestock feed supplement. In

Pakistan, cotton is cultivated mostly in the Indus valley of Sindh and Punjab provinces. It is the backbone of the Pakistan economy (Rahman et al, 2002). Cotton is also a major foreign exchange earner for Pakistan. It occupies the 2nd position with respect to cultivated area of all crops in the country (Ali, *et al,* 2005). It plays a significant role in national industrial development. Because of its clear importance to Pakistan and similar countries, any negative influence on yield is of importance. One such influence is excess salinity. Cotton is generally grown in mildly saline soils (Ashraf, 2002). However, at high salt levels its yield and quality is much lower compared with normal soils (Akhtar, *et al.,* 2005). Since much land in Pakistan is affected by such saline contamination, it is important to increase its yield per acre under such conditions.

1.3. Growth and development of cotton.

The main cotton growing countries are USA, Mexico, India, China, Australia, Argentina, Brazil, Pakistan and Uzbekistan (FAS, 2005). Cultivation of cotton depends on the climatic conditions. It is a warm-season crop, so it can be grown as a summer crop in high temperature zones. The temperature required from germination to harvest ranges from 20-30 ^{0}C (Reddy *et al.*, 1991 and Bhatti, 1975), but in Pakistan, the summer temperature often reaches 48-50 °C (Ashraf *et al.*, 1994) which is supra-optimal. The cotton crop also requires adequate moisture (Naheed and Rasul, 2009), but it is not a water loving plant; and produces best under cloudless condition. For high lint production, the requirement of water either by rainfall or irrigation for Sindh Province at least 183 mm per year (Naheed and Rasul, 2009). It is a sun loving plant and requires the high light intensity during most of the growing season for good growth and economic yield. The suitable soil for cotton crops is sandy loam (Torberi and Reeves, 1994).

1.4. Botany and genetics of cotton.

Cotton belongs to the genus *Gossypium* of the family Malvaceae. It is of tropical origin but is mostly cultivated in temperate climates. The genus *Gossypium* consists of 51 wild and cultivated species. Forty-five are diploid and six are allotetraploid (Fryxell, 1992). As noted earlier only four species of *Gossypium* are grown on a commercial scale. *G. hirsutum, G. barbadense* are New World species and *G. arboreum* and *G. herbaceum* are from old world species (Wendel and Richard, 2003). Four wild species of cotton (*G. danvinii, G. lanceolatum, G. mustelinum* and *G. tomentosum*) are perennial shrubs of arid and semi arid regions. Such wild species are of great interest from a plant breeding point of view because of their resistance quality. Some of them have already been used in hybridization programmes for improving the quality of cultivated cotton (Zhang, *et al.*, 2003).

1.4.1. Ploidy.

Polyploidy refers to a change in the numbers of the whole set of chromosomes (Sarah and Whitton, 2000). Polyploidy species are common in plants, which have mechanisms that allow them to tolerate extra chromosomes. Allotetraploid cotton is an example of this. As noted above forty five of the species of genus *Gossypium* are diploid, having a 2n chromosome number equal to 26. *G. arboreum* and *G. herbaceum* are both diploid species having the A genome, while *G. hirsutum* and *G. barbadense* are allotetraploids (Zhang *et al*, 2002) species (2n=4x=52) having (AADD) genomes.

The basic haploid number (n) of cotton is 13. But allotetraploid cotton have one complete set of diploid chromosomes (2n= 26) derived from each parental species (Wendel and Cronn 2003). Polyploid cotton shows a diversity of genomic responses to genome doubling. This includes the interaction between the two genomes in the polyploid nucleus (Adams and Wendel, 2004). Polyploidy (genome multiplication) can significantly effect gene expression. For example silencing can occur (Liu and Wendel, 2003).

1.4.2 Genome.

The genome of an organism is the complete DNA sequence of one set of chromosomes. Wheat is hexaploid and has as A, B and D genome. The genome size of eukaryotic organisms varies 200,000 fold (Hawkins, *et al.*, 2006). The genetic complement of the genus *Gossypium* can be categorised into A, B, C, D, E, F, G, and K genomes. (Table.1.1). These eight genomes have different sizes. The F genome has the largest chromosome (3.5 μm) and genome D has the smallest chromosome (900Mb) (Muranveko, *et al.,* 1998). A haploid (AD genome) plant of *Gossypium* was first reported by Harland (1936). *G. hirsutum* has an AD genome. Chromosomes 1-13 are derived from the A genome and 14-26 from the D genome. The A genome chromosomes are physically larger than those of the D genome (Fryxell, 1971). The size of cotton genome estimated approximately 2.5 Gb for *Gossypium hirsutum* (Chen *et al*,. 2007). Lin *et al.*, (2010) reported that the cotton genome contains about 400 DNA kb per cM. They also reported that the *Gossypium* genomes include respectively 2584 loci at 1.75 cM (~600kb) intervals based on 2007 probes (AtDt) and 1014 loci at 1.42 cM (~600kb) intervals detected by 809 probes (D). Four wild species *Gossypium darwinii, Gossypium lanceolatum, Gossypium mustelinum* and *Gossypium tomentosum* are tetraploid species. The wild species are good sources for resistance to diseases and pests as well as tolerance to harsh growing conditions.

Table 1.1. Cultivated wild species and sub species of genus *Gossypium* diploid (2n = 2x= 26). (Fryxell *et al* 1992, Khan *et al* 1999)

Species	**Genome**	**Distribution**
G. herbaceum L.	A_1	Old world
G.herbaceum var. africanum (Watt)	A_1	Africa (wild type)
G. arborium L.	A_2	Old world Pakistan & India.
G. anomalum	B_1	Africa
G. capitis viridis	B_3	Cape Verde Islands
G. sturtianum	C_1	Australia
G. robinsonii	C_2	Australia
G. costulatum	C_5	Australia
G. thurberi	D_1	Mexico
G. armourianum	$D_{2\text{-}1}$	Mexico
G. harknessii	$D_{2\text{-}2}$	Mexico
G. davidsonii	$D_{3\text{-}d}$	Mexico
G. aridum	D_4	Mexico
G. raimondii	D_5	Peru
G. lobatum	D_7	Mexico
G. laxum	D_9	Mexico
G. turneri	D_{10}	Mexico
G.schwendimanii	D_{12}	Islands
G. stocksii	E_1	Arabia
G. somalense (Gurke) Hutch	E_2	Africa
G. areysianum (Defl) Hutch.	E_3	Arabia
G.incanum (Schwartz) Hillc.	E_4	Arabia
G. longicalyx Hutch. & Lee	F_1	Africa
G. bickii	G_1	Australia
G. australe	G_2	Australia
G. nelsonii	G_3	Australia
G. populifolium	K	Australia
G.cunninghamii	-	Australia
G. pulchellum.	K	Australia
G. pilosum	K	Australia
G.enthyle	K	Australia
G. rotundifolium	K	Australia
G. nobile	K	Australia
G. exiguum	-	Australia
G. marchantii.	-	Australia
G. enthyle	-	Australia

Table1.2. Allotetraploids (2n =4x = 52) (Fryxell *et al* 1992, Khan *et al* 1999)

Species	**Genome**	**Distribution**
G. hirsutum L.	$(AD)_1$	New World Cultigen
G. barbadense L.	$(AD)_2$	New World Cultigen
G. tomentosum	$(AD)_3$	Hawaii
G.mustelinum	$(AD)_4$	Brazil
G. darwinii	$(AD)_5$	Galapagos Islands
G.lanceolatum	(AD)	Mexico

1.4.3. Classical genetics.

Plant breeding is a branch of applied genetics by which new improved varieties of cultivated plants can be produced. Homologies between the A and D chromosomes of tetraploid cotton have been the base of classical genetics in cotton (Endrizzi *et at,*. 1985). Breeders have produced many selective plant varieties of higher quality and yield.

Gossypium hirsutum has a low genetic diversity (Ulloa and Meredith, 2000). Therefore, there have been three main approaches for increasing that diversity. There are mutagenesis, germplasm introgression and transformation (Sukumar *et al.,* 2006). Most of the cotton linkage mapping populations currently available have been developed through the intra specific hybridization of the two cultivated species *G. hirsutum* (A_1D_1 genome) and *G. barbadense* (A_2D_2 genome) (Lacape *et al.,* 2003). High-yielding tetraploid cotton varieties of upland cotton of American origin were introduced into Pakistan where these varieties were crossed with local varieties (Desi Varieties). The use of a restricted genetic stock, however, resulted in a narrow genetic base (Iqbal *et al.*, 1997). This narrow genetic base of cultivated species seriously limits future breeding advances. Therefore, there is a need to use genes from wild species to meet future challenges. To this end, interspecific hybridization between *G. hirsutum* and *G. barbadense* has been initiated eg (Frelichowski, *et al.*, 2006).

1.5. Salinity in agriculture.

Salinity is caused by the excessive concentration in the soil of soluble electrolytes, such as chloride, sulphate, bicarbonate, sodium, calcium, magnesium and potassium. It is one of the most serious environmental problems that reduce plant growth in agriculture (Gorham, 1992) and is a major problem for agricultural productivity. It is estimated that 20% of artificially irrigated land in the world is affected by salinity (Flowers and Yeo, 1995). The degree of salinity in a soil is generally expressed in terms of electrical conductivity (EC). Electrical conductivity of soil water

is due to the ions dissolved in it as pure water is a very poor electrical conductor. A saline soil is described as sodic if a high EC is accompanied by a high pH (> 8.5).

It is estimated that about 6.68 million hectares are affected by salinity or sodicity in Pakistan (Rashid, *et al.,* 2009). Salinity is primarily due to the formation of soil by weathering from a parent rock which is rich in soluble salts (Ashraf, 1994). Secondary salinity is due to changes land uses which disturb the hydrology of the landscape and redistribute salts so that they accumulate in a location of importance to farming. Secondary salinity may also result from the human addition of water and mismanagement of irrigation system. The yield of cotton and wheat is reduced in saline and sodic soil (Murtaza *et al.,* 2006). High salinity can damage soil structure and can affect the chemical properties of soil by changing the cat ion exchange capacity (Singh and Chatrath 1992). In Pakistan, salinity is also a problem because of the semi arid climate. Due to each of these factors, many areas are becoming saline and going out of production. Introduction of salt tolerant plants and the selection and breeding of varieties for salt tolerance can play a major role for increasing the agricultural productivity under these conditions.

1.5.1. Salt tolerance.

Different plants respond to saline conditions in different ways, some plants have an ability to grow in saline soil, such as Suaeda (seep weeds), those plants have been categorised into four types, (1) strongly high salt tolerant plants grow at high salt concentrations (more than 24 dSm^{-1}) (2) high salt tolerant plants grow at high salt concentrations (15-24 dSm^{-1}) (3) moderately salt tolerant plants grow at (8-15 dSm^{-1}) and (4) slightly salt tolerant plants grow at (4-8 dSm^{-1}). Salt tolerant plants can absorb saline water from soil due to the ability of special physiological adaptation. In contrast salt sensitive plants are unable to tolerate under salt stress.

The growth of plants, under saline conditions, depends upon a number of morphological, physiological, biochemical and anatomical adaptations which enable the plants to grow in the presence of high concentrations of toxic ions. In a screening of four upland cotton varieties,

genotypic difference in salt tolerance were observed; NIAB 78 variety was the most tolerant with B - 557, Sarmast and Qalandari showing least tolerance (Jafri, 1994). Noor *et al*, 2001 observed that CIM 1100 and CIM 448 are salt tolerant varieties. As a breeder we need to develop varieties acceptable for cultivation on a commercial scale which survive in saline soil and produce economic yield.

1.5.2. Salt tolerance in cotton.

Some plants are naturally more salt tolerant than others. On this basis, plants have been categorised into two types; glycophytes and halophytes. Glycophytes are those plants that cannot tolerate high salinity. Halophytes can tolerate high salinity (Flowers *et al.*, 1986). Cotton is the most important non-food crop in the world. It is halophytic (Akhtar *et al*, 2010). However, cotton is sensitive to salinity at the germination and seedling stages (Ahmad *et al.*, 2002). Varietal and specific variation exists. Increased salinity causes a significant loss of cotton yield. Its effects at different growth stages in cotton are reviewed by Akhtar *et al.*, (1999) and Ali *et al.*, (2004). Due to the presence of high concentration of salts, the interrelations between plants and environment are changed. Meloni *et al.* (2001) observed a significant reduction in root, shoot and leaf biomass and an increase in root/shoot ratio in cotton by salinity. Salt - induced growth reduction has been reported by Gorham and Bridges, (1995). Salt tolerance in cotton was related to K^+/Na^+ selectivity as reported by Kent and Lauchli (1985). Salinity affects the plant osmotic pressure which reduces the plant growth (Greenway and Munns, 1980).

1.5.3. Effect of salinity in whole plant.

Salinity affects plant growth in different ways (Glenn, *et al.*, 1999). During the germination stage, salinity reduces germination percentage. The general response of plants can be the reduction in extension growth due to salinity. Sometimes growth is completely inhibited and plants will die if the salt concentration is too high. Salinity reduces vegetative growth of cotton (Leidi and Saiz, 1997; Qadir and Shams, 1997) and high salinity reduces cotton crops growth

and yield (Ashraf, 2002). Salinity can affect growth, stomatal conductance, photosynthetic capacity of *G. hirsutum L* and *Phaseolus vulgaris L.* (Brugoli and Lauteri, 1991).

The growth of cotton is affected by salinity at different stages of whole plant life (Meloni *et al.*, 2001). This has been shown for growth (Brugnoli and Lauteri., 1991), leaf area (Hoffman *et al.,* 1971), callus growth (Akhtar *et al.*, 1999) and elongation of primary root of cotton seedlings (Zhong and Lauchli., 1993). In contrast, increase in plant growth at low concentration of salt was reported by (Ahmad *et al.,* 2002) in line with cotton's identification as a moderate halophyte.

1.5.4. Effect of salinity on photosynthesis.

The mesophyll of leaves is the principal photosynthetic tissue in higher plants. It has many chloroplasts. There are many reports of suppression of photosynthesis during salt stress (Kao *et al.*, 2001; Chaudhuri and Chaudhuri, 1997; Yeo *et al.*, 1991; Kawasaki *et al.,* 2001; Brugnoli and Lauteri, 1991). In salt tolerant plants, chlorophyll content increased, while in salt susceptible plants it decreased (Ashraf and McNeilly, 1988). Photosynthesis and respiration are each affected by salinity and salt stress has been shown to have direct effects on enzyme function (Seemann and Critchly, 1985). Photosynthesis is inhibited when high concentrations of Na^+ and Cl^- accumulate in chloroplasts (Gale, *et al.,* 1967).

1.5.5. Effect of salinity on cotton yield.

In plants, salinity causes morphological, anatomical and physiological changes. Salt affected plants generally become stunted and produce reduced amounts of biomass.

The yield of a crop is affected by salinity, but it can be increased through salt tolerant varieties. With such varieties, proper soil management and irrigation and drainage system increase the yield of plants (Eynard *et al.,* 2005; Ahmad *et al.*, 2002 and Ashraf and Saghir, 2000). In 14 cultivars of cotton, salinity brought about 18% decreases in biomass production in two of the four cotton cultivars tested by Kuznetsov *et al.,* (1990). Variation for salt tolerance was

also observed by (Ali *et al.*, 2004). In addition, salinity can increase fibre length and reduced thickness (Wuwei *et al.*, 1997), characteristics of improved quality.

1.5.6. Mechanisms of plant salt stress tolerance.

Plants are different in nature and have different ability to grow and survive in salt stress conditions. Salt tolerance is the ability of the plants to maintain growth under such conditions. Plants have several different mechanisms to tolerate salinity (Munns and Tester, 2008). These include osmotic adjustment, Na^+/K^+ discrimination, salt exclusion and solute sequestration mechanisms. Maintainance of a specific cell osmotic potential is necessary for many plant functions. The main role of osmotic adjustment is to maintain cell volume and turgor pressure. In the absence of such responses the tissue will lose turgor and dehydrate. Most nutritional imbalance studies involve Na^+ and Cl^- ions, as they are the most abundant in saline soils. Na^+ decreases the uptake of other cat ions such as K^+, Ca^+ and Mg^+. The interactions between the sodium and calcium have been reported by Greenway and Munns (1980). An important selection criterion for salt tolerance in cotton is the maintainance of high K^+/Na^+ and Ca^+/Na^+ ratios (Glenn *et al.*, 1999 and Ashraf, 2002). Due to its ability for Na^+ accumulation, the vacuole play key role in reducing the toxic level of Na^+ in cytosol and increasing the vacuolar osmotic pressure (Cixin *et al.*, 2004). In several crop plants, limited Na^+ uptake is a trait related to salinity tolerance (Gorham *et al.*, 1985). Salinity tolerance is related to exclusion of Na^+ and Cl^+ from plant shoots in glycophytes (Gorham *et al.*, 1990).

1.6. Application of Molecular techniques in cotton.

Plant breeders can use molecular methods in their breeding programmes as molecular markers can provide precise and reliable identification of plants (Asif *et al.*, 2009). These methods are more effective than phenotypic selection (Moreau *et al.*, 2000). Several DNA markers systems are used in cotton breeding research. These include SSR (simple sequence repeat), RAPD (random amplified polymorphic DNA), AFLP (amplified fragment length

polymorphism) and RFLP (restriction fragment length polymorphism). The first cotton linkage maps constructed with RFLP were reported by Reinisch *et al.* (1994) who used F_2 population of *G.hirsutum x G. barbadense.* Several genetic linkage maps of cotton have been developed with RAPD and SSR (Zhang *et al.*, 2002) and RFLP, RAPD and SSR (Zuo *et al.*, 2000). The genetic linkage maps of tetraploid *Gossypium* species were constructed using different DNA markers are listed in Table 1.3.

SSR or "microsatellites" are PCR based markers and are widely used in genetic diversity analysis of cotton (Lacape, *et al.*, 2007). The genes which are involved in related quantitative traits can be identified through association with a molecular marker to make a map of a region of genome. Quantitative traits can be controlled by many genes associated with more than one molecular marker in more than one linkage group (Zachary, *et al.,* 1998). For example Yu, *et al.* (2007) used F2 progeny from an interspecific cross between two commercially important cotton species to determine the location of QTLs for fiber strength and other fiber quality properties. Qureshi, *et al,* (2004), used 84 primer pairs of EST- SSR markers and observed 26% polymorphism within *G. hirsutum* and forty four primer pairs (52%) polymorphic between *G. hirsutum* and *G. barbadense.*

Increasingly, genetic manipulation is being investigated and exploited. For example, *Arabidopsis* gene AVP1 plays an important role to improve drought and salt tolerance in cotton and also increases the fiber length in field conditions (Pasapula et al., 2010). Cixin *et al*, 2004, introduced the *Arabidopsis* gene AtNHXI into cotton. This AtNHXI protein gene can be responsible for increased salt tolerance (Zhang and Blumwald, 2001). Wu *et al*, (2004) also reported that GhNHX1 gene function at the tonoplast plays an important role in salt tolerance of cotton.

1.6.1. Evolutionary studies.

Gossypium is very old species of unknown origin (Fryxell, 1971). It is an allotetraploid species with chromosomes that are homologues to each other. Brown (1980) reported 62 translocations in *Gossypium* species. The most duplicated genes evolved independently after polyploidization in allotetraploid cotton (Rong, *et al.*, 2010). Zhang, *et al.,* (2002) used monosomic and mono telodisomic lines of allotetraploid cotton and detected chromosome number 5 and chromosome number 8 as a new pair of homologous chromosome. Higher nucleotide diversity in the D sub - genome was reported by Randal and Wendel (2002). Reinisch, *et al.*, (1994) reported that the cotton genome contains about 400 DNA kb per cM. They also reported that the A and D sub - genomes have identical recombination length (2119 and 2140 cM respectively). Polymorphic amplified restriction fragment (PARF) genetic markers were developed by Nekrutenko and Baker (2003), that are specific to the A and D genome of cotton.

Table 1.3. Reported linkage maps for tetraploid cotton

Crosses	Varieties	Mapping Population and size	Molecular markers	Genome coverage (cM)	Linkage groups	References
G. hirsutum x *G. barbadense*	Handan 208 x Pima 90	F2 (69)	SSR, RAPD & SRAPS	5141	41	Lin *et al.*, 2005
G. hirsutum x *G. hirsutum*	Yumian 1 x T 586	F2 (117)	SSR, AFLP	525	20	Zhang *et al.*, 2005
G. hirsutum x *G. hirsutum*	MD 5678ne x Prema	F2 &F3 (119)	RFLP	700	17	Ulloa and Meredith, 2000
G. hirsutum x *G. barbadense*	CRI 36 x Hai 7124	F2 (186)	SSR, AFLP, TRAP & SRAP	4536	35	Yu *et al.*, 2007
G. hirsutum x *G. hirsutum*	PMAR-5 x Harukei	F2 (93)	RAPD & AFLP	1097	19	Fukino *et al.*, 2002
G. hirsutum x *G. hirsutum*	HQ-95-62 MD51ne and 119-5 sub Okra2 MD51ne	F2 &F3 (199 and 155)	RFLP	1502	47	Ulloa *et al.*, 2002
G. barbadense x *G. hirsutum*	Guazuncho 2 x VH 8	BC1 (75), BC2 (200) & BC3 (411)	SSR & AFLP	5597	26	Lacape *et al.*, 2005
G. hirsutum x *G. barbadense*	Pima S-7 x Acalla 44	F2 (110)	SSR	531	11	Bolek *et al.*, 2005

1.6.2. Marker assisted selection (MAS).

From many years plant breeders have used conventional breeding methods for selecting plant varieties. It is a long and time consuming process (Preetha and Raveendern, 2008). Marker assisted selection has been an important process since 1980. It has allowed the plant breeders to increase the efficiency of conventional techniques through molecular applications (Stephen and Rita, 2008). Homozygous and heterozygous plants cannot be separated by conventional phenotype screening (Bertrand and David, 2008). Through MAS application, we can use DNA markers based on their genotypes for phenotypic selection for thousands of individuals and traits. Two types of molecular markers can be used for genetic study (Neale, *et al.*, 1992). These are, (1) biochemical markers such as isozymes and (2) DNA - based markers. Now various DNA markers are available for use of the plant breeders such as restriction fragment length polymorphism (RFLP) was first DNA based marker used in crop improvement and the first RFLP map was constructed by (Bernatzky and Tanksley, 1986) for tomato. And then several studies were reported about random amplified polymorphic DNA (RAPD) (Zhang *et al.*, 2002), amplified fragment length polymorphism (AFLP) (Lascape, *et al.*, 2005) and simple sequence repeat (SSR) (Liu, *et al*,. 2000).

Bertrand and David (2008) reported five main considerations in marker assisted selection for the use of DNA markers. These are (1) reliability, (2) quantity and quality of DNA required, (3) technical procedure for marker assay, (4) level of polymorphism and (5) cost. The success of MAS also depends on the localization of the marker with respect to the target gene (Francia *et al.*, 2005). Francia *et al*, (2005) also emphasised that tracing favourable dominant and recessive alleles across generations, identifying the most suitable individuals among segregating population based on allelic composition of a part or of the entire genome and breaking the possible linkage of favourable alleles with undesirable loci are the most useful application of MAS for the plant breeder. Witcombe and Virk (2001) reported that the plant breeder usually grows hundred or

sometimes thousands of plant populations and many thousands or millions of individual plants for a typical breeding programme.

1.6.3. Simple sequence repeats (SSR).

Today, the ideal marker system involves Simple Sequence Repeats (SSR). These sequences are more informative genetic markers. SSR consist of 1-6 repeating nucleotide units that are tandomly arranged in a genome (Qurashi, *et al.* 2004). These markers are reliable, co dominant and have high levels of allelic diversity (Bajracharya *et al.* 2006). Farooq and Azam (2002) reported that the variability at the microsatellite loci is due to the differences in the number of repeat units. Microsatellites (SSR) are used for identification of genes, construction of linkage maps and analysis of genetic structure of population (Chistiakov, *et al.*, 2006). Liu *et al* (2000) used 65 SSR primers pairs to amplify 71 marker loci in monosomic and monotelodisomic cotton cytogenetic stocks. The use of EST (expressed sequence tag) SSR is common in molecular studies, which score the expressed region of the genome (Qureshi *et al.*, 2004). In this present study 17 SSR primers (genomic and EST) were used to screen cotton genotypes.

It is important to screen the different cotton genotypes which are phenotypic ally different to each other, and also to categorise the physiological responses of different cotton as salt tolerant or susceptible genotypes.

1.7. Aims and objectives.

The objectives of this study were:

To compare the physiological responses to salt treatment of 40 cotton genotypes from three cultivated species (*Gossypium hirsutum* L., *Gossypium barbadense* L. and (*G. sturtianum*) and one wild species (*G. stocksii*).

To determine a reliable measure of salt tolerance and susceptibility among genotypes to allow screening of salt tolerance.

To discover correlations between these physiological traits and the SSR pattern of the same genotypes with the aim to exploit these in future Marker selection programme.

To study the phenotypic characterization of traits for cotton breeding and genetics using a rudimentary segregating population developed by backcross of an inter specific cross (*G. hirsutum* x *G. barbadense*).

Chapter 2

Material and methods

2.1 Plant material and growth.

The seeds of different varieties were supplied by Dr. John Gorham, CAZS-Natural Resources and School of Biological Science, Bangor University, and by Agriculture Research Institute (ARI) Tando Jam (Sindh). Four species of *Gossypium* were used in this study i.e. *G. hirsutum, G. barbadense, G. sturtianum and G. stocksii.* The list of varieties used in this study are shown in (Table 2.1).The seed were five years old and stored in the plastic bags in Memorial building Laboratory F26. The seeds were germinated in seed plug (P-84) trays (Plantpak, Malvern, England) having John Inns compost No 1 (Corwen, Clwyd, U.K.). These trays were kept in the green house on a bench and watered regularly with tap water until transplantation.

All the chemicals and reagents used in this study were obtained from BDH, Sigma or Fisher Scientific.

2.2. Temperature and light.

Various green houses and growth chambers were used to grow plants. In general, the minimum temperature was maintained at 25°C during a16 hour photoperiod and 20°C during the dark period. Supplementary 400 Watt high pressure sodium vapour lamps were used to maintain a minimum photon flux density of photosynthetically active radiation (400 – 700 nm) of 350 μmol m^{-2} s^{-1} during the photoperiod).

Table.2.1. List of varieties used in this study.

Genotypes	species	Tolerance	Parentage	Origin	References
Rehmani	*G. hirsutum*	Not available	G-hirsutum 21x McNair	ARI, Tando Jam	Naheed Naz. 2009
DNH 40	*G. hirsutum*	Not available	Not available	Not available	Not available
Coker 201	*G. hirsutum*	Salt tolerant	Not available	USA (South east)	Zhang *et al.*, 2001
AC 134	*G. hirsutum*	Not available	Not available	Not available	Not available
BP 52 – 63	*G. hirsutum*	Salt sensitive	Not available	Not available	Bhatti and Azhar, 2002
Dhandra	*G. barbadense*	Salt sensitive	G31 selected from G3	Egypt	Rathert, 1982
G. stocksii	*G. stocksii*	Not available	Not available	Not available	Not available
NIAB 313	*G. hirsutum*	Not available	Not available	Not available	Not available
Mc. Nair 220	*G. hirsutum*	Not available	CKR 201 x PD 2165	USA (South east)	Guillermo, et al. 2005
CIM 448	*G. hirsutum*	Salt tolerant	(W1104_S12)_CP 15/2 (Sister line of CIM 1100)	CCRI Multan	Noor *et al*, 2001
Sarmast	*G. hirsutum*	Salt sensitive	M4X Acalla again back cross with M4.	ARI, Tando Jam	Jafri, A. Z. and Ahmed, R. 1994
S 12	*G. hirsutum*	Salt sensitive	MNH 93 x 7203-14-4-Arizona	CRS Multan	Ali *et al.*, 2005
149 F	*G. hirsutum*	Salt sensitive	Not available	Not available	Kuznetsav *et al.*, 1992
B 756	*G. hirsutum*	Not available	Not available	Not available	Not available
MNH 147	*G. hirsutum*	Salt sensitive	431/79 x 283/80	CRS Multan	Ahmad *et al*, 2002 and Ashraf and Saghir, 2000
NIAB 78	*G. hirsutum*	Salt tolerant	AC 134_Delta Pine 16	NIAB, Faisalabad	Khan *et al.*, 2004 and Jafri and Ahmed, 1994
CIM 1100	*G. hirsutum*	Salt tolerant	492/87_CP 15/2	CCRI Multan	Noor *et al.*, 2001
CIM 240	*G. hirsutum*	Salt tolerant	CIM 70 x W1106	CRS Multan	Noor *et al.*, 2001
Acalla SJ 2	*G. hirsutum*	Salt sensitive	Not available	Mexico, USA	Lira and Hernandez, 1988
Qalandari	*G. hirsutum*	Salt sensitive	M4 _g-Anamalum x karank	ARI, Tando Jam	Jafri, and Ahmed, 1994
CIM 443	*G. hirsutum*	Salt sensitive	CIM 109_LRA-5166	CCRI Multan	Noor *et al*, 2001
MNH 93	*G. hirsutum*	Salt tolerant	147 F_(MS 39_Mex 12)	CRS Multan	Khan, *et al.* 2004
B 496	*G. hirsutum*	Salt sensitive	Not available	Not available	Bhatti and Azhar, 2002
Prococe 1	*G. hirsutum*	Salt sensitive	Not available	Brazil	Jacome *et al*, 2003
Karishma	*G. hirsutum*	Salt tolerant	NIAB 86_W83.29MEX	NIAB, Faisalabad	Akhter and Azhar, 2001
Coker 312	*G. hirsutum*	Salt sensitive	CKR 100 Staple x DP 15	USA (South east)	Gossett *et al*, 1996
CIM 109	*G. hirsutum*	Salt tolerant	NIAB 78 x A89/FM	CRS Multan	Khan *et al.* 2001
Allepo 45	*G. hirsutum*	Salt tolerant	Not available	Mexico, USA	Khan *et al.* 2001
Stonvelle 312	*G. hirsutum*	Not available	Not available	Not available	Not available
Acalla 1517	*G. hirsutum*	Salt tolerant	Not available	Mexico, USA	Rajguru *et al.*, 1999

CCRI = Central Cotton Research Institute; CRS = Cotton Research Station; CRI = Cotton Research Institute; NIAB = Nuclear Institute for Agriculture & Biology.

2.3. Flood bench system for applying NaCl stress.

Most of experiments the cotton plants were treated with salt solutions as follows.

A flood bench system (Pen-y-Ffridd Research Station, Bangor University, Bangor, UK) was used (Fig 2.1 & 2.2). (The current supplier of equivalent equipment is Powerplants Australia Pty Ltd). Flood bench containers (1) and 300 litre reservoir (2) were made from plastic (Polypropylene). The size of each container was 35 x 80 x 60 cm. These containers were fitted with sections of 15 mm hosepipe (3) connected to an electric pump (4) (Draper tools LTD. England UK) as shown in fig 2.1. The pump was immersed in the reservoir solutions and the reservoirs were placed underneath the bench to pump solutions to a level 5 mm below the surface of the soil. This was determined by the height of the entry into the drain pipe (5). Plants were flooded from 8:30 to 8:45 am every morning. Once the pump was switched off, the solution in the upper containers drained back into the reservoirs through the pump.

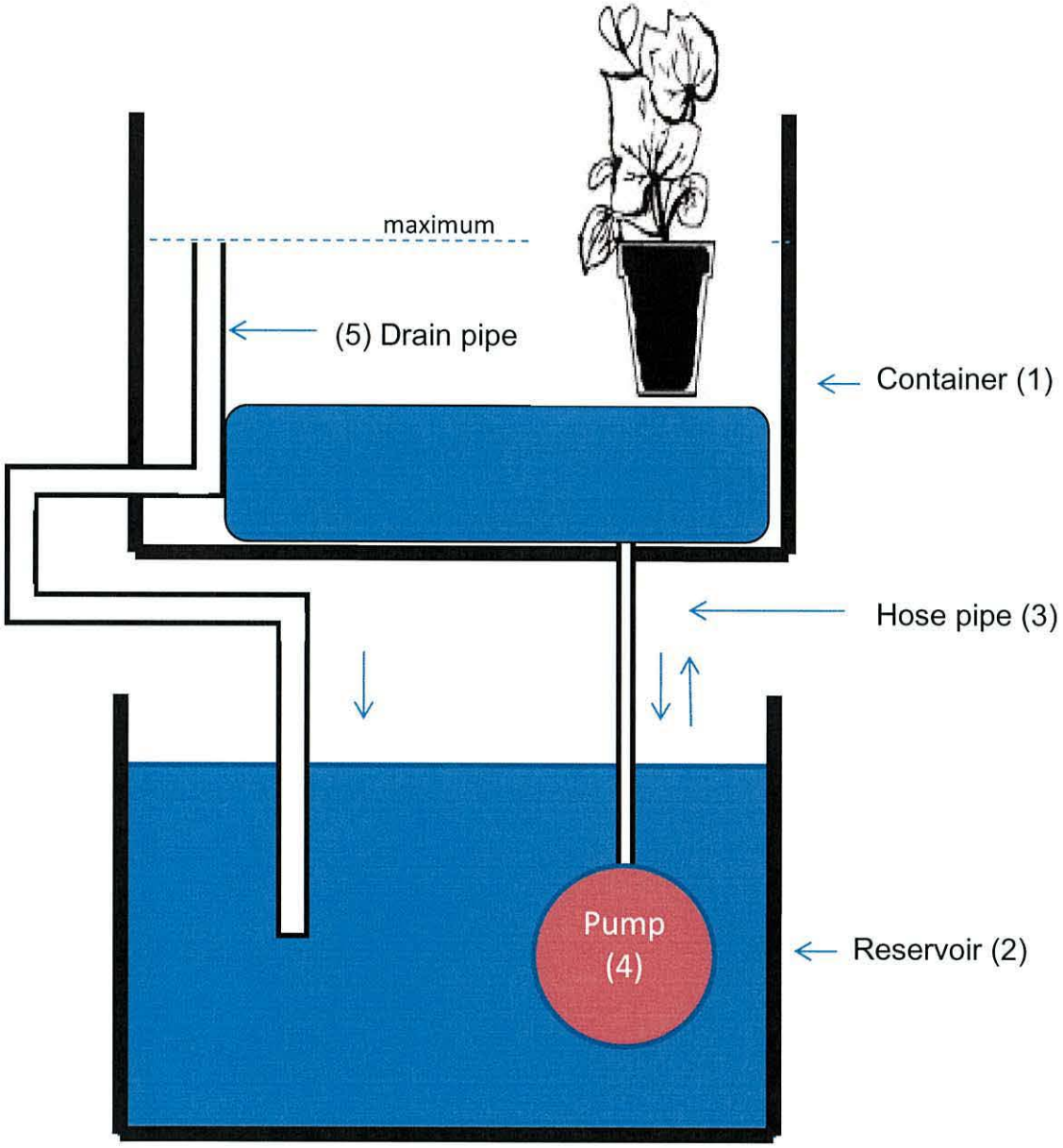

Fig 2.1. Flood bench system for assessment of phenotypic responses of control and salt treatments.

2.3.1: 150 mM NaCl Treatment.

For the first experiment (150 mM NaCl), there were a total of eight containers and five reservoirs. Three reservoirs fed two containers each (four containers for salt and two for control), whilst another two reservoirs fed one container each (for control treatment). Salt stress was started 15 days after sowing using (1 litre pots and John Innes No.1 soil) and the plants were treated with 150 mM NaCl and 7.5 mM $CaCl_2$ solution (Na: Ca ratio 20: 1) containing Phostrogen 1g l^{-1} (pbi Home & Garden Ltd, Middlesex), micronutrients 0.5 ml l^{-1} of stock solution (Table 2.3) and sodium silicate solution (BHD) 25% silicate 0.1 ml l^{-1} solution. The control plants were flooded with the same solution lacking the 150 mM NaCl and 7.5 mM $CaCl_2$ [in Phostrogen, which contributed 6 mM K^+ and 0.37 mM Ca^{2+}]. The list of concentrations of components at 1.045 g/l phostrogen is shown in (Table 2.2)

Figure 2.2: Flood bench system for assessment of phenotypic responses of control and salt treatments. (Pen-y-Ffridd Research Station, Bangor University, Bangor UK).

Table 2.2. The list of nutrient concentrations of 1.045 g/l Phostrogen.

Micro and macro nutrients	Percent (%)	Mwt
1.48 mM P	4.4 %	31
10 mM N	14 %	14
6 mM K	22.4 %	39
0.65 mM Mg	1.5 %	24
0.37 mM Ca	1.43 %	40
1.46 mM SO_4	4.5 %	32
0.0113 mM B	0.012 %	11
0.0008 mM Cu	0.0055 %	64
0.742 m M Fe	0.04 %	56
0.0037 mM Mn	0.02 %	55
0.0001 mM MO	0.0016 %	96
0.0008 mM Zn	0.055 %	65

2.3.2: 150/250 mM Treatment.

For a second experiment (150/ 250 mM NaCl) twenty plants per genotype were treated, ten plants as a control treatment and ten plants per genotypes as salt treatment. Twenty containers were used with ten reservoirs. After 15 days from sowing, the plants were treated for 15 days with 150 mM NaCl and 7.5 mM $CaCl_2$ solution as for section 2.3.1. At this point five plants per genotype of each treatment were harvested. The remaining five plants per genotype of each treatment were treated for a further 15 days of daily flooding for which the NaCl and $CaCl_2$ concentration were increased to 250 and 12.5 mM respectively (Note: the Na: Ca ratio was 20:1 throughout).

2.3.3: Constant $CaCl_2$ Treatment.

In a third experiment (constant $CaCl_2$). [Four containers and two reservoirs]. the NaCl treatment, micronutrients and Silicate was the same as section 2.3.1. [150 mM] but the $CaCl_2$ content of the control solutions was increased to 7.5 mM – to match the salt treatment.

Table 2.3: Micronutrients (Hoagland and Arnon, 1950)

Nutrients	**Concentration of stock solution g/L**	**ml stock solution / 1L**
$H_2MoO_4H_2O$	0.09	1
H_3BO_3	2.86	1
$MnCl_2.HO_2$	1.81	1
$ZnSO_4.{}_7H_2O$	0.22	1
$CUSO_4.{}_5H_2O$	0.051	1

2.4 Physiological parameters.

The following parameters were recorded at the time of harvesting.

2.4.1 Plant height.

Plant height from soil level to top leaf of shoot of each plant was measures using a ruler.

2.4.2 Nodes per plant. Nodes were counting per plant.

2.4.3 Fresh weight of plant shoots.

To determine fresh weight of the excised shoots, they were weighed using the balance (Mettler PB 300) immediately after recording the length of the plant.

2.4.4 Dry weight of plant shoots

The dry weight value was obtained after determining fresh weight of each shoot. The shoots were dried 48 hours in an oven (Binder GmbH Bergstr.) at 80 ^{0}C, and re-weighed to determine their dry weight.

2.5: Sap extraction.

Leaf, petiole and stem samples (approximately 1.5 g) of each genotype were cut and placed immediately in 1.5 cm^3 Eppendorf micro-centrifuge tubes. Cell sap was extracted according to Gorham *et al* (1984). The tubes were first frozen at -20^{0}C in a domestic freezer. After that the tubes were taken out and thawed at room temperature (approximately 20 ^{o}C) while still sealed to avoid condensation of moisture from the air on the cold plant material. Two holes were made (Fig 2.3), one at top and one in the bottom of the original tube. The lids of other tubes were removed and labelled. Each original micro centrifuge-tube was placed in one of the lid-less tube (Fig 2.3). The tubes were centrifuged (Eppendorf, Centrifuge 5810 R) at 15,000 xg rcf for 5 minutes. The upper tubes were discarded and the sap collected in the lower tube. That was stored at a -20 ^{o}C until further analysis.

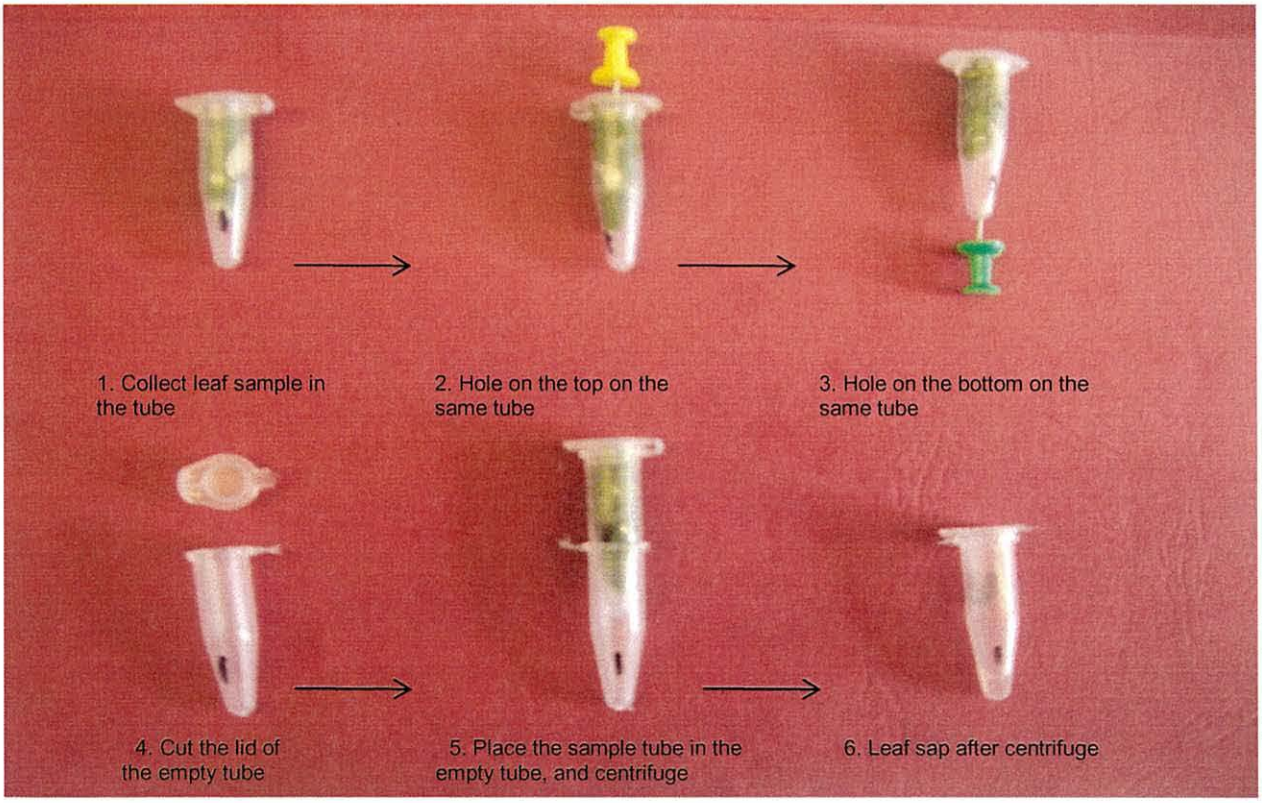

Fig: 2.3. Diagram for Sap extraction, shows in step 1. Collect the sample in the tube, 2. Hole on the top on the same tube, 3. Hole on the bottom on the same tube, 4. Cut the lid of the empty tube, 5, Place the sample tube in the empty tube and centrifuge, 6. Leaf sap after centrifuge.

2.6. Determination of Sodium (Na^+), Potassium (K^+) and Calcium (Ca^{++}).

Standard solutions, 0 -1 mM for K^+ and Ca^{2+} and 0 - 0.5 mM for Na^+, were used to contract standard curves. For each assay 20 µl of extracted sap was diluted with 5 ml de-ionized water. A Jenway PFP-7 flame photometer was used as per manufacturer's instructions.

2.7. Photosynthesis and Transpiration Rate measurement.

A TPS1 Portable Photosynthesis System (PP System Inc. USA) was used for photosynthesis (Pn), stomatal conductance (gs) and transpiration rate (E) measurement. The photosynthesis system was comprised of four basic units, (1) an IRGA (2) a narrow leaf chamber (2.5 cm^2 area), (3) air supply unit (4) a data logger.

The IRGA (Infra Red Gas Analyser) has an infrared detector that measures the CO_2 concentration of appropriate air samples. It works on the principle of measuring the difference of concentration of CO_2 going in and coming out of the leaf chamber. The difference between the amounts of CO_2 inside and outside the chamber, multiplied by the flow rate, is a measure of net carbon fixation rate. The IRGA and narrow leaf chamber were connected to an air supply unit taking air from outside the greenhouse. The second youngest fully expanded leaf from the top of each cotton plant was clamped in the narrow leaf chamber. The leaf chamber was provided with a seal to keep the chamber air tight. After putting the leaf in the chamber it was pressed gently to make it air tight. A DOS programme on a PC was used to download the data from the data logger as TPS file into a spread sheet (Excel).

2.8: Chlorophyll content (measured *in vivo*)

The leaf chlorophyll content was measured with portable chlorophyll equipment (Soil Plant Analyses Development) SPAD Minolta 502. The fully expanded 3rd or 4th leaf of cotton plant was measured for chlorophyll content. The values measured by the chlorophyll meter corresponded to the amount of chlorophyll present in the plant leaf. The value was calculated on based of the amount of light (red and infrared) transmitted by the leaf in two wavelength regions (650 & 940 respectively) in which the absorption of chlorophyll is different.

2.9: DNA extraction and Quantification

The DNA was isolated from approximately 100mg of the fresh young cotton leaf. Two methods were used according to the manufacturer's instructions.

2.9.1: DNeasy plant mini kits (Qiagen, UK) and Agarose gel electrophoresis.

The protocol of DNA extraction is given in Appendix 1. For quantification, an agarose gel method was used by running the cotton DNA samples with known quantities of standard Lambda DNA (Promega). Five different concentrations of Lambda DNA (6.25 ng/ µl, 12.5 ng/ µl, 25 ng/ µl, 50 ng/µ & 100ng/µl) were run along with the cotton DNA samples on a 1% agarose mini gel (100mm x 150mm) at 50 V for 30 minutes in a 1x TBE (Tris- borate-EDTA) buffer (89mM Tris- base, 89 mM Boric acid, 2 mM EDTA pH 8.0), containing 0.5 µg/ml Ethidium bromide (Promega). The genomic DNA was visualized and photographed under an ultra violet light source.

2.9.2: Qiagen Puregene Core kit A and Spectrophotometer.

The DNA was extracted using Qiagen Pure Core kit according to the manufacturer's instructions. A spectrophotometer (Thermo Spectronic, Unicam UV 500) was used for checking the purity and quantity of the DNA. The DNA was measured by recording the absorbance at 260 and 280 nm wavelengths. The ratio of A260/A280 was used to estimate the purity of the DNA (Glasel, 1995). DNA concentration was calculated using the formula as follows,

Absorbance 260 nm x DF X 50 (Where DF = Dilution factor) (Glasel, 1995).

2.10: PCR (Polymerase chain reaction) amplification.

PCR is a repetitive series of thermal cycles, which provide three different temperature steps for the amplification of a specific sequence of DNA. These steps are a denaturation, an annealing and an extension period. A successful PCR depends on three critical factors; these are the number of cycles, the primer annealing temperature and the time of the extension period. Ready mix^{M} PCR Master Mix (ABgene) and Go Taq polymerase (Promega) were used as PCR enzymes. A concentration of 3 mM $MgCl_2$ was used throughout the analysis and a concentration of the template DNA of 10ng/µl were used for all analyses.

Three different PCR (PTC-100 programmable thermal controller. MJ Research, Inc.)

Programmes and two enzyme kits were used for amplifying the cotton genomic DNA.

2.10.1. Programme 1 (Turner, 2003).

Go Taq polymerase (Promega) was used according to manufacturer's instructions. The temperature programme used was 1 min at 95^{0}C and 1 min at 93^{0}C, one cycle; 30s at 94^{0}C, 30s at 58^{0}C, 2 min 30 sec at 72^{0}C, 34 cycles; final extension at 72^{0}C for 5 mins.

2.10.2. Programme 2.

This was the same as programme 1, but with only 25 cycles (to reduce non - specific amplification).

2.10.3. Programme 3 (Touchdown programme 1; adopted from Cho *et al*, 2000).

Reddy mix^{M} PCR Master mix (ABgene) was used according to manufacturer's instructions. The programme was 5 min at 94^{0}C, one cycle; 1 min at 94^{0}C, 1 min at 57^{0}C and 2 min at 72^{0}C, two cycles; 1 min at 94^{0}C, 1 min at 56^{0}C and 2 min at 72^{0}C, two cycles; 1 min at 94^{0}C, 1 min at 55^{0}C and 2 min at 72^{0}C, five cycles; 1 min at 94^{0}C, 1 min at 54^{0}C and 2 min at 72^{0}C, 26 cycles; final extension 72^{0}C for 5 mins.

2.11. Gel electrophoresis.

The PCR product was run on gel electrophoreses for separation of amplified DNA. A gel was made using 3% Metaphor Super Fine Resolution (SFR) agarose (Amresco). The TBE was used as a running buffer. Different known DNA ladders (100bp and 1kb from Promega; and Hyper ladder from Bioline) were used to determine the size of unknown DNA. The 3% gel (100mm x 150mm) was run on 90 V for 3 hours. Ethidium bromide (0.5µg/ml) or 2.5µl/ml Safe View (NBS) was used as a stain. The DNA fragment was visualized under UV illumination (UVP) and a Mitsubishi video copy processor with Camera (UVP) was used to record the result.

2.12. Scoring of SSR fragments through capillary electrophoresis.

For separation of PCR product, capillary electrophoresis in a CEQ 8000 Genetic Analysis System (Backman Coulter, Inc) was used and analysed with the included software. A size standard (PA 400) was used to size fragments. The undiluted PCR products 0.5 µl of each sample were added separately into each wells on a 98 well loading plate with 0.5 µl size standard (Backman Coulter) and 40 µl of sample loading solution (SLS) and covered with 2 -3 drops of mineral oil to avoid the evaporation of samples during running on sequencer. The size standard is given as a red trace seen in chapter 4 (Fig. 4.3.1.2b). The tail primers for each primer set were used for fragment analysis which were designed for polymorphic primers by adding the complementary M13 forward sequence to the 5` end of the forward primer. The use of dye labelled M13 primer enables us for fragment analysis (Robert *et al.*, 2005). If these are present in

all samples they are termed monomorphic, whereas if they are present in some traces yet absent in others they are termed polymorphic.

2.12.1. Binary data matrix.

A binary data matrix was constructed in Microsoft office excel 2003, on the bases of presence or absence of peaks. A "1" represents the presence of the allele and a "0" its absence. A "-"indicates the variety was not tested. The matrix data is given in Appendix Table 19.

2.13. Ranking data.

Not unexpectedly the genotypes ranked differently according to the different parameters analysed.

An attempt was made to bring them rankings together in order to generate a "universal rank". To do this a mean value was calculated for each parameter (e.g. plant height), this was subtracted from each individual value and the sum divided by the standard deviation of the value of each parameter (Table 3.5.1).

2.14: Experiment layout and Statistical analysis.

All experiments were laid out in randomized design. The statistical analysis was done using the SPSS 14.2 software package to assess significant difference among the genotypes, treatments and genotypes x treatment. The data analysed by one way ANOVA with Tukeys test and two ways ANOVA. For principle component analysis (MINITAB – 15 and NTSYS pc version 1.8) statistical packages were used.

Chapter 3

To measure the effect of salt on physiological traits of different cotton varieties.

Introduction.

Cotton is a cone shaped crop. The height of cotton plant varies between 3 to 6 feet, which depends on the species, origin or where it is grown. The leaves of cotton are broad having 3 to 5 lobes. Cotton is a kharif season crop; it is grown in high temperature zones. The temperature required from germination to harvest ranges from 20-30 ^{0}C (Bhatti, 1975), but in Pakistan, the summer temperature often reaches 48-50 °C (Ashraf, *et al.*, 1994) which is supra-optimal.

Salinity is a major problem all over the world. Munis, *et al,* (2010) reported that 800 million hectares of land are affected by salt in the world. All plants response differently during salt stresses. Plants have the ability to tolerate salinity up to a certain threshold levels, without any yield reduction, when increase in salinity level plants reduced the yield significantly. Cotton is a moderately salt tolerant crop with a salinity threshold level of 7.7dSm^{-1}, however, cotton yield decreases with a response to high salinity of 5.2 % per dSm^{-1} over the threshold (Ashraf, 2002). The conductivity 1 dSm^{-1} is equivalent to 10 mM NaCl (Cramer, 2004). In this study I use 150 and 250 mM NaCl, to 15 and 25 dSm^{-1}. Many studies have investigated the effect of salinity on phenotypic and physiological responses of cotton (Akhtar et al., 1999 and Ali *et al.*, 2004). However, salinity causes a significant loss of cotton yield. Munns, (2005) reported that salinity effects plant metabolism due to ionic and osmotic imbalances which result in the reduction of plant growth. Qadir and Shams (1997) observed that salinity increased Na^{+} and Cl^{-} concentration in leaves, stem and root.

Plants have adopted several mechanisms to cope with these stresses. An important selection criterion for salt tolerance in cotton is the maintainance of high K^+/Na^+ and Ca^+/Na^+ ratios (Glenn *et al.*, 1999 and Ashraf, 2002).

Due to its ability for Na^+ accumulation, the vacuoles play key roles in reducing the toxic level of Na^+ in cytosol and increase the vacuolar osmotic pressure (Cixin *et al.*, 2004). Salinity tolerance is related to exclusion of Na^+ and Cl^- from plant shoots in glycophytes (Gorham *et al.,* 1990). Salt stress reduced the K^+ and Ca^{++} concentration in root and shoot (Kent and Lauchli 1985). However, Khan *et al* (2004) observed that the cotton variety MNH 93 showed increase in K^+ concentration. Similar results for K^+ and Ca^{++} were also reported by Abd - Ella and Shalaby (1993) and Meloni *et al.*, (2001).

The results of previous studies showed that varieties of wheat (Gurmani *et al.,* 2009, Khan *et al.*, 2009), rice (Hakim *et al.*, 2010), barley (Bagci, 2003, Belkhodja *et al.,* 1994) and sorghum (Nawaz *et al.*, 2010) showed different response to salinity from each other.

Previous studies conducted on salt tolerance in cotton showed the genotypic variability within the species (Khan *et al.*, 1995, Akhtar and Azhar 2001). Some varieties of cotton were considered salt tolerant and some were considered susceptible (see chapter one Table 1.4a and 1.4b). I decided to investigate to these phenomenons by measuring anatomical or physiological and chemical parameters. Salt tolerance might be expected to be exemplified by high K^+, little effect on height and weight and photosynthesis. The objectives of this study were to compared the physiological responses of 40 cotton genotypes from three cultivated species (*Gossypium hirsutum* L.) and (*Gossypium barbadense* L.) and (*G. sturtianum*) and one wild species (*G. stocksii*), and to determine a reliable measure of salt tolerance and susceptibility among genotypes for screening of salt tolerance. These descriptions would then be compared with a SSR – based genomic study of the same genotypes with the aim of discovering correlations, and possible QTLs for the relevant traits.

3.1.1. The effect of 150 mM NaCl on cotton.

This initial experiment was to screen a range of cotton varieties obtained from various sources as to their physiological responses in the presence of salt. The varieties used are listed in Table 2.1. The purpose of this experiment was to study the genotypic variation in 22 cotton varieties with regards to salt tolerance and sensitivity, and also to study the relations between ion accumulation and salt tolerance ability of cotton varieties.

3.1.2. Material and methods.

Twenty two different cotton varieties were sown on 1-3-2007. For growth, temperature and light see chapter 2 (section 2.1 and 2.2). Ten seeds per variety were grown. The seed was five years old. Because of its age, and previous storage under non ideal conditions, germination percentage was low with only 73 plants germinating from of 220 seeds. Salinity treatment (150 mM of NaCl, 7.5 mM $CaCl_2$) was started 15 days after germination see chapter 2 (sections 2.3. and 2.3.1). Plants were analysed 15 days after addition of salt. For methods used in further analysis see chapter 2 (sections 2.4, 2.5, 2.6 and 2.7). No control (non – salt) plants were grown in this experiment.

3.1.3 Results.

A wide range of parameter values was observed across the various varieties. This suggested that the cotton material provided would a useful source of variety with respect to salt tolerance in general and salt tolerance mechanisms in particular.

3.1.3.1. Plant height, fresh weight, dry weight and Na^+ in leaf, petiole and stem of cotton genotypes.

The significant differences ($P<0.000$) in the plant shoot height, fresh weight, dry weight and Na^+ in leaf were observed between genotypes, however, no significant differences were observed between genotypes in Na^+ of petiole and stem (Table 3.1.3.3). The variety Acalla SJ 2 had tallest height (579 mm) which was significantly different from MNH 93 (320 mm). However, Acalla SJ 2 was not significantly different from CIM 443, Coker 201, Precoce 1, CIM 240 and Acalla 1517. This result showed that 150 mM NaCl had similar effect on most of varieties except of Karishma and MNH 93 (Table 3.1.3.1). It was observed from the data presented from Table 3.1.3.4 showed that plant height showed the highly significant correlation with fresh weight, dry weight and K^+ concentration in petiole.

The varieties CIM 240, Sarmast, Acalla SJ 2 and Coker 201 had highest fresh weight (68 – 87 g), which was significantly different from MNH 93 (13 g). The result as expected that MNH 93 had lowest weight due to its lowest plant height (Table 3.1.3.1). It was observed from the data presented from Table 3.1.3.4 showed that fresh weight showed the highly significant correlation with dry weight and Na^+ concentration in leaf and stem and K^+ concentration in petiole.

The varieties Acalla SJ 2, CIM 240, Sarmast, AC 134 and Coker 201 had highest dry weight (13.8 – 21.2 g) significantly different from MNH 93 (2 g). The result as expected that MNH 93 had lowest weight due to its lowest plant height and fresh weight (Table 3.1.3.1). It was observed from the data presented from Table 3.1.3.4 showed that dry weight showed the highly significant correlation with Na^+ concentration in leaf and stem and K^+ concentration in petiole.

The highest Na^+ concentration in leaf was observed in Sarmast (400mM) which was significantly different from MNH 93 which had lowest Na^+ concentration (60.6 mM) (Table 3.1.3.1). However, this result suggests that MNH 93, Stonvelle 213, Karishma, Qalandari, Acalla SJ 2 and 149 F were salt tolerant varieties due to lower uptake of Na^+ and Sarmast was salt sensitive variety due to higher uptake of Na^+. The highest Na^+ concentration in petiole was observed in Sarmast, CIM 109 and CIM 240 (244 -270 mM) which was significantly different from Stoneville 213 which had lowest Na^+ concentration (83.3 mM) (Table 3.1.3.1). However, this result suggests that Stonvelle 213, Coker 312, CIM 1100 and Acalla SJ 2 were salt tolerant varieties due to lower uptake of Na^+ and higher uptake of K^+ in petiole. The varieties Stonvelle 213 and Acalla SJ 2 were also showed the lower uptake of Na^+ in leaf and Sarmast also showed the similar response in leaf. It was observed from the data presented from Table 3.1.3.4 showed that Na^+ concentration in leaf showed the highly significant correlation with Na^+ concentration in petiole and stem and K^+ concentration in petiole and K^+/Na^+ ratio in leaf and stem.

The highest Na^+ concentration in stem was observed in Sarmast, NIAB 78, B 496, Precoce 1 and CIM 240 (202 -229 mM) and lowest Na^+ concentration was observed in MNH 93 (83.6 mM) (Table 3.1.3.1). However, this result suggests that MNH 93, Stonvelle CIM 109 and Allepo 45 were salt tolerant varieties due to lower uptake of Na^+ and higher uptake of K^+ in petiole. It was observed from the data presented from Table 3.1.3.4 showed that Na^+ concentration in petiole showed the highly significant correlation with K^+/Na^+ ratio in petiole.

The highest Na^+ concentration was observed in Sarmast, NIAB 78, B 496, Precoce 1 and CIM 240 (202 -229 mM) and lowest Na^+ concentration was observed in MNH 93 (83.6 mM) in petiole (Table 3.1.3.1). It was observed from the data presented from Table 3.1.3.4 showed that Na^+ concentration in stem showed the highly significant correlation with K^+/Na^+ ratio in leaf and stem.

Table 3.1.3.1. Means and standard deviation of plant height, fresh weight, dry weight and Na^+ in leaf, petiole and stem of cotton genotypes.

Genotypes	Plant height (mm)	FW (g)	DW (g)	Na^+ (leaf)	Na^+ (petiole)	Na^+ (stem)
Krishma	358±14	42±19	9±40	98.3±21	129.1±35	162.0±26
CIM 1100	443±47	45±39	9±80	139.0±67	95.2±55	131.8±60
Acalla 1517	446±37	39±22	8±50	127.3±19	140.8±67	132.1±60
Precoce 1	477±95	62±50	15±2	146.8±48	151.0±37	202.7±52
B 496	481±19	65±50	16±1	176.1±40	170.8±54	211.9±68
CIM 448	479±22	55±10	12±2	118.0±48	145.0±80	125.5±44
CIM 109	440±22	49±50	12±2	120.7±27	250.4±79	116.0±29
BP 52-63	468±66	66±10	15±2	168.1±56	150.2±68	178.6±53
NIAB 78	423±16	62±17	13±4	136.7±75	143.4±57	223.7±96
Stonvelle 213	442±24	61±70	13±2	70.4±52	83.3±10.2	83.8±23
Allepo 45	452±63	48±50	12±2	142.7±57	132.0±34	116.7±43
Coker 312	464±52	63±50	16±2	120.3±41	92.7±19	152.3±50
CIM 443	506±39	57±13	14±3	157.5±19	153.1±49	164.9±82
MNH 147	444±63	45±16	10±3	120.1±45	104.7±38	165.8±50
Qalandari	438±66	44±90	9±40	133.4±46	106.4±24	156.1±46
Coker 201	503±23	68±18	15±5	154.9±67	187.1±126	170.2±74
AC 134	472±48	63±25	14±6	149.0±35	174.7±93	177.6±41
CIM 240	470±97	87±28	19±2	199.4±96	270±27	198.9±34
149 F	499±61	54±40	12±2	168.6±2	158.3±36	131.4±56
Acalla SJ 2	579±91	84±26	21±6	109.0±11	104.6±3	118.3±40
Sarmast	440±24	85±90	18±4	400.2±19	243.7±20	230±29
MNH 93	320±18	13±10	2±40	60.6±40	161±40	83.7±80

3.1.3.2. K^+ concentration and K^+/Na^+ ratio in leaf, petiole and stem of cotton genotypes.

No significant differences in leaf, petiole and stem of K^+ concentration were observed between genotypes (Table 3.1.3.3). The significant differences in the leaf ratio of K^+/ Na^+ ($P<0.050$) was observed between genotypes, however, no significant differences in the petiole and the stem ratio of K^+/ Na^+ were observed between genotypes.

Highest K^+ concentrations in leaves were observed in Stoneville 213, 149 F and B.P 52-63 approximately (167.9 mM) which was significantly different from Sarmast (99.2 mM) (Table 3.1.3.2). However, this result suggests that MNH 93, Stonvelle 213, Karishma, Qalandari, Acalla SJ 2 and 149 F were salt tolerant varieties due to lower uptake of Na^+ and higher uptake of K^+ in leaf and Sarmast was salt sensitive variety due to higher uptake of Na^+ and lower uptake

of K^+ in leaf. It was observed from the data presented from Table 3.1.3.4 showed that K^+ concentration in leaf showed the highly significant correlation with K^+/Na^+ ratio in leaf.

Highest K^+ concentration in petiole was observed in Sarmast (307 mM) which was significantly different from Qalandari (192 mM) (Table 3.1.3.2). However, this result suggests that Stonvelle 213, Coker 312, CIM 1100 and Acalla SJ 2 were salt tolerant varieties due to lower uptake of Na^+ and higher uptake of K^+ in petiole.

Highest K^+ concentration in stem was observed in Sarmast, 149 F, CIM 1100 and Acalla SJ 2 (208 - 238 mM) which was significantly different from NIAB 78 (132 mM) (Table 3.1.3.2). However, this result suggests that MNH 93, Stonvelle CIM 109 and Allepo 45 were salt tolerant varieties due to lower uptake of Na^+ and higher uptake of K^+ in stem. It was observed from the data presented from Table 3.1.3.4 showed that K^+ concentration in stem showed the highly significant correlation with K^+/Na^+ ratio in stem.

The highest K^+/ Na^+ ratio in leaves was observed in Stonvelle 213 (3.6) which was significantly different from Sarmast which had lowest K^+/ Na^+ ratio (0.3) (Table 3.1.3.2). However, this result suggests that Stonvelle 213, 149 F, CIM 240 and MNH 93 were salt tolerant varieties due to higher ratio of K^+/Na^+. It was observed from the data presented from Table 3.1.3.4 showed that K^+/ Na^+ ratio in leaf showed the highly significant correlation with K^+/Na^+ ratio in stem.

The highest K^+/ Na^+ ratio in petiole was observed in Coker 312, CIM 1100, Coker 201 (2.9 – 3.5) and lowest K^+/ Na^+ ratio was observed in CIM 240 (1) (Table 3.1.3.2). However, this result suggests that Coker 312, CIM 1100, Coker 201, Acalla SJ 2 and Stonvelle 213 were salt tolerant varieties due to higher ratio of K^+/Na^+. It was observed from the data presented from Table 3.1.3.4 showed that K^+/ Na^+ ratio in petiole showed no significant correlation with any traits.

The highest K^+/ Na^+ ratio in stem was observed in Stonvelle 213 (2.4) lowest K^+/ Na^+ ratio was observed in NIAB 78 (0.7) (Table 3.1.3.2). However, this result suggests that Stonvelle 213,

MNH 93, Allepo 45, CIM 109 and 149 F were salt tolerant varieties due to higher ratio of K^+/Na^+. It was observed from the data presented from Table 3.1.3.4 showed that K^+/ Na^+ ratio in stem showed no significant correlation with any traits.

Table 3.1.3.2. Means and standard deviation of K^+ and K^+/Na^+ in leaf, petiole and stem of cotton genotypes.

Genotypes	**K^+ (leaf)**	**K^+ (petiole)**	**K^+ (stem)**	**K^+/Na^+ (leaf)**	**K^+/Na^+ (petiole)**	**K^+/Na^+ (stem)**
Krishma	109.2±28	229.0±32	145.7±37	1.1±0.1	1.8±0.5	0.9±0.2
CIM 1100	107.0±23	236.1±26	215.7±35	0.9±0.5	3.2±2.1	1.9±1.1
Acalla 1517	143.1±36	238.4±31	146.4±32	1.1±0.1	1.9±0.5	1.4±0.5
Precoce 1	124.9±46	235.4±47	188.5±48	1.0±0.6	1.6±0.5	1.0±0.5
B 496	124.3±17	263. ±62	150.7±24	0.7±0.2	1.6±0.5	0.8±0.2
CIM 448	108.2±10	244.9±30	177.5±15	1.0±0.3	2.0±0.8	1.5±0.4
CIM 109	159.9±15	236.8±62	212.2±25	1.4±0.4	1.3±0.8	2.0±0.8
BP 52-63	167.9±25	256.8±40	188.3±26	0.8±0.2	2.1±1.4	1.1±0.5
NIAB 78	149.1±2	231.8±83	131.7±21	1.4±0.8	1.6±0.1	1.1±0.5
Stonvelle 213	167.0±30	221.6±25	191.4±22	3.6±2.7	2.7±0.6	2.4±0.9
Allepo 45	154.5±47	277.6±42	208.5±11	1.3±0.9	2. ±0.6	2.0±1.1
Coker 312	142.3±22	299.6±33	165.2±12	1.3±0.5	3.5±2.4	1.2±0.5
CIM 443	120.0±14	288.0±26	178.7±30	0.8±0.2	2.0±0.5	1.4±0.8
MNH 147	153.0±32	208.1±53	211.0±21	1.5±1.0	2.5±1.9	1.4±0.4
Qalandari	135.9±21	191.9±30	177.7±20	1.3±0.3	1.9±0.7	1.2±0.4
Coker 201	138.0±14	271.0±61	178.9±23	1.1±0.7	2.9±2.9	1.3±0.7
AC 134	142.6±25	268.0±35	199.0±16	1.0±0.3	1.8±0.7	1.2±0.4
CIM 240	150.2±94	258.1±31	174.0±76	1.9±2.4	2.0±0.5	1.3±1.3
149 F	169.0±2	253.5±13	227.7±15	2.3±1.4	1.6±0.3	1.9±0.9
Acalla SJ 2	154.3±2	289.7±51	203.8±49	1.4±0.1	2.8±0.3	1.7±0.3
Sarmast	99.2±10	307.5±12	238.1±13	0.2±0.1	1.3±0.9	1.0±0.8
MNH 93	115.4±34	193.4±15	204±28	1.9±0.2	1.3±0.1	2.4±0.9

Table 3.1.3.3. Mean square, degree of freedom and P values of cotton genotypes treated with 150mM NaCl.

Variables	Genotypes	DF
Plant height	5841.995**	21
FW	5713.0**	21
DW	377.38**	21
Na^+ (leaf)	6679.0**	21
Na^+ (petiole)	7789.0 ^{NS}	21
Na^+ (stem)	1.22^{NS}	21
K^+ (leaf)	1202.1^{NS}	21
K^+ (petiole)	7789.0 ^{NS}	21
K^+ (stem)	4062.0 ^{NS}	21
K^+/Na^+ (leaf)	1.24*	21
K^+/Na^+ (petiole)	3287.0 ^{NS}	21
K^+/Na^+ (stem)	1951.3 ^{NS}	21

Table 3.1.3.4. Correlation of all physiological traits.

	Plant height	FW	DW	Na^+ (leaf)	K^+ (leaf)	Na^+ (petiole)	K^+ (petiole)	Na^+ (stem)	K^+ (stem)	K^+/Na^+ (leaf)	K^+/Na^+ (petiole)	K^+/Na^+ stem)
Plant height	1	.688(**)	.762(**)	0.094	0.327	-0.008	.557(**)	0.143	0.129	-0.116	0.342	-0.131
FW		1	.971(**)	.575(**)	0.172	0.349	.654(**)	.550(**)	0.032	-0.142	0.042	-0.404
DW			1	.467(*)	0.244	0.288	.701(**)	.477(*)	0.013	-0.134	0.075	-0.365
Na+ (leaf)				1	-0.351	.558(**)	.549(**)	.692(**)	0.198	-.594(**)	-0.327	-.489(*)
K+ (leaf)					1	-0.07	-0.059	-0.225	0.074	.564(**)	0.128	0.257
Na+ (petiole)						1	0.273	0.399	0.159	-0.243	-.691(**)	-0.171
K+ (petiole)							1	0.281	0.154	-0.396	0.139	-0.202
Na+ (stem)								1	-0.276	-.569(**)	-0.292	-.886(**)
K+ (stem)									1	0.085	0.027	.560(**)
K+/Na+ (leaf)										1	0.151	.638(**)
K+/Na+ (petiole)											1	0.147
K+/Na+ (stem)												1

3.1.4. Principal component analysis of phenotypes.

Principal component analysis (PCA) is a procedure which changes a number of possibly related variables into a smaller number of unrelated (uncorrelated) variables. These uncorrelated variables are known as principle components. The first principal component shows high significance and other components show the remaining significance between the variables.

In this project I have been trying to correlate the differences of the values of such components (derived from physiological data) with known genetic (and hence breeding history) differences. The genetic differences have been described quantitatively according to the SSR (simple sequences repeat) pattern at 17 loci of each variety (see in chapter 4). The aim was to be able to predict the physiological behaviour of a variety from its SSR pattern. In the context of this thesis, this is a model exercise for the processes involved in predicting a breeding trait from a genetic marker – marker assisted breeding. Far larger data sets would be required for this approach to be fully affective.

This experiment was designed to perform this analysis on twenty two independent varieties for convenience; Stonvelle 213, Allepo 45, CIM 109, CIM 448, CIM 240, CIM 443, 149 F, Karishma, Qalandari, CIM 1100, B 496, Sarmast, NIAB 78, Acalla SJ 2, Acalla 1517, AC 134, MNH 147, Coker 201, Coker 213, Precoce 1, B 52- 63 and MNH 93.

The SSR analysis provides evidence of seven main clusters of varieties at the root of dandrogram. One clade includes CIM 1100 and CIM 448 a second clade includes MNH 147, CIM 443, Karishma, CIM 109 and Coker 201. The third clade includes Coker 312, Sarmast, Allepo 45, Acalla 1517, B 52 – 63, Qalandari and Precoce 1 and the fourth clade includes MNH 93 and fifth includes NIAB 78. Twelve physiological parameters (plant height, fresh weight, dry weight, Na^+, K^+ and K^+/Na^+ in leaf, petiole and stem) generated Fig.-3.1.4.1. The clearest groups are generated by PC1 and PC2, which accounts for 84.3% and 10.5% of the total variance respectively. Here we see varieties Acalla SJ 2, Coker 312, Stonvelle 213, CIM 109, Allepo 45,

149 F, Qalandari, MNH 147, CIM 448, CIM 1100, Karishma, Acalla 1517 and MNH 93 (group I) grouping apart from Sarmast, Precoce 1, B 496, NIAB 78, Coker 201, B P 52- 63, AC 134, CIM 443 and CIM 240 (group II).

Principal component 1 (PC1) is a function of plant height, fresh weight, dry weight and Na^+ in leaf, petiole and stem and K^+ in stem. Principal component 2 (PC2) is a function of K^+ in leaf and stem, K^+/Na^+ ratio in leaf, petiole and stem, plant height, fresh weight, dry weight

The Ward linkage correlation coefficient distance for these individual parameters is shown in Fig.-3.1.4.2. It is, perhaps, an expected division between the two PCs – for example plant height, fresh weight, dry weight and Na^+ in leaf petiole and stem being dependent of each other, and K^+ and K^+/Na^+ being independent.

Comparison of the PCA groups I and II with the genetic dendrogram is interesting. Members of the MNH 147, Karishma, CIM 109, clade are found in same groups, as are members of the Sarmast, Precoce 1, B P 52 – 63, clade. Generating a relationship map based on the physiology (Fig.-4.3.3.1) confirms that relationship in between CIM 443, Coker 201 and B 496 and also the relationship in between Allepo 45, Qalandari and Acalla 1517 (seen in the SSR dendrogram).

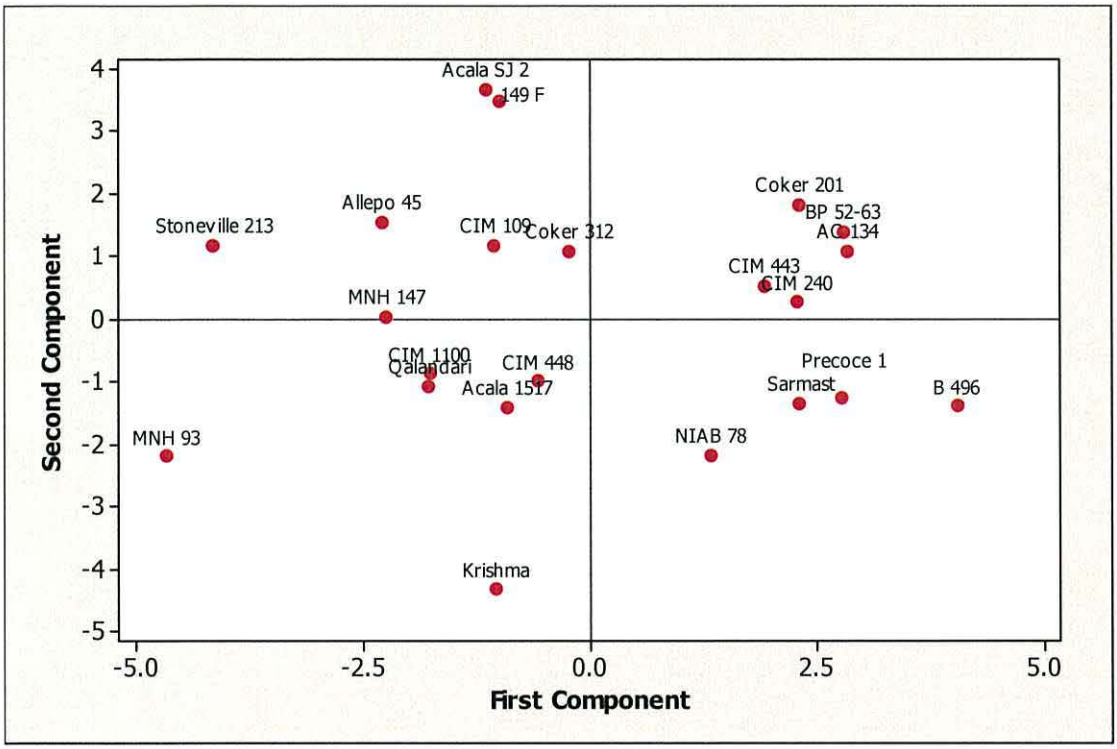

Fig.3.1.4.1. Scatter plot of genotypes based on the principal component analysis of physiological traits.

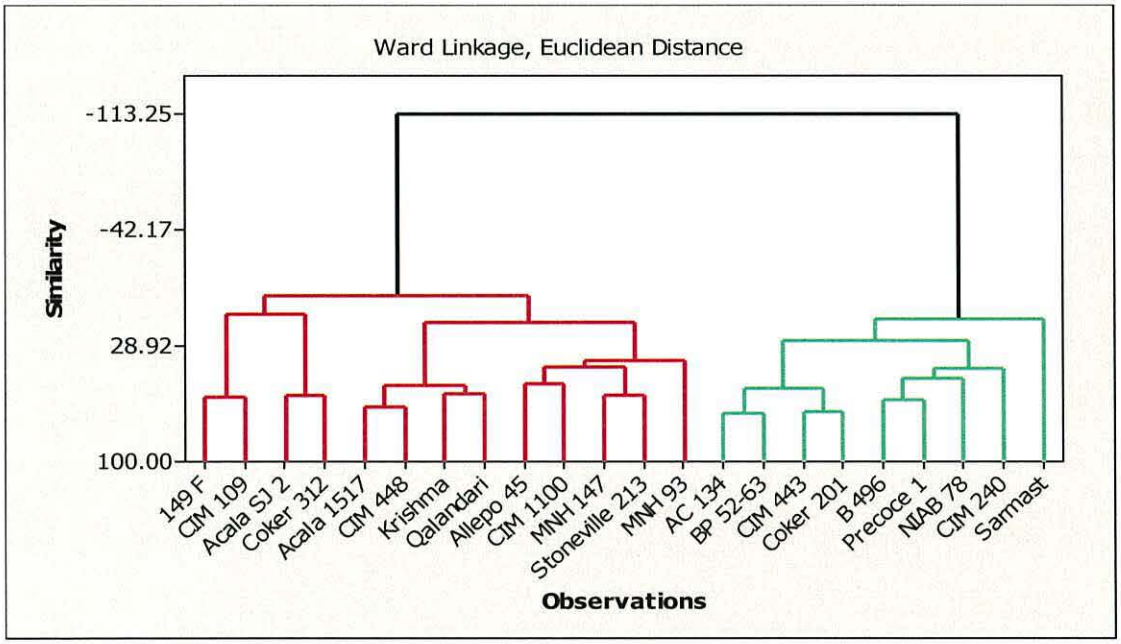

Fig 3.1.4.2. Dendrogram of cotton varieties based on principal component analysis generated by significant physiological traits.

3.2.1. Effect of two different treatments of salt (150 - 250 mM NaCl) on cotton.

This experiment was designed to perform this analysis on six independent varieties (called "parents" for convenience; CIM 448, Dandara, DNH 40, MNH 147, *G. stocksii* and NIAB 313) and three hybrids derived from crosses between four (CIM 448, DNH 40, MNH 147, and NIAB 313) of these "parents". This models the real-world situation where selection for traits would be made at each generation. However, seven other hybrids were also used in this study, the values of the result for these was diminished since the three of their "parents" were not available (S 6, *G. hirsutum* and *G.sturtanium*). In this experiment the purpose of this work was to select salt tolerant and sensitive varieties along with their hybrids and to assess the physiological and growth responses under 150 – 250 mM salt stress. In the previous experiment salt tolerance was screened without a control treatment, but in this experiment control was used to see the effect of salt with increased salt treatment to 250 mM. In addition, the effect of NaCl on Na^+ and K^+ concentration of young and old tissues were also analysed.

3.2.2. Material and methods.

In this study 16 genotypes of cotton were sown on 23-5-07 in Pen-y-Ffridd Research Station see in chapter 2 (2.1 and 2.2). Two different treatments of salt, moderate salt (150Mm NaCl), and high salt (250mM NaCl) were used see in chapter 2 (2.3.1, 2.3.2 and 2.3). After fifteen days the genotypes were treated with 150 mM of NaCl, 7.5 mM $CaCl_2$. For methods used in further analysis see chapter 2 (sections 2.4, 2.5, 2.6 and 2.7).

3.2.3 Results.

3.2.3.1. Plant height.

The effect of salt after 15 days of 150 mM salt treatment and after a further 15 days of 250 mM salt treatment reduced plant height significantly ($P<0.000$) (see Appendix Table 3 and 4). The relative differences in plant height were in the range of 9.7% - 37% and 30 % - 58.3% at the end of the 150 mM and 250 mM treatment periods, respectively. However, for most varieties almost no further growth was observed in the 250mM period (Fig. 3.2.3.1). Significant ($P<0.000$) differences were observed between genotypes. The interaction between treatments and genotypes was not significant after 15 days of 150 mM salt treatment, however, the interaction between treatments and genotypes was significant ($P<0.000$) in (30 days x 250 mM). This significance, however, was entirely due to the different behaviour of *G. stocksii*. Dandara x S6 and S6 x Dandara had the tallest plants under both conditions (salt and control) and had the highest absolute reduction in height (271 & 243 mm respectively) under 150 mM NaCl stress. The lowest absolute reduction was observed in *G. stocksii* (28 mm). This was significantly different from all other genotypes.

At 15 days x 250 mM, Dandara and S6 x Dandara had the tallest plants under both salt and control conditions and had an absolute reduction in height (527.5 & 476.7 mm respectively) in the 250 mM NaCl stress periods. These were significantly different from other varieties. The lowest absolute reduction was observed in *G. stocksii* (71.7 mm) which was significantly different from all other genotypes. The genotype Dandara showed the highest relative difference (33.7%) in both stresses than other genotypes, which had relative difference with a range of -3% – 20%.

It was observed from the data presented from Table 1 (see in Appendix) that the plant height showed highly significant positive correlation with fresh weight, dry weight, Na^+ in old leaf and young leaf and petiole, and significant positive correlation with K^+ in old petiole and young

stem. However, highly significant negative correlation K^+/Na^+ ratio in young and old leaf and petiole and significant negative correlation was observed with K^+/Na^+ ratio in young stem at 15 day x 150 mM.

It was observed from the data presented from Table 2 (see in Appendix) that the plant height showed highly significant correlation with fresh weight, dry weight, Na^+ in old and young leaf and K^+ in young petiole and stem, K^+/Na^+ ratio in young petiole and significant correlation with K^+/Na^+ ratio in young stem. However, highly significant negative correlation was observed with Na^+ in young petiole, K^+ in old leaf and K^+/Na^+ ratio in old and young leaf and significant negative correlation was observed with K^+ in young leaf at 15 day x 250 mM.

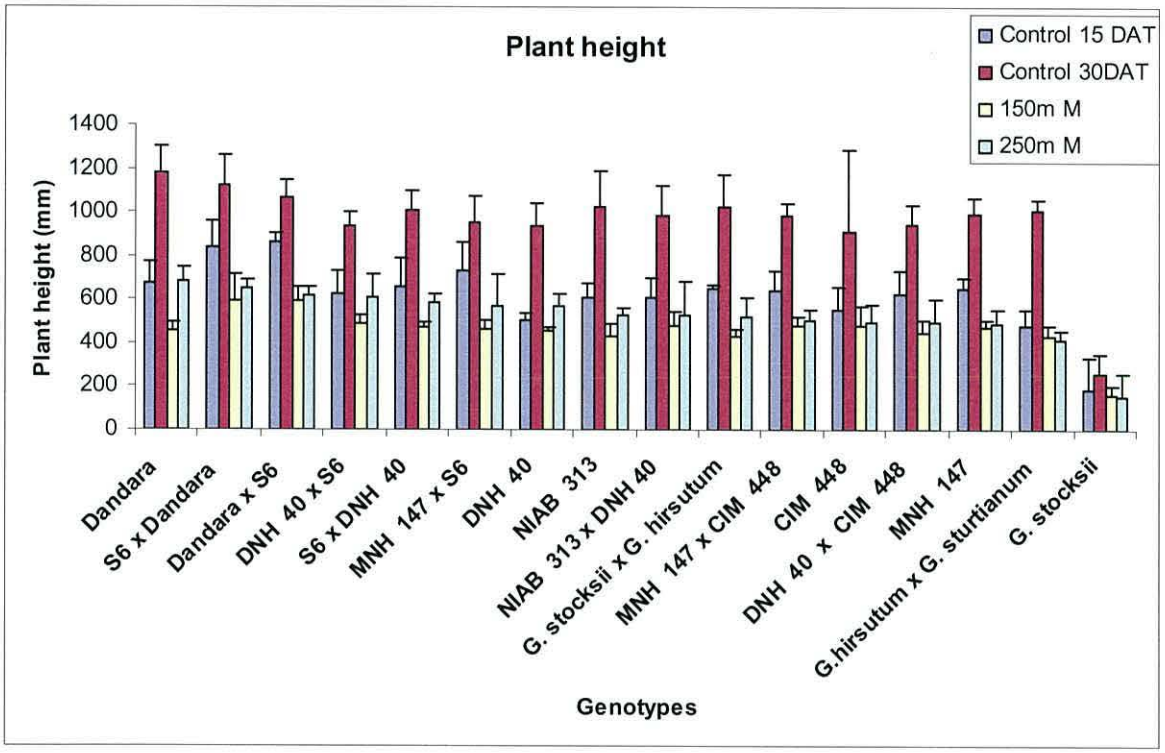

Fig.3.2.3.1. Effect of 150 followed by 250 mM NaCl on plant shoots height of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd. DAT= days after treatments (see in Appendix Table 5 and 6).

3.2.3.2. Nodes per plant.

The effect of salt after 15 days of 150 mM salt treatment and after a further 15 days of 250 mM salt treatment reduced the number of nodes per plant significantly ($P<0.000$) in genotypes (see Appendix Table 3 and 4). However, in G. *stocksii* the number of nodes per plant was increased under both stresses (Fig. 3.2.3.2). Significant ($P<0.000$) differences were observed between genotypes after 15 days of 150 mM salt treatment. The interaction between treatments and genotypes was not significant after 15 days of 150 mM salt treatment; however, the interaction between treatments and genotypes was significant ($P<0.000$) in (30 days x 250 mM). The genotypes DNH 40, NIAB 313 x DNH 40 and CIM 448 showed the highest relative differences (40% - 48%) in the numbers of nodes per plant than other genotypes in control treatments. Whereas the genotypes NIAB 313 x DNH 40, MNH 147 x S6, DNH 40 x CIM 448, MNH 147 x CIM 448, Dandara x S6 and CIM 448 showed the highest relative differences (31% - 36%) in the numbers of nodes per plant than other genotypes in salt treatments.

It was observed from the data presented from Table 1 (see in Appendix) that the nodes per plant showed highly significant correlation with fresh weight, dry weight, and significant correlation with K^+ in old stem. However, highly significant negative correlation was observed with K^+/Na^+ ratio in old leaf and young petiole and stem 15 day x 150 mM.

It was observed from the data presented from Table 2 (see in Appendix) that the nodes per plant showed highly significant positive correlation with K^+/Na^+ ratio in old stem and significant correlation with dry weight, K^+ in old stem. However, highly significant negative correlation was observed with Na^+ in old stem and K^+ old leaf and significant negative correlation was observed with Na^+ in old petiole at 15 day x 250 mM.

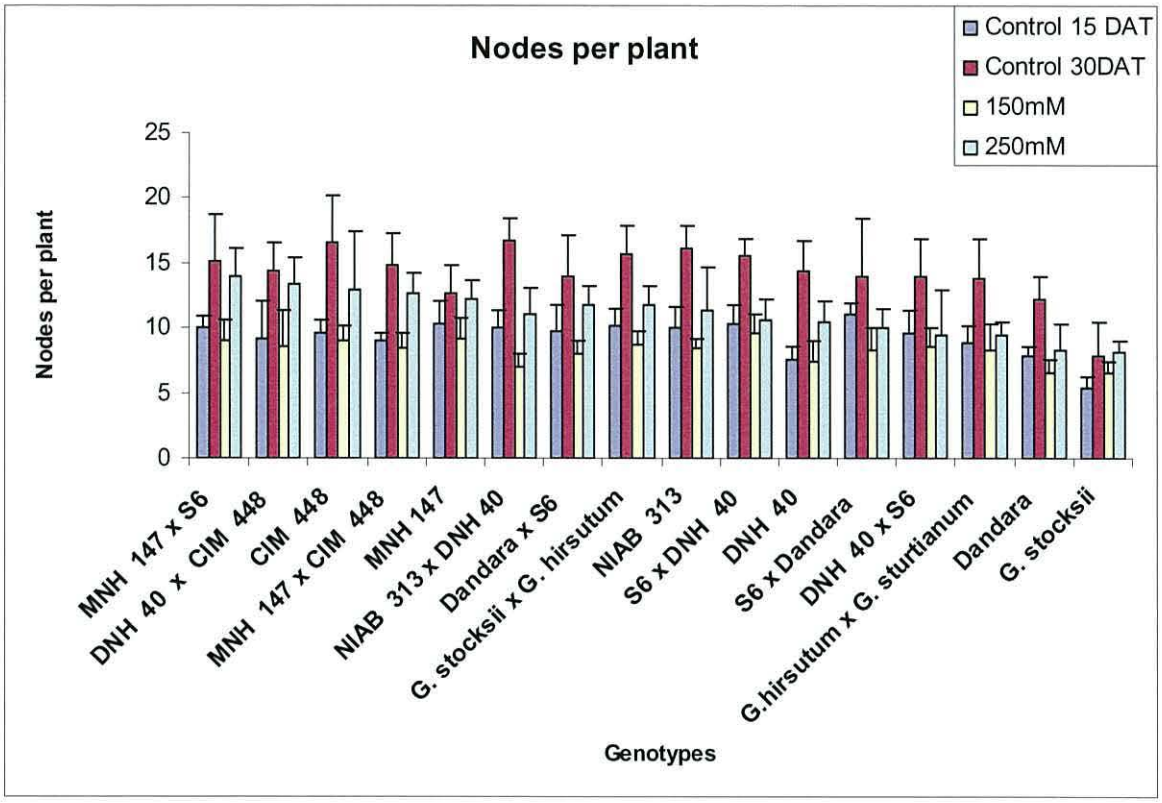

Fig.3.2.3.2. Effect of 150 followed by 250 mM NaCl on nodes per plant of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd. DAT= days after treatments (see in Appendix Table 5 and 6).

3.2.3.3. Fresh weight.

The effect of salt after 15 days of 150 mM salt treatment and after a further 15 days of 250 mM salt treatment reduced fresh weight significantly ($P<0.000$) (see in Appendix Table 3 and 4). The relative differences in fresh weight were in the range of 21% - 76.5% and 42.5 % - 72.6% at the end of the 150 mM and 250 mM NaCl treatment periods. However, for most varieties almost no further increase in fresh weight was observed in the 250mM period (Fig. 3.2.3.3). Significant ($P<0.000$) differences were observed between genotypes. The interaction between treatments and genotypes was not significant after 15 days of 150 mM salt treatment; however, the interaction between treatments and genotypes was significant ($P<0.000$) in (30 days x 250 mM). This significance, however, was largely due to the different behaviour of *G. stocksii*. The genotypes CIM 448, *G.hirsutum x G.sturtainum*, Dandara, MNH 147 x CIM 448 and DNH 40 showed the highest relative differences (45% - 55.5%) compared to other varieties in control treatments. Whereas the genotypes DNH 40 x S6, Dandara x S6 and S6 x Dandara, *G. stocksii* x *G.hirsutum* and DNH 40 showed the highest relative differences (10% - 15.5%) than other varieties in salt treatments.

It was observed from the data presented from Table 1 (see Appendix) that the fresh weight showed highly significant positive correlation with dry weight, Na^+ in young leaf and petiole, and significant correlation with Na^+ in old leaf and young stem. However, highly significant negative correlation was observed with K^+/Na^+ ratio in old petiole and in young leaf, petiole and stem and significant negative correlation was observed with and K^+/Na^+ ratio in old leaf at 15 day x 150 mM.

It was observed from the data presented from Table 2 (see Appendix) that the fresh weight showed highly significant positive correlation with dry weight, Na^+ in old leaf K^+ in young petiole and stem, K^+/Na^+ ratio in young leaf and significant correlation with K^+/Na^+ ratio in young petiole and stem. However, highly significant negative correlation was observed with Na^+

in young petiole, K^+ in old leaf and K^+/Na^+ ratio in old leaf and significant negative correlation was observed with and K^+/Na^+ ratio in young leaf at 15 day x 250 mM..

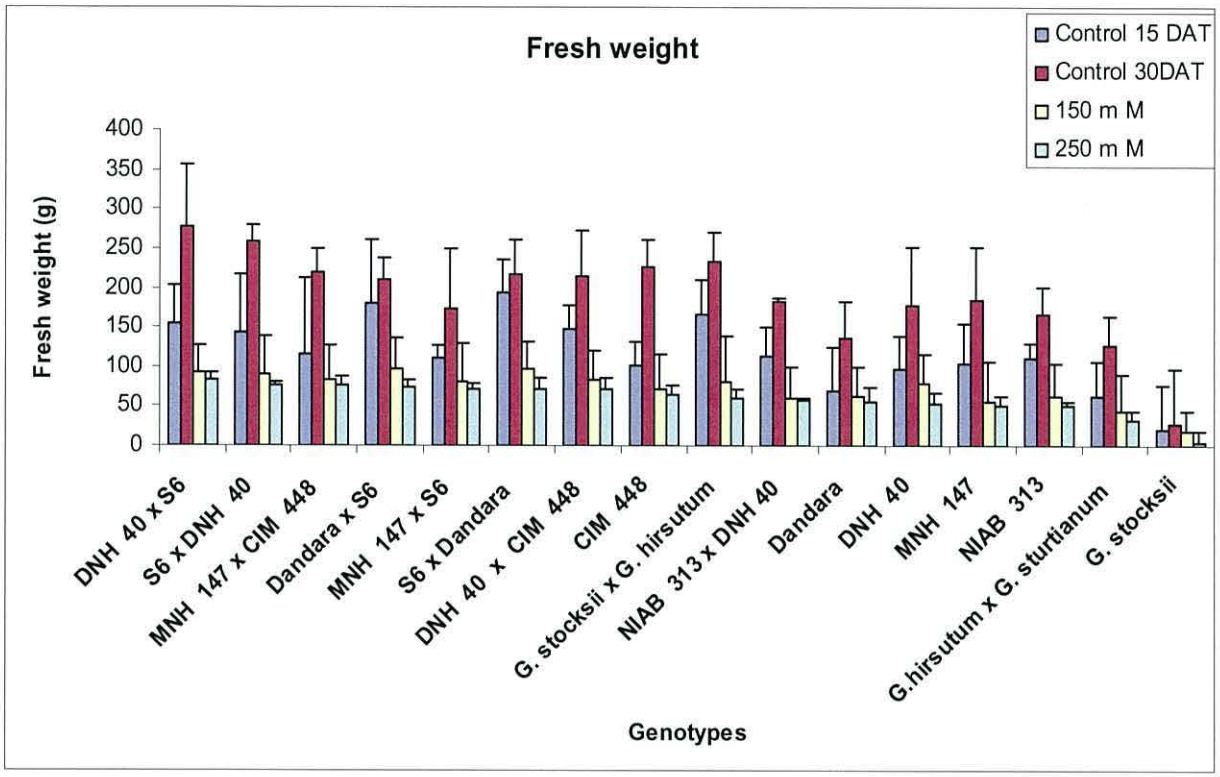

Fig.3.2.3.3. Effect of 150 followed by 250 mM NaCl on fresh weight of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd. DAT= days after treatments (see in Appendix Table 5 and 6).

3.2.3.4. Dry weight.

The effect of salt after 15 days of 150 mM salt treatment and after a further 15 days of 250 mM salt treatment reduced fresh weight significantly ($P<0.000$) (see in Appendix Table 3 and 4). The relative differences in dry weight were in the range of 0.4% - 64% and 47% - 71% at the end of the 150 mM and 250 mM NaCl treatment periods (Fig. 3.2.3.4). Significant ($P<0.000$) differences were observed between genotypes. The interaction between treatments and genotypes was not significant in (15days x 150 mM: 30days x 250 mM). All genotypes showed the relative differences in the range of 66% - 90% in control treatments. Whereas in salt treatments the genotypes DNH 40 x CIM 448, MNH 147 x CIM 448, DNH 40 x S6, *G. stocksii* x *G.hirsutum*, S6 x DNH 40, MNH 147 x S6 and CIM 448 showed the highest dry weight 19.5 – 23.5 g in 250 mM NaCl, and their relative differences of both stresses were with a range of 29% - 52%.

It was observed from the data presented from Table 1 (see Appendix) that the dry weight showed highly significant positive correlation with Na^+ in old leaf, young leaf and petiole, and significant correlation with Na^+ in young stem. However, highly significant negative correlation was observed with K^+/Na^+ ratio in old leaf, petiole and in young leaf, petiole and stem at 15 day x 150 mM.

It was observed from the data presented from Table 2 (see Appendix) that the dry weight showed highly significant positive correlation with K^+ in young stem and significant correlation with K^+ in old stem and K^+/Na^+ ratio in old and young stem. However, highly significant negative correlation was observed with K^+ in old leaf and K^+/Na^+ ratio in old leaf and significant negative correlation was observed and K^+/Na^+ ratio in old petiole and young petiole and stem at 15 day x 250 mM..

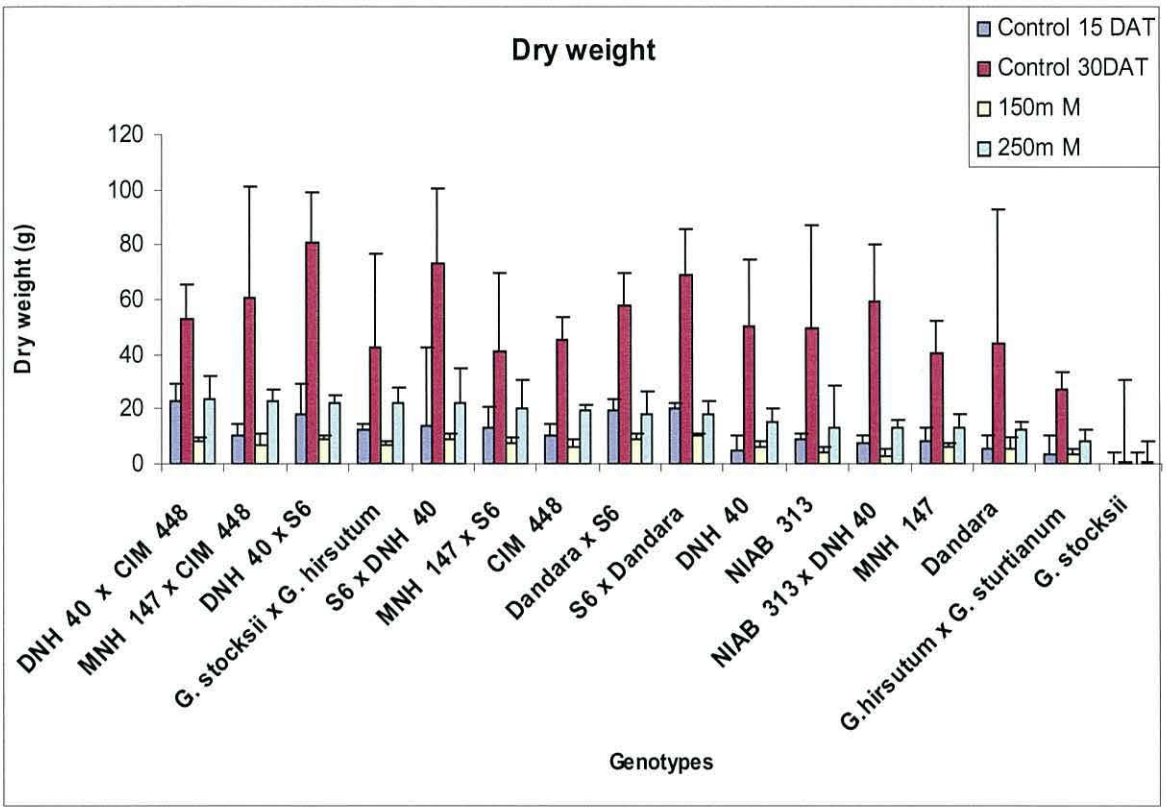

Fig.3.2.3.4. Effect of 150 followed by 250 mM NaCl on dry weight of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd. DAT= days after treatments (see in Appendix Table 5 and 6).

3.2.3.5. Na^+ concentration in the young leaf.

The effect of salt after 15 days of 150 mM salt treatment and after a further 15 days of 250 mM salt treatment increased Na^+ concentration in young leaf significantly (P<0.000) (see in Appendix Table 3 and 4). The young leaf showed an increase in Na^+ concentration in the range of 15% - 94% in 150 mM which was increased with increasing NaCl stress to 250 mM in the range of 79% - 96.4% as compared to control plants (Fig. 3.2.3.5). Significant (P<0.000) differences were observed between genotypes. The interaction between treatments and genotypes was significant (P<0.000) in (15days x 150 mM: 30days x 250 mM). This significance, however, was entirely due to the different behaviour of *G. stocksii*. The genotypes Dandara x S6, Dandara, S6 x Dandara, DNH 40 x S6 and CIM 448 showed highest increase of Na^+ 50% - 84% in 250 mM compared with 150 mM NaCl.. Whereas the genotypes S6 x DNH 40, MNH 147 x S6 and MNH 147 showed lowest increase of Na^+ in 250 mM with a range of 10% - 31% compared 150 mM NaCl. The result shows that the genotypes MNH 147 and MNH 147 x S6 are salt tolerant genotypes due to low uptake of Na^+ in young leaf during high stress as compared to other genotypes.

It was observed from the data presented from Table 1 (see Appendix) that the Na^+ concentration in young leaf showed highly significant positive correlation with Na^+ in young petiole and stem and significant positive correlation with K^+ in young petiole and stem and K^+/Na^+ ratio in old stem. However, highly significant negative correlation was observed with K^+/Na^+ ratio in old petiole and significant negative correlation was observed with K^+/Na^+ ratio in young leaf, petiole and stem at 15 day x 150 mM. It was observed from the data presented from Table 2 (see Appendix) that the Na^+ concentration in young leaf showed highly significant positive correlation with K^+ in young petiole and significant correlation with K^+ in old petiole. However, highly significant negative correlation was observed with K^+ in young leaf and K^+/Na^+ ratio in young leaf at 15 day x 250 mM.

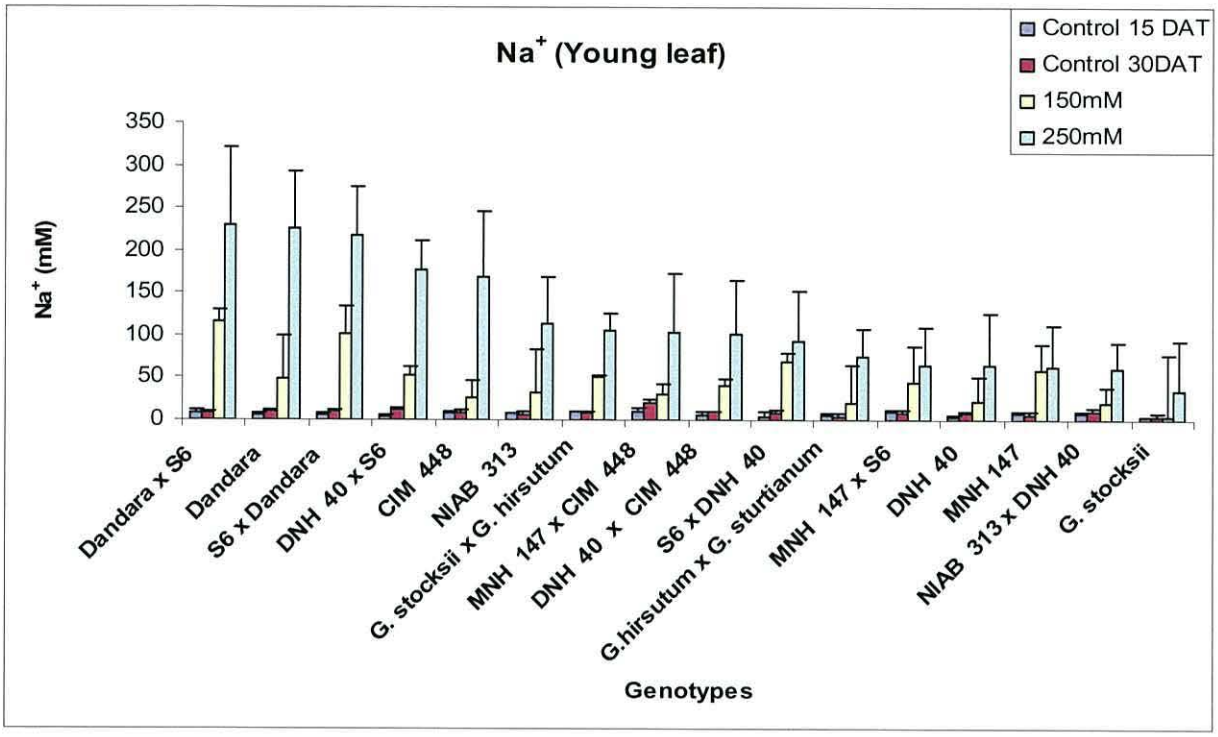

Fig.3.2.3.5. Effect of 150 followed by 250 mM NaCl on Na^+ concentration in young leaf of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd. DAT= days after treatments (see in Appendix Table 7 and 8).

3.2.3.6. K^+ concentration in the young leaf.

The effect of salt after 15 days of 150 mM salt treatment and after a further 15 days of 250 mM salt treatment on K^+ concentration in young leaf was not significant (see Appendix Table 3 and 4). The young leaf K^+ showed a relative difference was in the range of -28% - 50% and -1% - 48% at the end of the 150 - 250 mM NaCl treatment periods (Fig.3.2.3.6). No significant differences were observed between genotypes. The interaction between treatments and genotypes was significant ($P<0.000$) in (15days x 150 mM: 30days x 250 mM). In control plants young leaf K^+ showed increase with increasing age of plants. Most genotypes showed a decrease in young leaf K^+ (MNH 147 x CIM 448, CIM 448, NIAB 313 x DNH 40, S6 x DNH 40, Dandara x S6 and S6 x Dandara, DNH 40 x S6 and Dandara) in both stresses relative to control. But the genotypes MNH 147, MNH 147 x S6, DNH 40, DNH 40 x CIM 448, NIAB 313 and *G. stocksii* x *G.hirsutum* showed a decrease in 150 mM and an increase in the young leaf K^+ in the 250 mM NaCl period relative to control. However, *G. stocksii* and *G.hirsutum x G.sturtainum* showed an increase of young leaf K^+ in both stresses of NaCl compared to control. It was observed from the data presented from Table 1 (see Appendix) that the K^+ concentration in young leaf showed significant positive correlation with K^+ in young leaf at 15 day x 150 mM. It was observed from the data presented from Table 2 (see Appendix) that the K^+ concentration in young leaf showed highly significant positive correlation with of K^+/Na^+ ratio in young leaf, highly significant negative correlation with K+ in young petiole and significant negative correlation with K^+/Na^+ ratio in young petiole at 15 day x 250 mM.

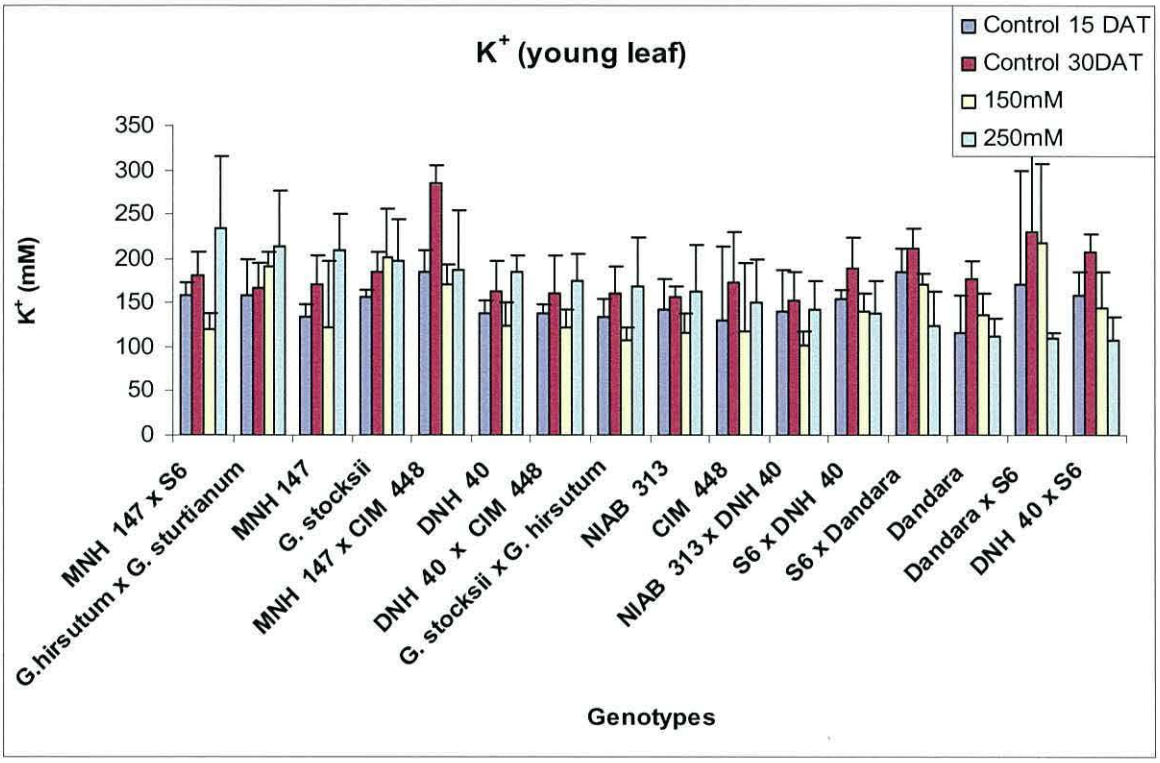

Fig.3.2.3.6. Effect of 150 followed by 250 mM NaCl on K^+ concentration in young leaf of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd. DAT= days after treatments (see in Appendix Table 9 and 10).

3.2.3.7. K^+/Na^+ ratio in the young leaf.

The effect of salt after 15 days of 150 mM salt treatment and after a further 15 days of 250 mM salt treatment reduced K^+/Na^+ ratio significantly ($P<0.000$) (see Appendix Table 3 and 4). The young leaf K^+/Na^+ ratio showed a relative difference were in the range of 73% - 98% under both stresses of NaCl (Fig. 3.2.3.7). The interaction between treatments and genotypes was not significant (15days x 150 mM: 30days x 250 mM). Significant ($P<0.000$) differences were observed between genotypes. This significance, however, was largely due to the different behaviour of *G. stocksii*. In control plants, the young leaf K^+/Na^+ ratio showed an increase with increasing age of plants in most of genotypes except for DNH 40, DNH 40 x CIM 448, DNH 40x S6, *G. stocksii*, NIAB 313 x DNH 40, S6 x Dandara and S6 x DNH 40.

In salt treatments, the highest K^+/Na^+ ratio was observed in 150 mM treatment. Most genotypes showed the highest increase in 150 mM with a range of 25% - 83% compared with 250 mM. However, MNH 147 and MNH 147 x S6 showed the highest increase in high salt stress (250mM) in the range of 35% & 65%. The highest K^+/Na^+ ratio in the young leaf was observed in *G. stocksii* in both stresses. MNH 147 and MNH 147 x S6 also showed the highest increase in 250 mM. In this result, *G. stocksii,* MNH 147 and MNH 147 x S6 maintain the highest young leaf K^+/Na^+ ratio in high salt level relative to other genotypes. It was observed from the data presented from Table 1 (see Appendix) that the K^+/Na^+ ratio in young leaf showed highly significant positive correlation with K^+/Na^+ ratio in young petiole and significant correlation with K^+/Na^+ ratio in young stem at 15 day x 150 mM.

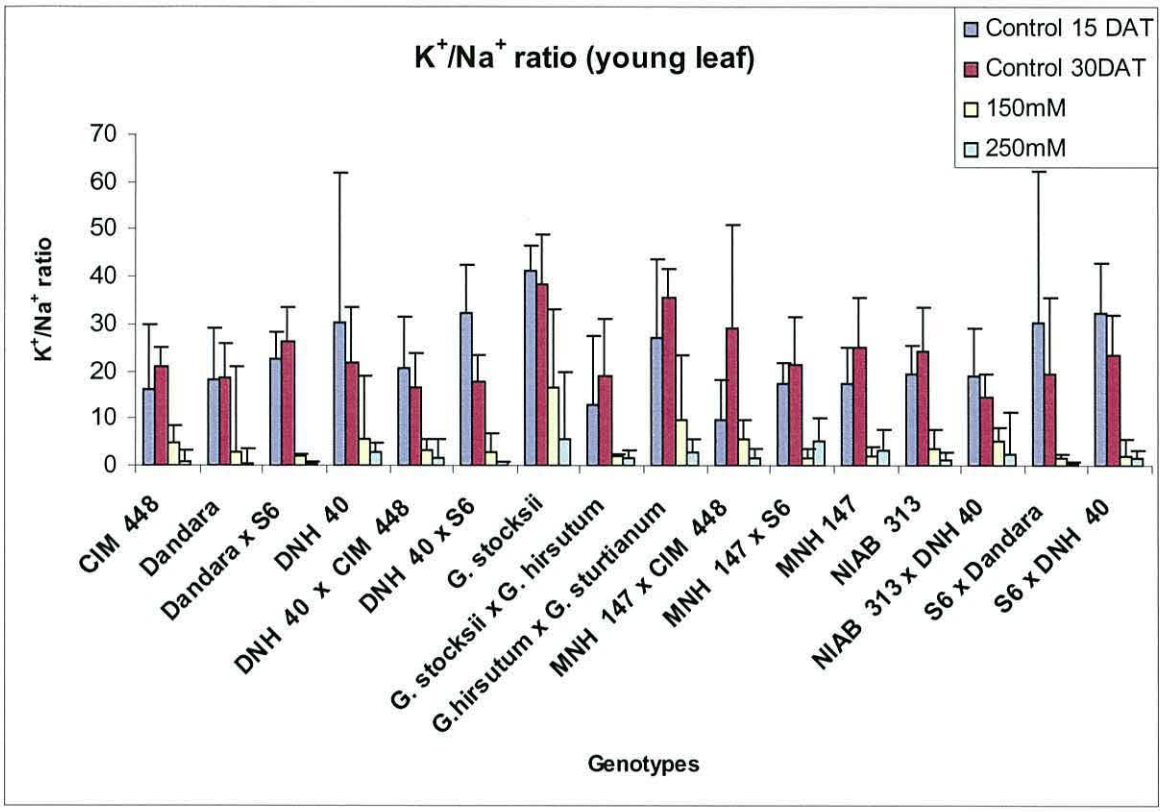

Fig.3.2.3.7. Effect of 150 followed by 250 mM NaCl on K^+/Na^+ ratio in young leaf of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd. DAT= days after treatments (see in Appendix Table 11 and 12).

3.2.3.8. Na^+ concentration in the young petiole.

The effect of salt after 15 days of 150 mM salt treatment and after a further 15 days of 250 mM salt treatment increased Na^+ concentration in young petiole significantly ($P<0.000$) (see Appendix Table 3 and 4). The young petiole showed an increase in Na^+ concentration in the range of 49.5% - 94% in 150 mM which was increased with increasing NaCl stress to 250 mM in the range of 89% - 97% as compared to control plants (Fig. 3.2.3.8). No significant differences were observed between genotypes. The interaction between treatments and genotypes was not significant in (15days x 150 mM: 30days x 250 mM). The genotypes *G. stocksii* and CIM 448 showed highest increase of Na^+ 90% - 96% in 250 mM compared with 150 mM NaCl. Whereas the genotypes S6 x DNH 40, Dandara x S6 and S6 x Dandara showed lowest increase of Na^+ in 250 mM in the range of 53% - 63% compared with 150 mM NaCl. In this result S6 x DNH 40, Dandara x S6 and S6 x Dandara showed salt tolerance on low Na^+ accumulation bases than other genotypes. S6 x DNH 40 also showed a low Na^+ accumulation in the young petiole (Fig. 3.2.3.8). It was observed from the data presented from Table 1 (see Appendix) that the Na^+ concentration in young petiole showed highly significant positive correlation with Na^+ in young stem and significant positive correlation with K^+ in young stem at 15 day x 150 mM. It was observed from the data presented from Table 2 (see Appendix) that the Na^+ concentration in young petiole showed highly significant positive correlation with Na^+ in young stem and K^+/Na^+ ratio in old leaf and significant correlation with K^+ in old leaf. However, highly significant negative correlation was observed with K^+ in young petiole and stem and K^+/Na^+ ratio in young petiole and stem at 15 day x 250 mM.

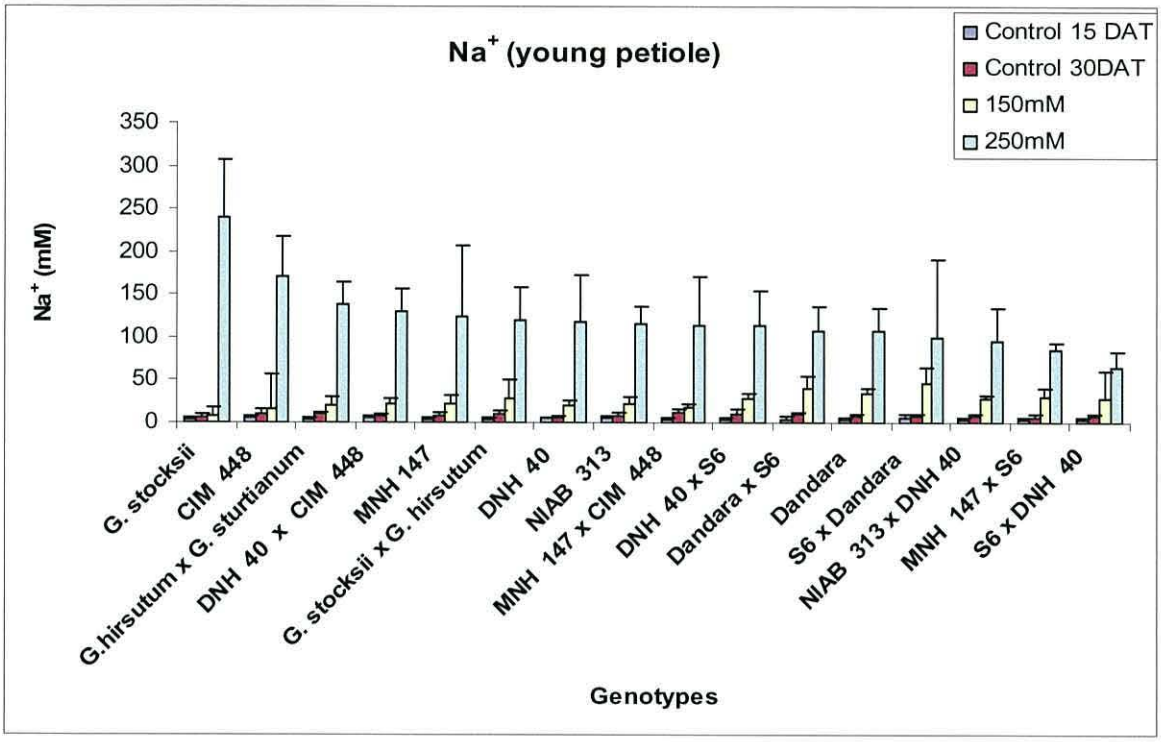

Fig.3.2.3.8. Effect of 150 followed by 250 mM NaCl on Na^+ concentration in young petiole of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd. DAT= days after treatments (see in Appendix Table 7 and 8).

3.2.3.9. K^+ concentration in the young petiole.

The effect of salt after 15 days of 150 mM salt treatment and after a further 15 days of 250 mM salt treatment on K^+ concentration in young petiole was significant ($P<0.000$) (see Appendix Table 3 and 4). The young petiole K^+ showed a relative difference was in the range of -12% - 53% and -3% - 42% at the end of the 150 - 250 mM NaCl treatment periods (Fig.3.2.3.9). Significant ($P<0.000$) differences were observed between genotypes. The interaction between treatments and genotypes was not significant in (15days x 150 mM: 30days x 250 mM). In control plants the young petiole K^+ showed an increase with increasing age of plants. Most genotypes showed an increase in leaf K^+ except for MNH 147 x S6 in 150 mM compared with control plants. However, most of genotypes showed increase young petiole K^+ with increasing salt level, except for *G. stocksii* which decreased (9%) compared with 150 mM. The relative differences showed between 150 – 250 mM in the range of 7% - 53%. The highest differences were observed between MNH 147 x S6, Dandara, DNH 40 x S6 and S6 x DNH 40 in the range of 42% - 53% and the lowest differences were observed between *G.hirsutum x G.sturtainum*, MNH 147, CIM 448 and MNH 147 x CIM 448 in the range of 7% - 19%. It was observed from the data presented from Table 2 (see Appendix) that the K^+ concentration in young petiole showed highly significant positive correlation with K^+ in young stem and K^+/Na^+ ratio in young petiole and significant positive correlation with K^+/Na^+ ratio in young stem. However, there was highly significant negative correlation with K^+/Na^+ ratio in young leaf and significant negative correlation with K^+/Na^+ ratio in old leaf at 15 day x 250 mM.

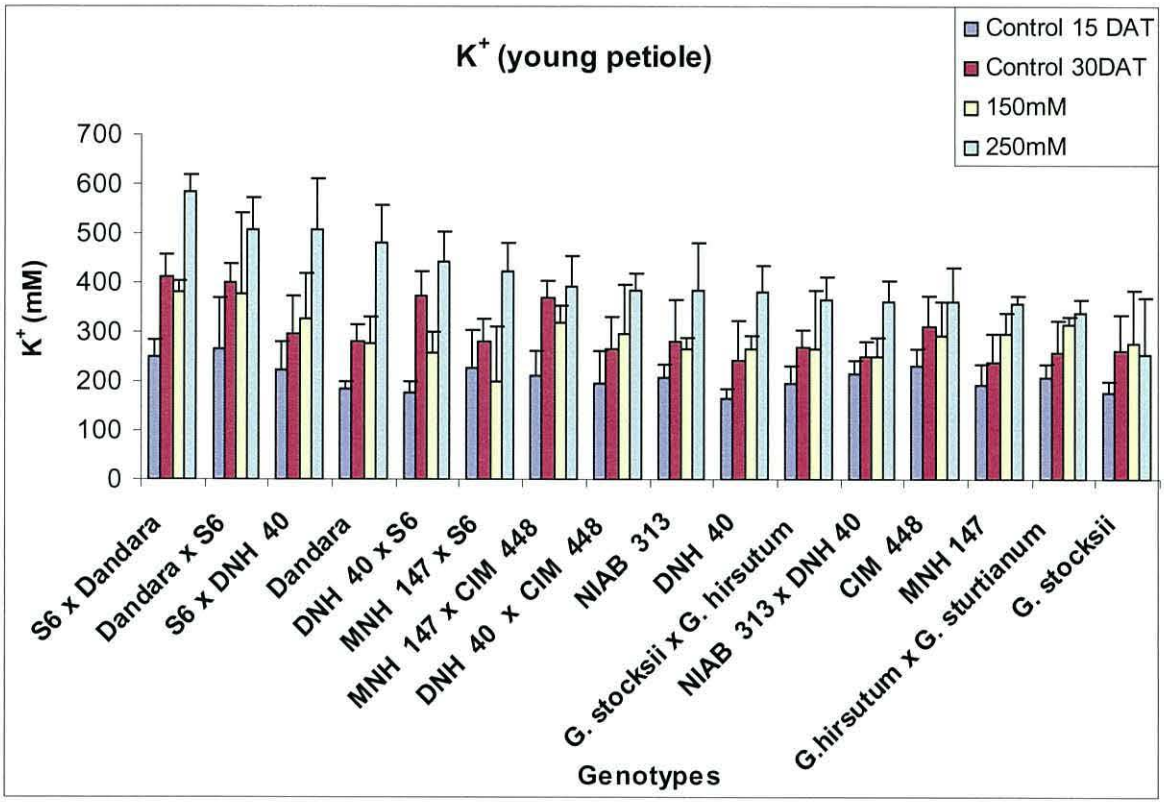

Fig.3.2.3.9. Effect of 150 followed by 250 mM NaCl on K^+ concentration in young petiole of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd. DAT= days after treatments (see in Appendix Table 9 and 10).

3.2.3.10. K^+/Na^+ ratio in the young petiole.

The effect of salt after 15 days of 150 mM salt treatment and after a further 15 days of 250 mM salt treatment reduced K^+/Na^+ ratio significantly ($P<0.000$) (see Appendix Table 3 and 4). The young petiole K^+/Na^+ ratio showed a relative difference in the range of 25% - 83% and 59% - 91 under both stresses of NaCl (Fig. 3.2.3.10). No significant ($P = 0.372$) differences were observed between genotypes. The interaction between treatments and genotypes was not significant in (15days x 150 mM: 30days x 250 mM). In control plants, the young petiole K^+/Na^+ ratio showed a decrease with increasing age of plants in most of genotypes except for S6 x Dandara, DNH 40 and *G. stocksii*. In salt treatments, the highest K^+/Na^+ ratio was observed in 150 mM treatment. Most of the genotypes showed the highest increase in 150 mM in the range of 27% - 97% compared with 250 mM. However, NIAB 313 x DNH 40 showed an increase in young petiole K^+/Na^+ ratio (47%) in high salt stress (250mM).The genotypes CIM 448, MNH 147 x CIM 448, *G. stocksii* and *G.hirsutum x G.sturtainum* showed the highest increase of K^+/Na^+ ratio 81% - 97% in 150 mM compared with 250 mM NaCl. These genotypes did not maintain the same ratio in high salt. Whereas the genotypes DNH 40 x S6, Dandara x S6, Dandara, S6 x DNH 40, S6 x Dandara and MNH 147 x S6 showed the lowest relative differences of K^+/Na^+ ratio in 150 mM with a range of 27% - 56% compared with 250 mM NaCl. In this experiment, NIAB 313 x DNH 40, DNH 40 x S6, Dandara x S6, Dandara, S6 x DNH 40, S6 x Dandara and MNH 147 x S6 maintain the highest young petiole K^+/Na^+ ratio in high salt level relative to other genotypes. It was observed from the data presented from Table 1 (see Appendix) that the K^+/Na^+ ratio in young petiole showed highly significant positive correlation with K^+/Na^+ ratio in young stem at 15 day x 150 mM. It was observed from the data presented from Table 2 (see Appendix) that the K^+/Na^+ ratio in young petiole showed highly significant positive correlation with K^+/Na^+ ratio in young stem at 15 day x 250 mM.

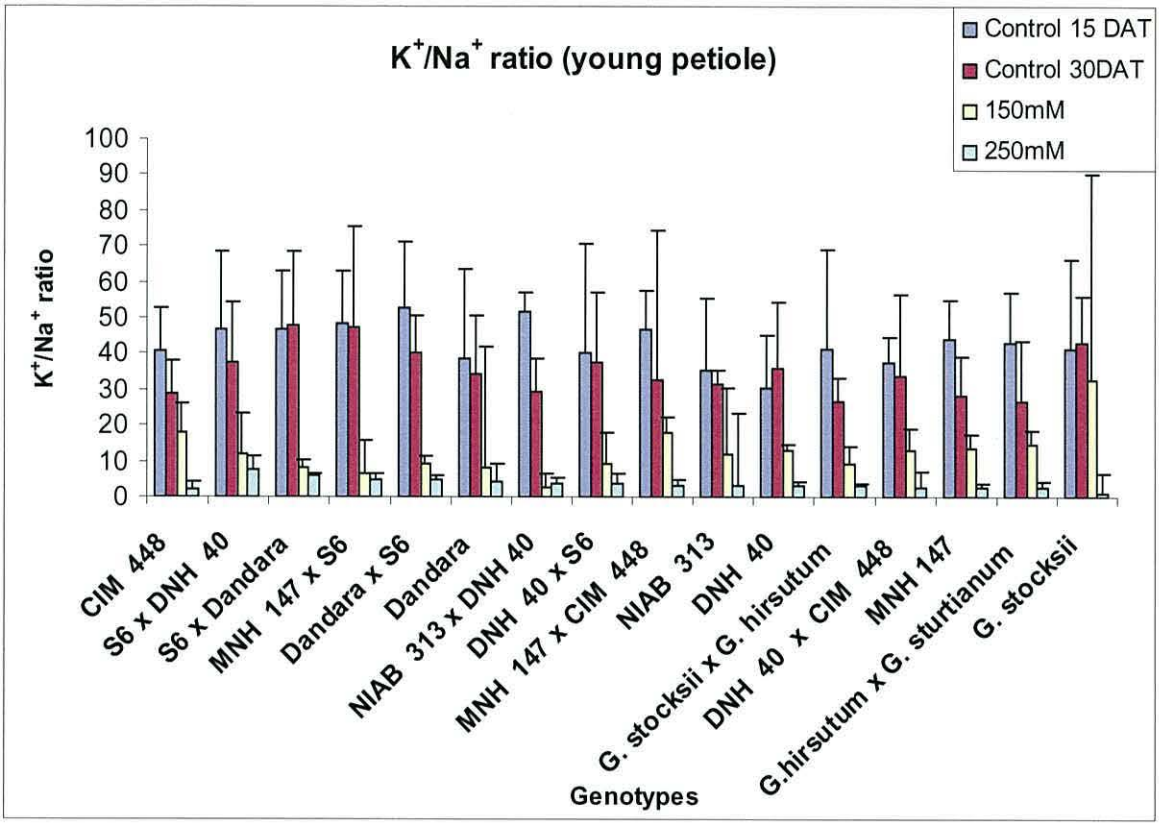

Fig.3.2.3.10. Effect of 150 followed by 250 mM NaCl on K^+/Na^+ ratio in young petiole of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd. DAT= days after treatments (see in Appendix Table 11 and 12).

3.2.3.11. Na^+ concentration in the young stem.

The effect of salt after 15 days of 150 mM salt treatment and after a further 15 days of 250 mM salt treatment increased Na^+ concentration in young stem significantly ($P<0.000$) (see Appendix Table 3 and 4). The young stem showed an increase in Na^+ concentration (44.3% - 92.3%) in the 150 mM treatment. This increased with increasing NaCl stress to 250 mM in the range of 91% - 98% as compared to control plants (Fig. 3.2.3.11). Significant ($P<0.000$) differences were observed between genotypes. The interaction between treatments and genotypes was not significant after 15 days of 150 mM salt treatment; however, the interaction between treatments and genotypes was significant ($P<0.000$) in (30 days x 250 mM). This significance, however, was largely due to the different behaviour of *G. stocksii*. The genotypes CIM 448, MNH 147, NIAB 313, DNH 40, MNH 147 x CIM 448, NIAB 313 x DNH 40, *G. stocksii* and *G.hirsutum* x *G.sturtainum* showed the highest increase of Na^+ 79% - 88% in 250 mM compared with 150 mM NaCl. Whereas the genotype S6 x DNH 40, Dandara, S6 x Dandara showed lowest increase of Na^+ in 250 mM in the range of 46% - 67% compared with 150 mM NaCl. The genotypes S6 x DNH 40 showed low Na^+ in all young plant parts and S6 x Dandara also showed the low Na^+ accumulation in young petiole (Fig. 3.2.3.8).

It was observed from the data presented from Table 1 (see Appendix) that the Na^+ concentration in young stem showed significant positive correlation with K^+ in old leaf and significant negative correlation with K^+/Na^+ ratio in old petiole at 15 day x 150 mM.

It was observed from the data presented from Table 2 (see Appendix) that the Na^+ concentration in young petiole showed highly significant negative correlation with K^+ in young stem and K^+/Na^+ ratio in young petiole and stem at 15 day x 250 mM.

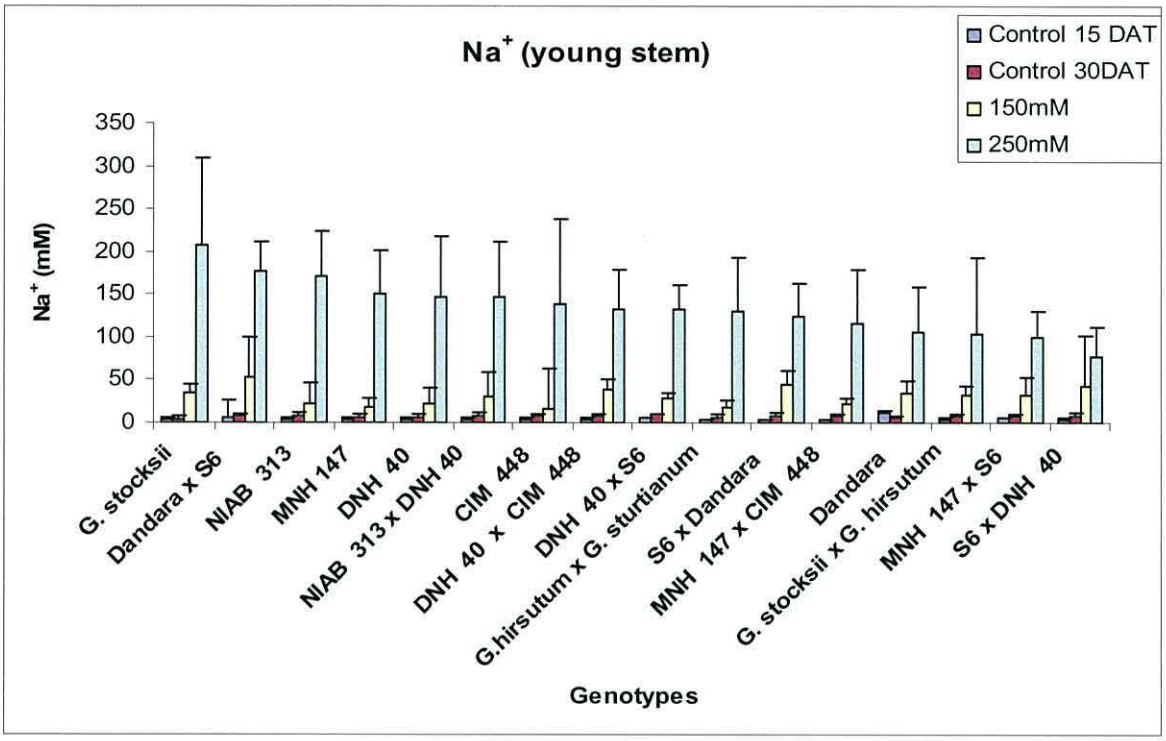

Fig.3.2.3.11. Effect of 150 followed by 250 mM NaCl on Na^+ concentration in young stem of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd. DAT= days after treatments (see in Appendix Table 7 and 8).

3.2.3.12. K^+ concentration in the young stem.

The effect of salt after 15 days of 150 mM salt treatment and after a further 15 days of 250 mM salt treatment in stem K^+ was significant ($P<0.000$) (see Appendix Table 3 and 4). The young stem K^+ showed a relative difference were in the range of -4% - 45% and -2% - 66% at the end of 150 - 250 mM NaCl treatment periods (Fig.3.2.3.12). Significant ($P<0.000$) differences were observed between genotypes. The interaction between treatments and genotypes was not significant after 15 days of 150 mM salt treatment; however, the interaction between treatments and genotypes was significant ($P<0.000$) in (30 days x 250 mM). In control plants, the young stem K^+ showed an increase with increasing age of plants in most genotypes except for Dandara x S6. Most genotypes except for *G. stocksii* showed an increase in young stem K^+ in the additional 250 mM compared with control plants. However, most of genotypes showed an increase in young stem K^+ with increasing salt level, except of *G.hirsutum* x *G.sturtainum* and *G. stocksii* which decreased 13% - 47% from the 150 mM result. The relative differences between both salt stresses were in the range of 4% - 47%. The highest differences were observed between MNH 147 x S6, Dandara and MNH 147 x CIM 448 with a range of 34% - 4% and the lowest differences were observed between NIAB 313, CIM 448, S6, Dandara and Dandara x S6 with a range of 4% - 16%. It was observed from the data presented from Table 1 (see Appendix) that the K^+ concentration in young stem showed significant negative correlation with K^+/Na^+ ratio in old petiole at 15 day x 150 mM. It was observed from the data presented from Table 2 (see Appendix) that the K^+ concentration in young stem showed highly significant positive correlation with K^+/Na^+ ratio in young petiole and stem and highly significant negative correlation with K^+/Na^+ ratio in old leaf at 15 day x 250 mM.

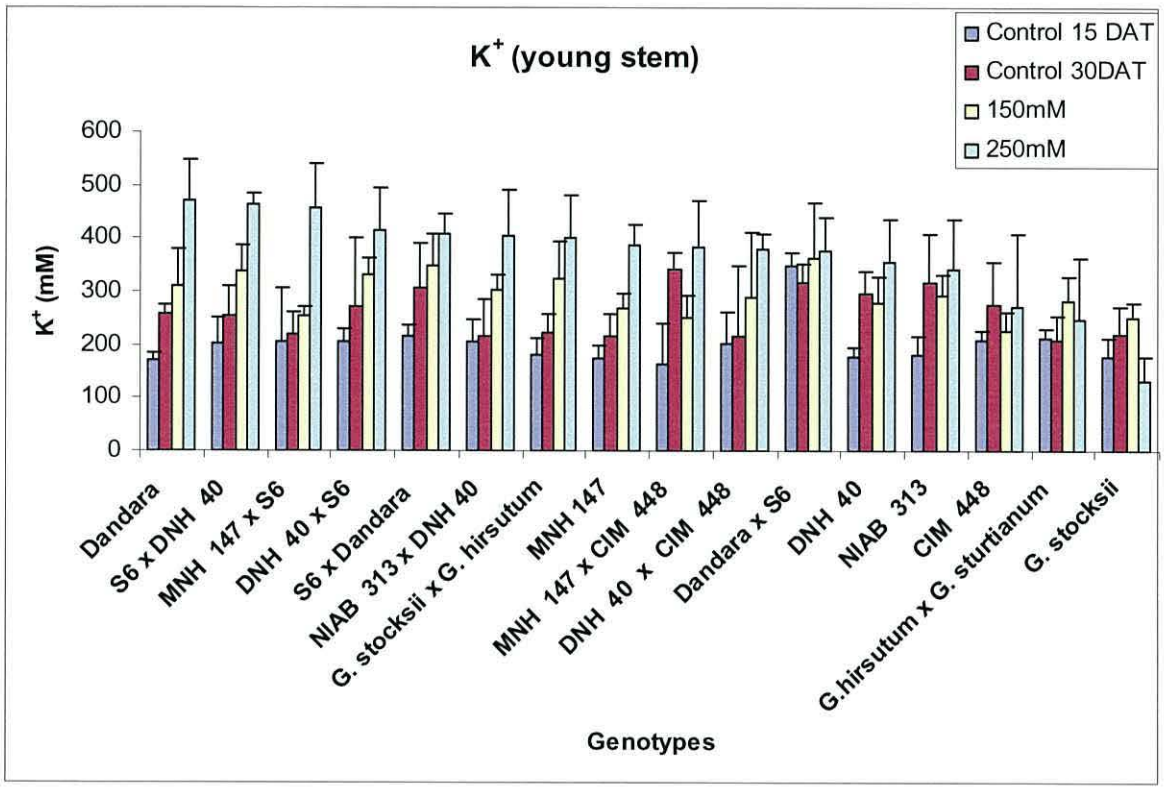

Fig.3.2.3.12. Effect of 150 followed by 250 mM NaCl on K^+ concentration in young stem of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd. DAT= days after treatments (see in Appendix Table 9 and 10).

2.3.13. K^+/Na^+ ratio in the young stem.

The effect of salt after 15 days of 150 mM salt treatment and after a further 15 days of 250 mM salt treatment reduced the K^+/Na^+ ratio significantly ($P<0.000$) (see Appendix Table 3 and 4). The young stem K^+/Na^+ ratio showed a relative difference in the range of 17% - 86% and 79% - 98% for both stresses of NaCl (Fig. 3.2.3.13). No significant ($P = 0.418$) differences were observed between genotypes. The interaction between treatments and genotypes was not significant ($P = 0.831$) in (15days x 150 mM: 30days x 250 mM). In control plants, the young stem K^+/Na^+ ratio showed a decrease with increasing age of plants in most of genotypes except for Dandara and *G. stocksii*. In salt treatments, the highest K^+/Na^+ ratio was observed in 150 mM treatment. All genotypes showed the highest increase in 150 mM in the range of 25% - 91% compared with 250 mM. The genotypes CIM 448, *G. stocksii* and *G.hirsutum* x *G.sturtainum*, NIAB 313, MNH 147 and DNH 40 showed the highest increase of K^+/Na^+ ratio 80% - 91% in 150 mM compared with 250 mM NaCl. These genotypes did not maintain the same ratio in high salt. Whereas the genotypes S6 x DNH 40, Dandara, *G. stocksii* x *G.hirsutum*, S6 x Dandara and MNH 147 x S6 showed the lowest relative differences of K^+/Na^+ ratio in 150 mM with a range of 27% - 56% compared with 250 mM NaCl. In this result S6 x DNH 40, Dandara, *G. stocksii* x *G.hirsutum*, S6 x Dandara and MNH 147 x S6 maintain the highest young stem K^+/Na^+ ratio in high salt level relative to other genotypes.

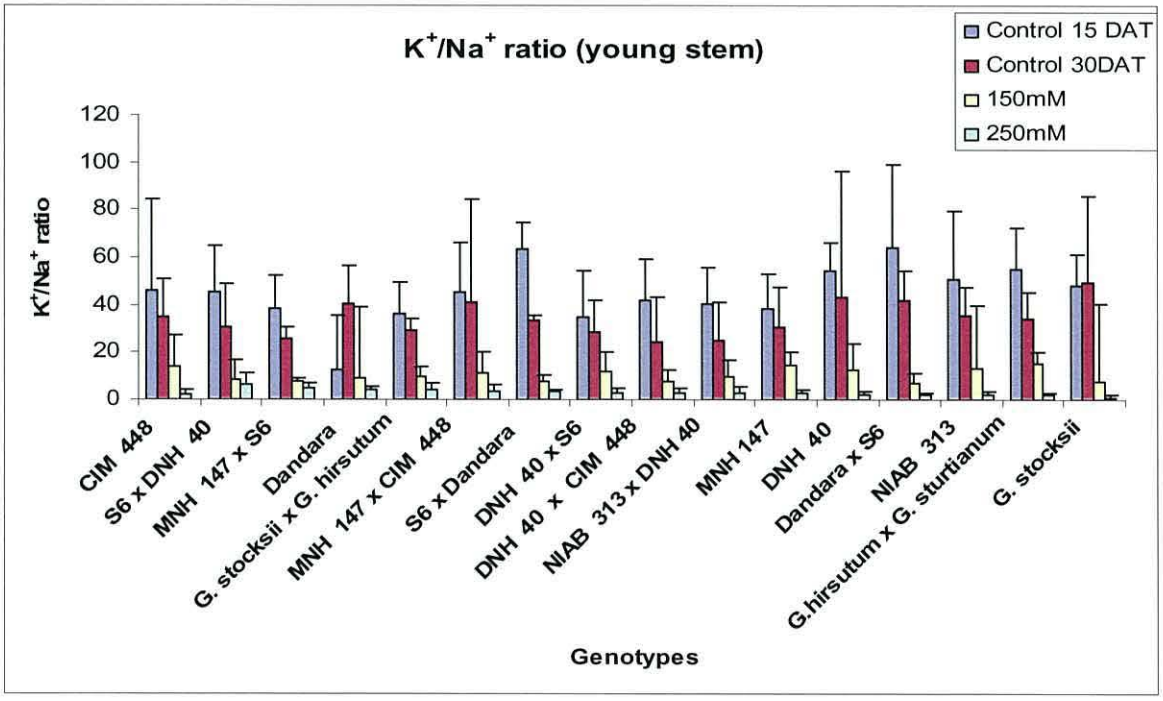

Fig.3.2.3.13. Effect of 150 followed by 250 mM NaCl on K^+/Na^+ ratio in young stem of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd. DAT= days after treatments (see in Appendix Table 11 and 12).

3.2.3.14. Na^+ concentration in the old leaf.

The effect of salt after 15 days of 150 mM salt treatment and after a further 15 days of 250 mM salt treatment increased Na^+ concentration in old leaf significantly (P<0.000) (see Appendix Table 3 and 4). The old leaf showed an increase in Na^+ concentration 38.5% - 84.5% in 150 mM which was increased with increasing NaCl stress to 250 mM in the range of 68% - 93% as compared to control plants (Fig. 3.2.3.14). Significant (P<0.000) differences were observed between genotypes. The interaction between treatments and genotypes was not significant after 15 days of 150 mM salt treatment; however, the interaction between treatments and genotypes was significant (P<0.000) in (30 days x 250 mM). This however, was largely due to the different behaviour of *G. stocksii*. The genotypes CIM 448, MNH 147, NIAB 313, DNH 40, DNH 40 x S6 and *G.hirsutum x G.sturtainum* showed the highest increase of Na^+ 76% - 83% in 250 mM compared with 150 mM NaCl. Whereas the genotypes MNH 147 x CIM 448, Dandara x S6, DNH 40 x CIM 448, *G. stocksii* x *G.hirsutum*, S6 x DNH 40 and MNH 147 x S6 showed the lowest increase of Na^+ in 250 mM in the range of 57% - 67% compared with 150 mM. The genotypes S6 x DNH 40 and MNH 147 x S6 showed low Na^+ accumulation in young leaf.

It was observed from the data presented from Table 1(see Appendix) that the Na^+ concentration in old leaf showed highly significant positive correlation with Na^+ in young leaf and petiole and K^+ in young stem and significant correlation with Na^+ in old petiole. However, highly significant negative correlation was observed with K^+/Na^+ ratio in old leaf, petiole and in young petiole, whilst significant negative correlation was observed with K^+/Na^+ ratio in young leaf at 15 day x 150 mM.

It was observed from the data presented from Table 2 (see Appendix) that the Na^+ concentration in old leaf showed highly significant positive correlation with Na^+ in young leaf and K^+ in young petiole and stem, and significant correlation with K^+/Na^+ ratio in young petiole. However, highly significant negative correlation was observed with K^+ in young leaf and K^+/Na^+ ratio in old and

young leaf, and significant negative correlation was observed Na^+ in young petiole and K^+ in old leaf at 15 day x 250 mM.

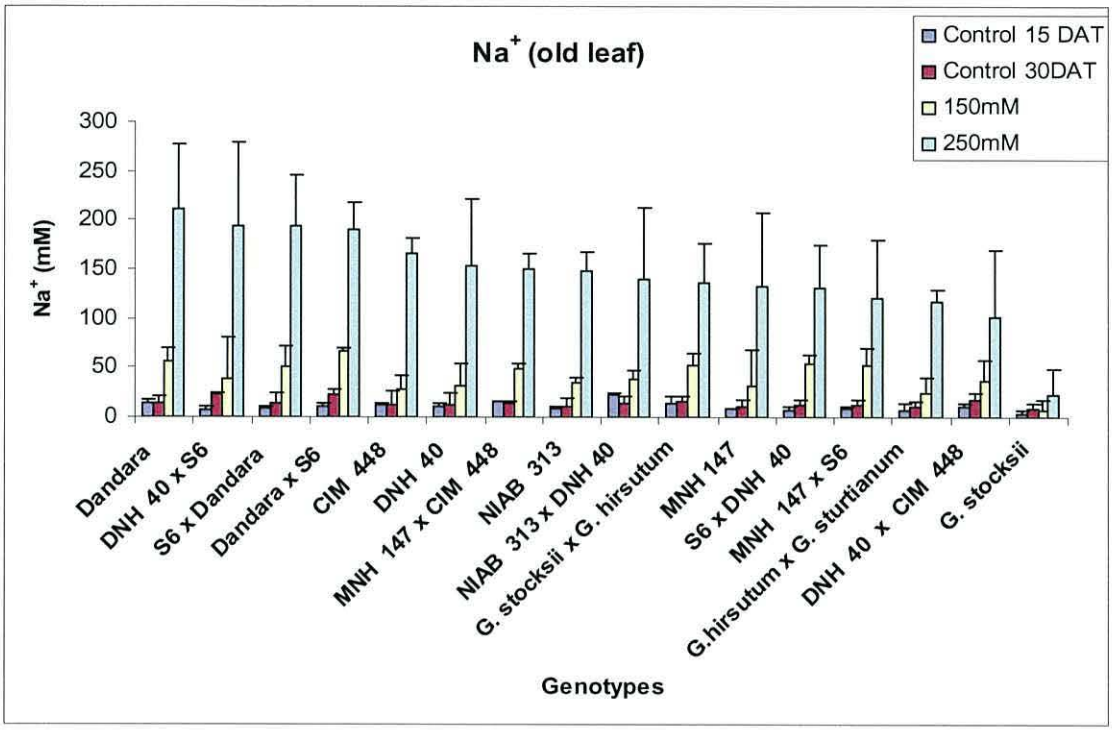

Fig.3.2.3.14. Effect of 150 followed by 250 mM NaCl on Na^+ concentration in old leaf of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd. DAT= days after treatments (see in Appendix Table 7 and 8).

3.2.3.15. K^+ concentration in the old leaf.

The effect of salt after 15 days of 150 mM salt treatment was not significant and after a further 15 days of 250 mM salt treatment on old leaf K^+ was significant ($P<0.000$) (see Appendix Table 3 and 4). The old leaf K^+ showed a relative difference in the range of -0.2% - 96.7% and -18% - 77.4% at the end of 150 - 250 mM NaCl treatment period (Fig.3.2.3.15). Significant ($P<0.000$) differences were observed between genotypes. The interaction between treatments and genotypes was not significant after 15 days of 150 mM salt treatment; however, the interaction between treatments and genotypes was significant ($P<0.000$) in (30 days x 250 mM). In control plants, the old leaf K^+ showed an increase with increasing age of plants in most genotypes except for *G.hirsutum x G.sturtainum* and MNH 147. Most genotypes showed a decrease in leaf K^+ except for *G. stocksii* and *G.hirsutum x G.sturtainum* in both stresses from control plants and S6 x Dandara, Dandara, Dandara x S6, NIAB 313, *G. stocksii* x *G.hirsutum*, and MNH 147 x CIM 448 showed an increase in 150 mM from control. However, most of the genotypes showed a decrease in old leaf K^+ with increasing salt level, except for *G.hirsutum x G.sturtainum* and *G. stocksii,* DNH 40, MNH 147, MNH 147 x S6, NIAB 313 and NIAB 313 x DNH 40 which increase 4% - 29% compared with 150 mM. The relative differences between both salt stresses were in the range of -4% - 66%. The highest differences were observed between *G. stocksii*, *G.hirsutum* x *G.sturtainum,* MNH 147 x S6 and DNH 40 in the range of 21% - 29%. It was observed from the data presented from Table 1 (see Appendix) that the K^+ concentration in old leaf showed significant positive correlation with K^+ in young petiole and significant correlation with K^+/ in young leaf at 15 day x 150 mM.

It was observed from the data presented from Table 2 (see Appendix) that the K^+ concentration in old leaf showed highly significant positive correlation with of K^+/Na^+ ratio in old leaf and highly significant negative correlation with K^+ in old and young stem, and significant negative correlation with K^+/Na^+ ratio in old stem at 15 day x 250 mM.

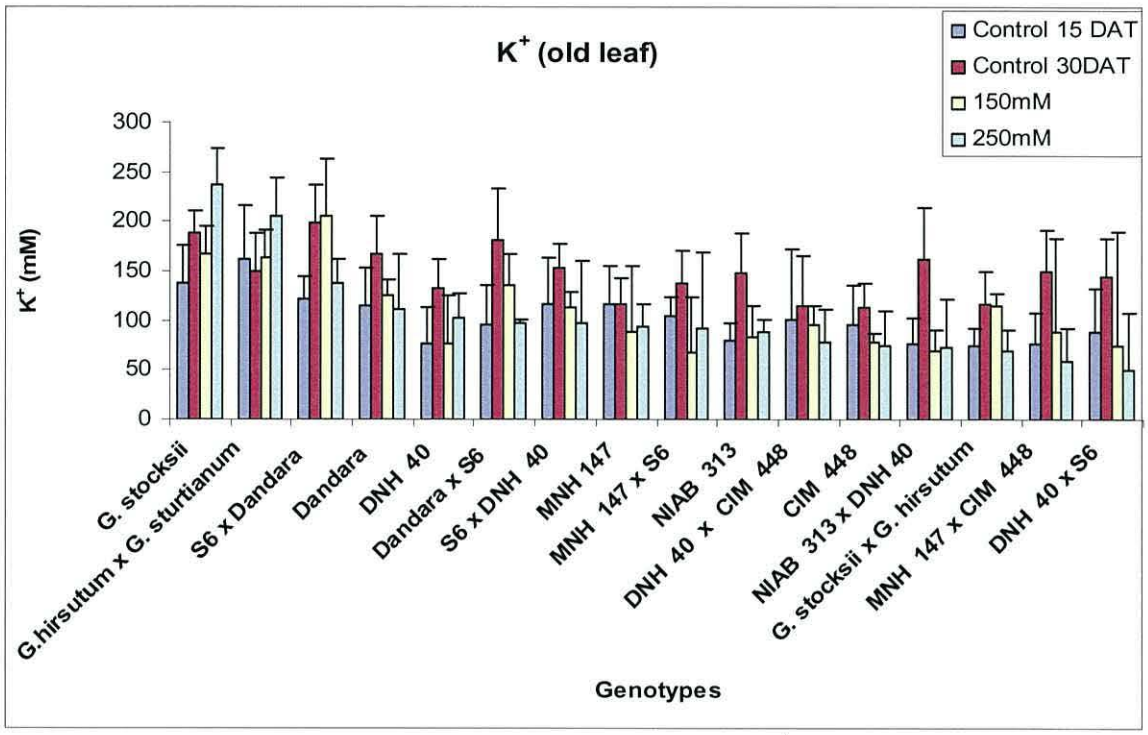

Fig.3.2.3.15. Effect of 150 followed by 250 mM NaCl on K^+ concentration in old leaf of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd. DAT= days after treatments (see in Appendix Table 9 and 10).

3.2.3.16. K^+/Na^+ ratio in the old leaf.

The effect of salt after 15 days of 150 mM salt treatment and after a further 15 days of 250 mM salt treatment reduced the K^+/Na^+ ratio significantly (P<0.000) (see Appendix Table 3 and 4). The old leaf K^+/Na^+ ratio showed a relative difference in the range of 11% - 90% and 33% - 97% under both stresses of NaCl (Fig. 3.2.3.16). Significant (P<0.000) differences were observed between genotypes. The interaction between treatments and genotypes was not significant (P = 0.297) in (15days x 150 mM: 30days x 250 mM). In control plants, the old leaf K^+/Na^+ ratio showed an increase with increasing age of plants in most genotypes except for DNH 40 x CIM 448, S6 x DNH 40, MNH 147, Dandara x S6, DNH 40 x S6, *G.hirsutum* x *G.sturtainum* and *G. stocksii*. In salt treatments, the highest K^+/Na^+ ratio was observed in 150 mM treatment. All genotypes showed the highest increase in 150 mM in the range of 41% - 87% compared with 250 mM. The genotypes CIM 448, DNH 40 x S6, S6 x Dandara showed the highest increase of K^+/Na^+ ratio 82% - 87% in 150 mM compared with 250 mM NaCl. Whereas the genotypes *G. stocksii* and *G.hirsutum* x *G.sturtainum* maintained the old leaf K^+/Na^+ ratio of 60% & 73% compared with 250 mM NaCl. In this result, *G. stocksii* and *G.hirsutum* x *G.sturtainum* maintain the highest old leaf K^+/Na^+ ratio in high salt level than other genotypes. No correlation was observed with any trait in both salt treatments. It was observed from the data presented from Table 1 (see Appendix) that the K^+/Na^+ ratio in old leaf showed highly significant positive correlation with K^+/Na^+ ratio in old petiole and young leaf and petiole at 15 day x 150 mM.

It was observed from the data presented from Table 2 (see Appendix) that the K^+/Na^+ ratio in old leaf showed highly significant positive correlation with K^+/Na^+ ratio in young leaf significant negative correlation with K^+/Na^+ ratio in young stem at 15 day x 250 mM.

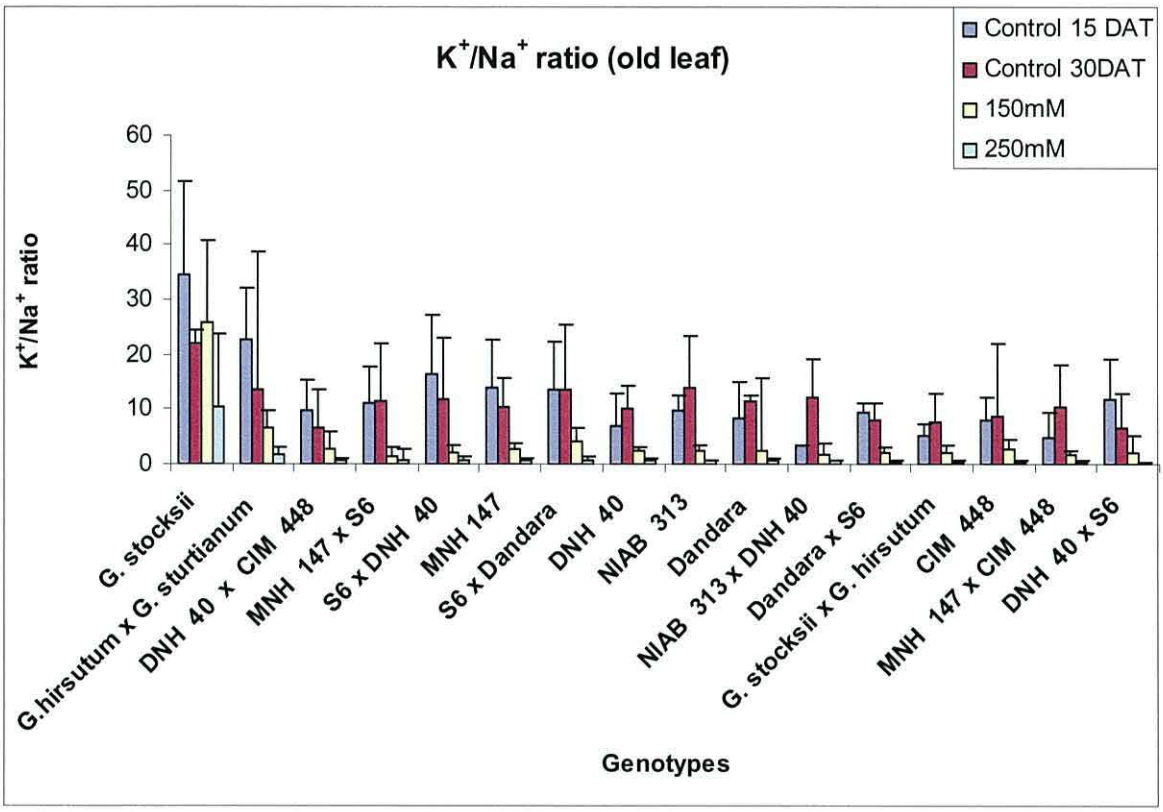

Fig.3.2.3.16. Effect of 150 followed by 250 mM NaCl on K^+/Na^+ ratio in old leaf of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd. DAT= days after treatments (see in Appendix Table 11 and 12).

3.2.3.17. Na^+ concentration in the old petiole.

The effect of salt after 15 days of 150 mM salt treatment and after a further 15 days of 250 mM salt treatment increased Na^+ concentration in old petiole significantly ($P<0.000$) (see Appendix Table 3 and 4). The old petiole showed an increase in Na^+ concentration (58% - 91%) at 150 mM was increased with increasing NaCl stress to 250 mM in the range of 87% - 97% as compared to control plants (Fig. 3.2.3.17). No significant differences were observed between genotypes. The interaction between treatments and genotypes was not significant ($P = 0.206$) in (15days x 150 mM: 30days x 250 mM). The genotypes CIM 448, MNH 147, DNH 40 and *G. stocksii* showed the highest increase of Na^+ 77% - 82% in 250 mM compared with 150 mM NaCl. Whereas the genotypes MNH 147 x CIM 448, Dandara, Dandara x S6, S6 x Dandara, *G. stocksii x G.hirsutum*, S6 x DNH 40 and MNH 147 x S6 showed lowest increase of Na^+ in 250 mM with a range of 51% - 67% compared with 150 mM. The genotype S6 x DNH 40 showed the similar response in all young parts and old leaf and MNH 147 x S6 also showed similar result in young and old leaf. Dandara, Dandara x S6, S6 x Dandara had lower Na^+ in young petiole and MNH 147 x CIM 448 and *G. stocksii* x *G.hirsutum* showed a similar response in old leaf. It suggests that the salt tolerance of genotypes is due to low Na^+ accumulation.

It was observed from the data presented from Table 1 (see Appendix) that the Na^+ concentration in old petiole showed highly significant positive correlation with Na^+ in young leaf, petiole and stem at 15 day x 150 mM.

It was observed from the data presented from Table 2 (see Appendix) that the Na^+ concentration in old petiole showed highly significant positive correlation with Na^+ in old stem and K^+/Na^+ ratio in old leaf and significant correlation with Na^+ in young petiole. However, highly significant negative correlation was observed with K^+ in old stem and K^+/Na^+ ratio in old stem at 15 day x 250 mM.

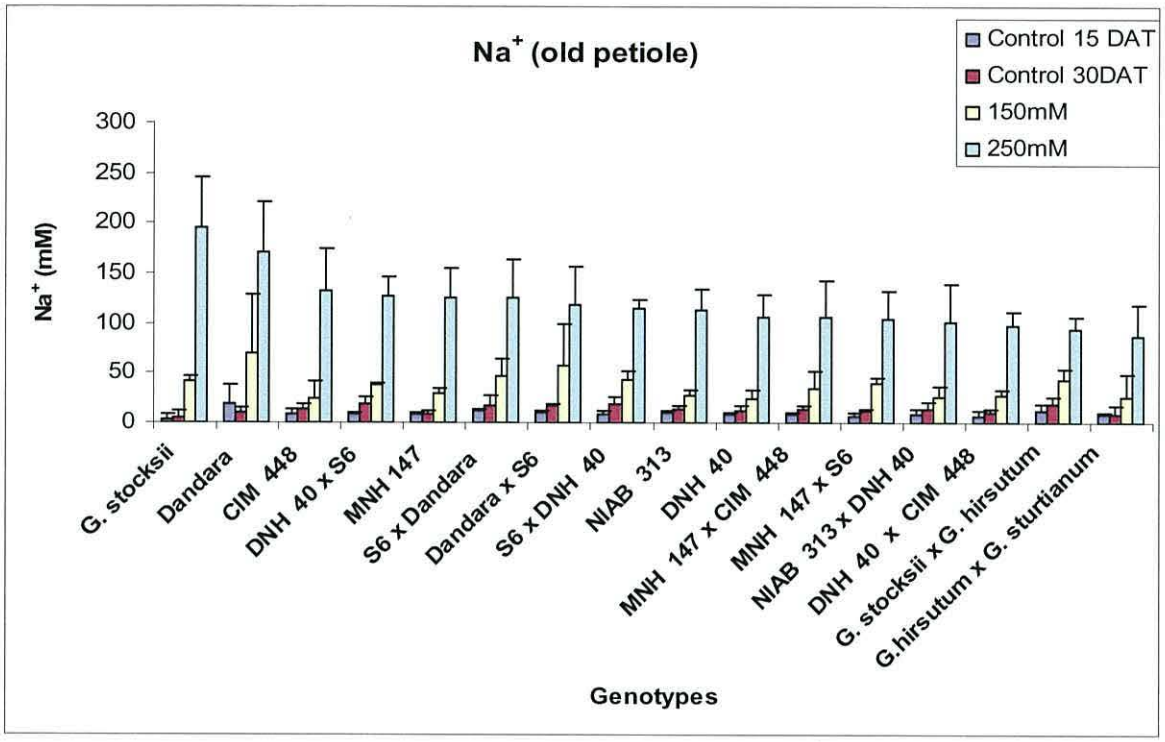

Fig.3.2.3.17. Effect of 150 followed by 250 mM NaCl on N^+ concentration in old petiole of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd. DAT= days after treatments (see in Appendix Table 7 and 8).

3.2.3.18. K^+ concentration in the old petiole.

The effect of salt after 15 days of 150 mM salt treatment and after a further 15 days of 250 mM salt treatment in old petiole K^+ was significant (P<0.000) (see Appendix Table 3 and 4). The old petiole K^+ showed the relative difference in the range of 6.4% - 53% and –0.4% - 34% at the end of 150 - 250 mM NaCl treatment period (Fig.3.2.3.18). Significant (P<0.000) differences were observed between genotypes. The interaction between treatments and genotypes was not significant (P = 0.637) in (15days x 150 mM: 30days x 250 mM). In control plants, the petiole K^+ showed an increase with increasing the age of plants in most of genotypes except for DNH 40 x CIM 448. Most of genotypes showed an increase in petiole K^+ except for MNH 147 x CIM 448, *G. stocksii* and DNH 40 x S6 in 150 mM from control plants. However, most of genotypes showed decrease in old leaf K^+ with increasing salt level, except for DNH 40 x S6 which decrease (1.8%) from 150 mM. The relative differences between both salt stresses were in the range of -1.8% - 47.5%. The highest differences were observed between *G. stocksii* x *G.hirsutum*, Dandara and S6 x DNH 40 in the range of 47.5% - 38.7%. The lowest differences were observed between NIAB 313, MNH 147, DNH 40 x CIM 448 and MNH 147 x S6 in the range of 9.7% - 15%.

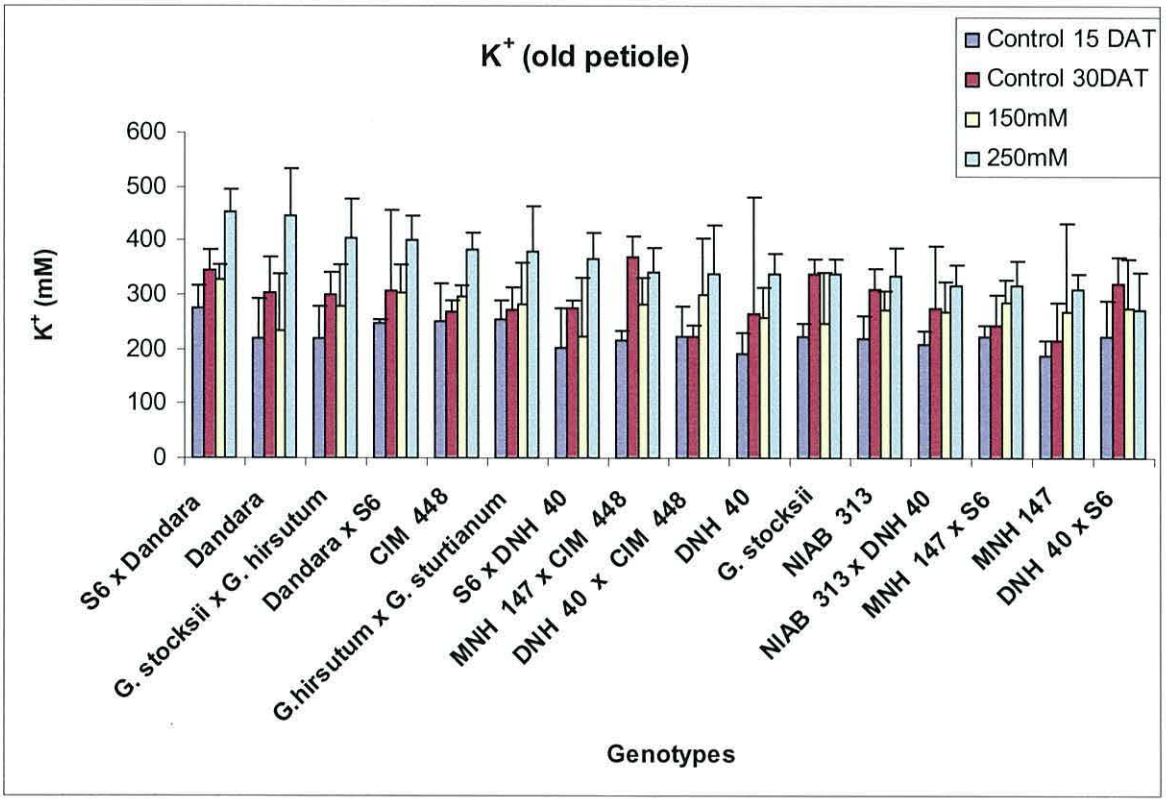

Fig.3.2.3.18. Effect of 150 followed by 250 mM NaCl on K^+ concentration in old petiole of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd. DAT= days after treatments (see in Appendix Table 9 and 10).

3.2.3.19. K^+/Na^+ ratio in the old petiole.

The effect of salt after 15 days of 150 mM salt treatment and after a further 15 days of 250 mM salt treatment reduced the K^+/Na^+ ratio significantly ($P<0.000$) (see Appendix Table 3 and 4). The old petiole K^+/Na^+ ratio showed the relative difference with a range of 51% - 78% and 87% - 97% by both stresses of NaCl (Fig. 3.2.3.19). Significant ($P<0.000$) differences were observed between genotypes. The interaction between treatments and genotypes was not significant after 15 days of 150 mM salt treatment; however, the interaction between treatments and genotypes was significant ($P<0.000$) in (30 days x 250 mM). In control plants, the old petiole K^+/Na^+ ratio showed a decrease with increasing the age of plants in most of genotypes except for NIAB 313, Dandara, MNH 147 and *G. stocksii*.

In salt treatments, the highest K^+/Na^+ ratio was observed in 150 mM treatment. All genotypes showed the highest increase in 150 mM with a range of 21% - 77% compared with 250 mM. The genotypes CIM 448, MNH 147, *G. stocksii*, DNH 40, NIAB 313, NIAB 313 x DNH 40, *G.hirsutum* x *G.sturtainum* and DNH 40 x S6 showed highest increase of K^+/Na^+ ratio 70% - 77% in 150 mM compared with 250 mM NaCl. In this result CIM 448, *G. stocksii* and *G.hirsutum* x *G.sturtainum* maintain the highest old petiole K^+/Na^+ ratio in the range of 61% & 71% at high salt level relative to other genotypes. It was observed from the data presented from Table 1 (see Appendix) that the K^+/Na^+ ratio in old petiole showed highly significant positive correlation with K^+/Na^+ ratio in young leaf and petiole and significant correlation with K^+/Na^+ ratio in young stem at 15 day x 150 mM.

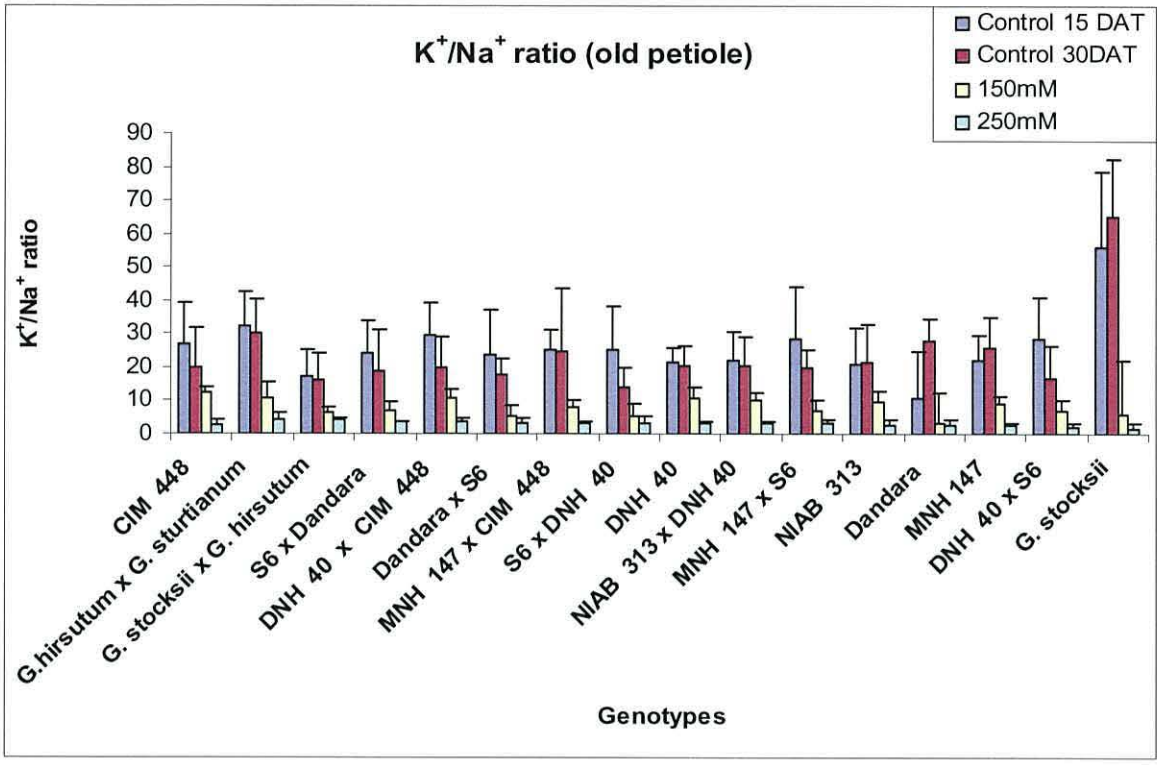

Fig.3.2.3.19. Effect of 150 followed by 250 mM NaCl on K^+/Na^+ ratio in old petiole of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd. DAT= days after treatments (see in Appendix Table 11 and 12).

3.2.3.20. Na^+ concentration in the old stem.

The effect of salt after 15 days of 150 mM salt treatment and after a further 15 days of 250 mM salt treatment increased Na^+ concentration in old stem significantly (P<0.000) (see Appendix Table 3 and 4). The old stem showed an increase in Na^+ concentration (74% - 92%) by 150 mM was increased with increasing NaCl stress to 250 mM in the range of 65.7% - 96.5% as compared to control plants (Fig. 3.2.3.20). Significant (P<0.000) differences were observed between genotypes. The interaction between treatments and genotypes was not significant after 15 days of 150 mM salt treatment; however, the interaction between treatments and genotypes was significant (P<0.000) in (30 days x 250 mM). This significance, however, was entirely due to the different behaviour of *G. stocksii*. The genotypes Dandara, DNH 40 x S6, *G. stocksii* x *G.hirsutum* and *G. stocksii* showed the highest increase of Na^+ 53% - 56% in the additional 250 mM compared with 150 mM NaCl. Whereas the genotypes MNH 147 x CIM 448, DNH 40 x CIM 448, *G. stocksii* x *G.hirsutum*, Dandara x S6, NIAB 313, CIM 448, MNH 147 and S6 x DNH 40 showed lowest increase of Na^+ in 250 mM in the range of 1% - 41% and MNH 147 x S6 and S6 x Dandara showed a decrease in Na^+ concentration (13% &17%) compared with 150 mM. This result suggests that MNH 147 x S6 and S6 x Dandara are salt tolerant genotypes due to low Na^+ accumulation.

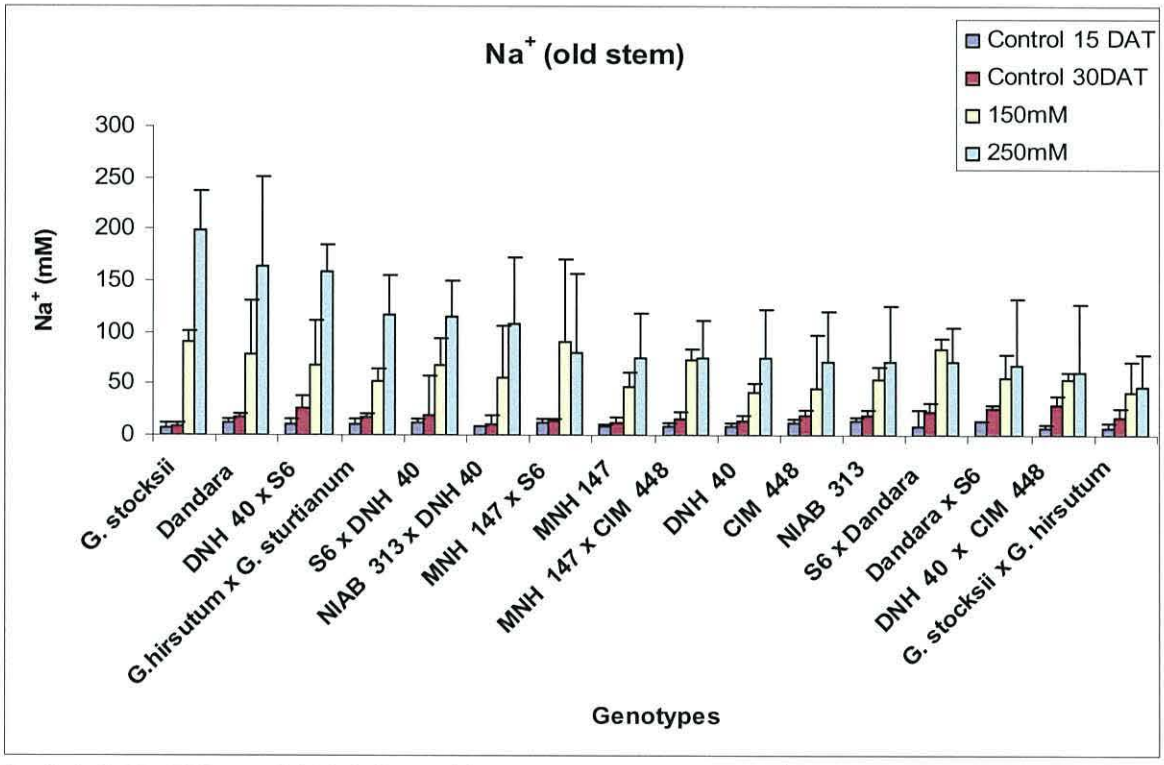

Fig.3.2.3.20. Effect of 150 followed by 250 mM NaCl on Na^+ concentration in old stem of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd. DAT= days after treatments (see in Appendix Table 7 and 8).

3.2.3.21. K^+ concentration in the old stem.

The effect of salt after 15 days of 150 mM salt treatment and after a further 15 days of 250 mM salt treatment in old stem K^+ was significant (P<0.000) (see Appendix Table 3 and 4). The old stem K^+ showed relative differences in the range of -4% - 36.6% and –7% - 61% at the end of the 150 - 250 mM NaCl treatment periods (Fig.3.2.3.18). Significant (P<0.000) differences were observed between genotypes in both treatments. The interaction between treatments and genotypes was significant (P <0.000) in (30days x 250 mM). In control plants, the old stem K^+ showed an increase with increasing the age plants in most of genotypes except for MNH 147, MNH 147 x S6 and Dandara. Most genotypes showed an increase in old stem K^+ except Dandara x S6 and *G. stocksii* in 150 mM and MNH 147 x CIM 448, DNH 40 x CIM 448, Dandara and S6 x Dandara in 250 mM compared with control plants. However, most genotypes showed an increase in old stem K^+ with increasing salt level, except for NIAB 313, Dandara, Dandara x S6, S6 x Dandara and *G. stocksii* which decrease (1% - 37%) compared with 150 mM. The relative differences between both stresses were in the range of -1% - 29%. The highest differences were observed between MNH 147 x CIM 448, NIAB 313 x DNH 40 and DNH 40 in the range of 23% - 29%. The lowest differences were observed between DNH 40 x CIM 448, *G.hirsutum x G.sturtainum* and MNH 147 in the range of 2% - 5%. It was observed from the data presented from Table 1 (see Appendix) that the K^+ concentration in old stem showed highly significant negative correlation with K^+/Na^+ ratio in old leaf and young petiole and significant negative correlation with K^+ in young leaf and at 15 day x 150 mM.

It was observed from the data presented from Table 2 (see Appendix) that the K^+ concentration in old stem showed highly significant positive correlation with of K^+/Na^+ ratio in old stem and significant negative correlation with K^+/Na^+ ratio in old leaf at 15 day x 250 mM.

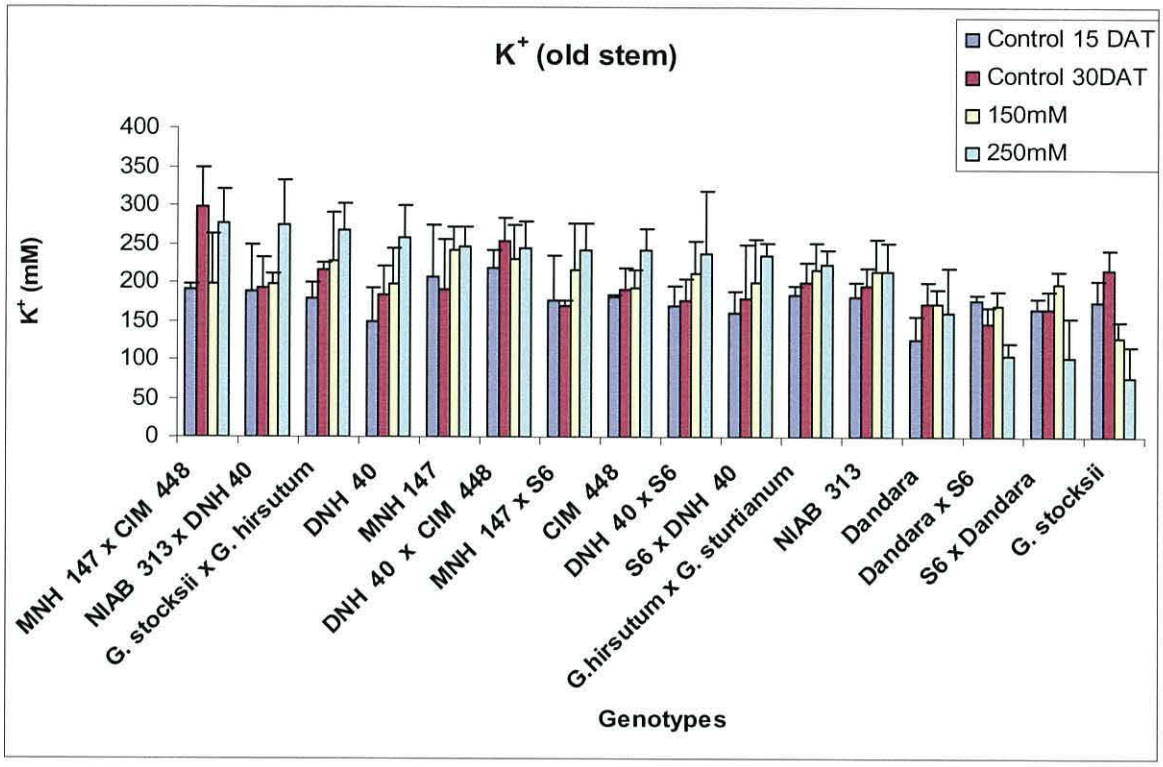

Fig.3.2.3.21. Effect of 150 followed by 250 mM NaCl on K^+ concentration in old stem of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd. DAT= days after treatments (see in Appendix Table 9 and 10).

3.2.3.22. K^+/Na^+ ratio in the old stem.

The effect of salt after 15 days of 150 mM salt treatment and after a further 15 days of 250 mM salt treatment reduced the K^+/Na^+ ratio significantly ($P<0.000$) (see Appendix Table 3 and 4).. The old stem K^+/Na^+ ratio showed a relative difference in the range of 51% - 78% and 87% - 97% under both stresses of NaCl (Fig. 3.2.3.22). Significant ($P<0.000$) differences were observed between genotypes in 30 day x 250 mM. The interaction between treatments and genotypes was not significant in (15days x 150 mM: 30days x 250 mM). In control plants, the old stem K^+/Na^+ ratio showed a decrease with increasing age of plants in most genotypes except for Dandara and *G. stocksii*.

In salt treatments, the highest K^+/Na^+ ratio was observed in 150 mM treatment. Most genotypes showed the highest increase in 150 mM in the range of 4% - 72% compared with 250 mM. The genotypes *G. stocksii*, Dandara, *G.hirsutum* x *G.sturtainum*, DNH 40 x S6 40 and Dandara x S6 showed highest increase of K^+/Na^+ ratio 50% - 72% in 150 mM compared with 250 mM NaCl. Whereas the genotypes *G. stocksii* x *G.hirsutum,* MNH 147 x S6 and MNH 147 x CIM 448 showed the highest increase in 250 mM with a range of (6% - 39%). In this result CIM 448, MNH 147, DNH 40, NIAB 313, DNH 40 x CIM 448, *G. stocksii* x *G.hirsutum* and MNH 147 x S6 maintained the highest old stem K^+/Na^+ ratio in high salt level than other genotypes.

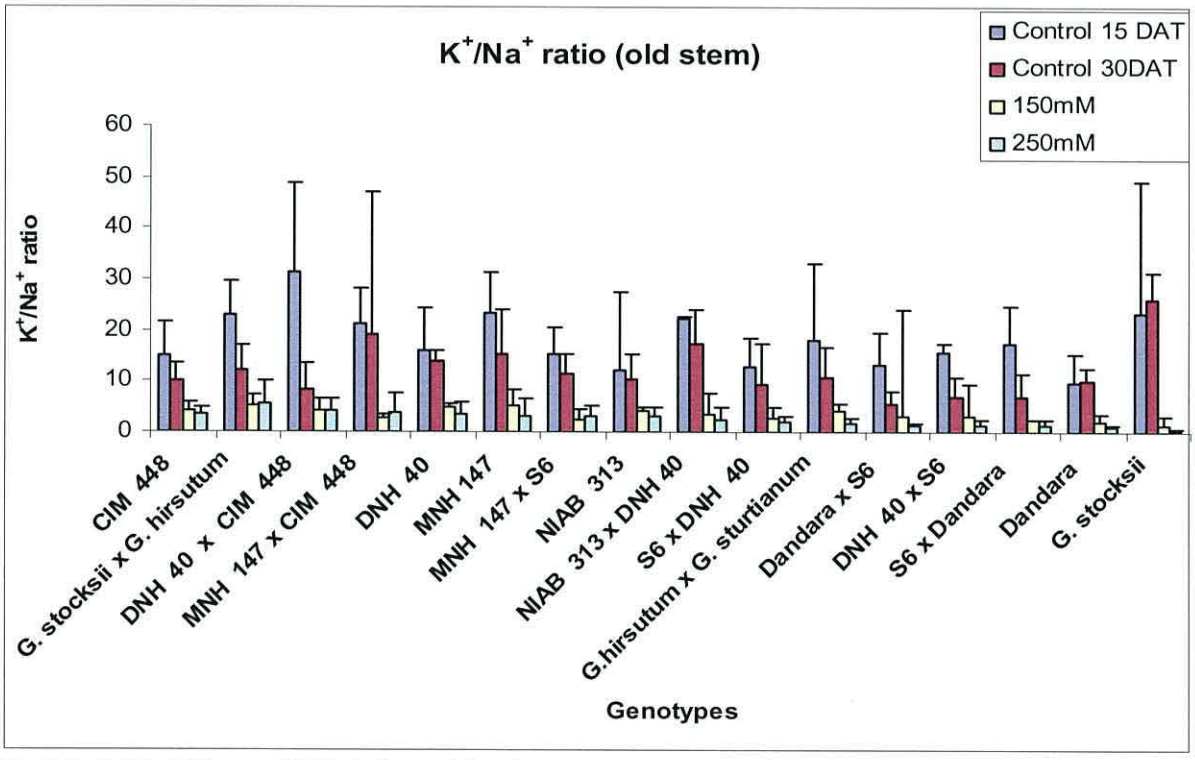

Fig.3.2.3.22. Effect of 150 followed by 250 mM NaCl on K^+/Na^+ ratio in old stem of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd. DAT= days after treatments (see in Appendix Table 11 and 12).

3.2.5. Principal component analysis of phenotypes.

Application of PCA to all the mean values of twelve physiological parameters (plant height, fresh weight, and dry weight, Na^+ concentration in all young part and old leaf and stem, K^+ concentration in young petiole and stem and K^+/Na^+ ratio in young leaf and old petiole) generated Fig.3.2.5.1. This reveals several grouping according to physiological trait bases. The clearest are generated by PC1 and PC2 (which accounts for 89% and 28% of the total variance respectively). Here we see in PC1, the genotypes NIAB 313 x DNH 40, MNH 147 x CIM 448, *G. stocksii x G.hirsutum*, DNH 40 x CIM 448, S6 x DNH 40, MNH 147, DNH 40 x S6, Dandara, S6 x Dandara and Dandara x S6 (group I) grouping apart from DNH 40, MNH 147 x S6, *G.hirsutum* x *G.sturtainum*, NIAB 313, CIM 448 and *G. stocksii* (group II). Principle component two (PC2) shows the genotypes NIAB 313 x DNH 40, MNH 147 x CIM 448, *G. stocksii x G.hirsutum*, DNH 40 x CIM 448, S6 x DNH 40, MNH 147, DNH 40, MNH 147 x S6, *G.hirsutum* x *G.sturtainum* and NIAB 313 (group I) and DNH 40 x S6, Dandara, S6 x Dandara, Dandara x S6, CIM 448 and *G. stocksii* (group II).

Principal component 1 (PC1) is a function of plant height, fresh and dry weight, Na^+ in young and old leaf and K^+ in young petiole and stem, which shows the positive correlation and in this component Na^+ in young petiole and in young and old stem, K^+/Na^+ ratio in young leaf and old petiole shows the negative correlation. The Ward Linkage correlation coefficient distance for twelve individual parameters is shown in Fig.3.2.5.2. The genotypes were grouped into three main clusters. It is, perhaps, cluster one that showed an expected results – for example cluster one include responses of plant height, fresh weight, dry weight, Na^+ in young and old leaf, K^+ in young petiole and stem and K^+/Na^+ ratio in young leaf and old petiole being dependent of each other, and cluster two is a response of Na^+ in young petiole and stem due to low accumulation of Na^+ in genotypes Dandara, Dandara x S6, S6 x Dandara, S6 x DNH 40 and DNH 40 x S6 (Fig. 3.2.3.8 &

3.2.3.11) and cluster three is a result of Na^+ in old stem, due to the high uptake of Na^+ in old stem (Fig. 3.2.3.20).

The SSR analysis provides evidence of seven main clusters of varieties at the root of the dandrogram. One cluster includes CIM 448, DNH 40 and Dandara, the second includes MNH 147 and DNH 40 x CIM 448. NIAB 313 is includes in a third cluster. In this analysis, the one cross involving parents from the same cluster (CIM 448 x DNH 40) maps far from both parents (CIM 448 and DNH 40). (SSR analysis was not applied to another crosses used in this analysis (CIM 448 x MNH 147 and NIAB 313 x CIM 448).

Comparison of the PCA groups I and II with the genetic dendrogram is interesting. Members of the CIM 448 and DNH 40 clusters are found in same groups, but the cross of both was in another group in both SSR and physiological study. Dandara was responding differently due to uptake of Na^+ from CIM 448 and DNH 40, but genetically it was close to both varieties. Two crosses included in group I have one parent in the same group (Dandara). The second parent (S6) was not available for this study. Moreover, the MNH 147 and their cross with CIM 448 are found in another clade close to each other, in SSR analysis the cross was not used. (DNH 40 x S 6 and S 6 x DNH 40) crosses are found in another clade from which one parent is missing.

Generating a relationship map based on the physiology (Fig.3.2.5.1) confirms a relationship between SSR dendrogram (Fig. 4.3.3.1) between CIM 448 and DNH 40, but not between these and NIAB 313.

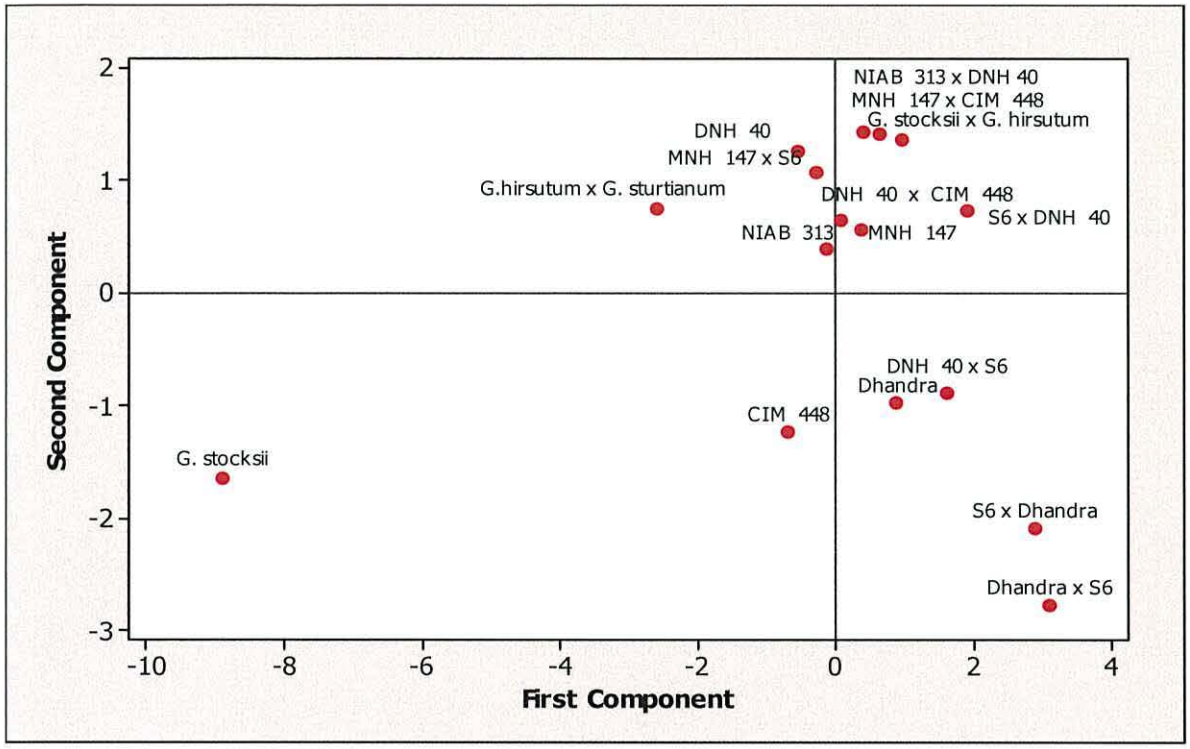

Fig 3.2.5.1. Scatter plot of genotypes based on principal component analysis of physiological traits.

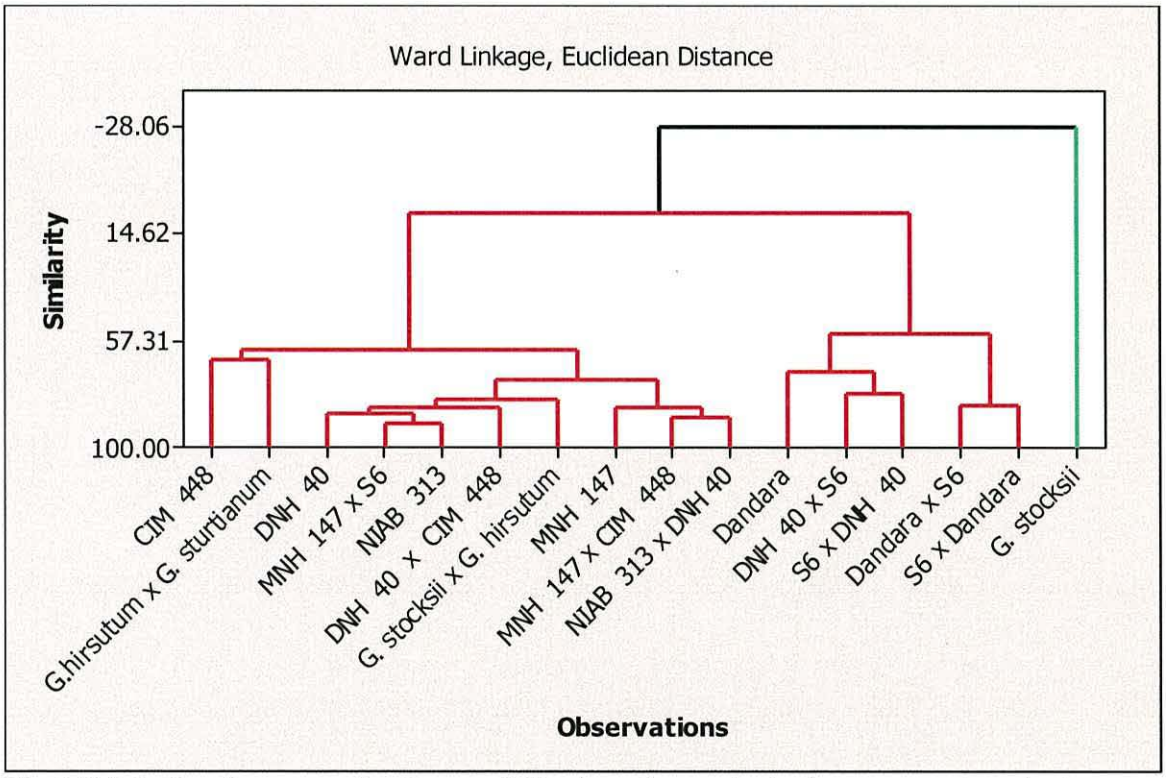

Fig 3.2.5.2. Dendrogram of cotton varieties based on principal component analysis generated by significant physiological traits.

3.3.1. The effect of 150 mM NaCl on cotton genotypes with constant $CaCl_2$.

A number of researchers have reported that supplemental Ca^{++} reduced the adverse effects of salinity (Kwon, *et al.*, 2009 and Sima, *et al.*, 2009). For example calcium content increased significantly in 10 mM $CaCl_2$ but reduced significantly in the control treatment (0 $CaCl_2$) in *Brassica rapa* reported by (Kwon, *et al.*, 2009). In previous experiments the plants were treated with (Na: Ca ratio 20: 1) containing Phostrogen 1g l^{-1}, but for control only Phostrogen 1g l^{-1} was used. In this experiment it was also decided to analysis more parameters including Ca^{++} content in all young and old plant parts. This experiment was design to screen ten cotton varieties as to their physiological responses to salinity with constant $CaCl_2$ treatment.

3.3.2. Material and methods.

Ten different cotton varieties (Karishma, Rehmani, Qalandari, NIAB 78, B 765, Mc Nair 220, CIM 1100, Sarmast, S 12 and MNH 93) were sown on 27-4-2009 in a green house, salinity treatment was (150 mM) NaCl and constant $CaCl_2$ treatment was started two weeks after germination see chapter 2 (section 2.1, 2.2, 2.3.3 and 2.3). Plants were measured 15 days after addition of salt. For methods used in further analysis see chapter 2 (sections 2.4, 2.5, 2.6, 2.7, 2.8 and 2.9)

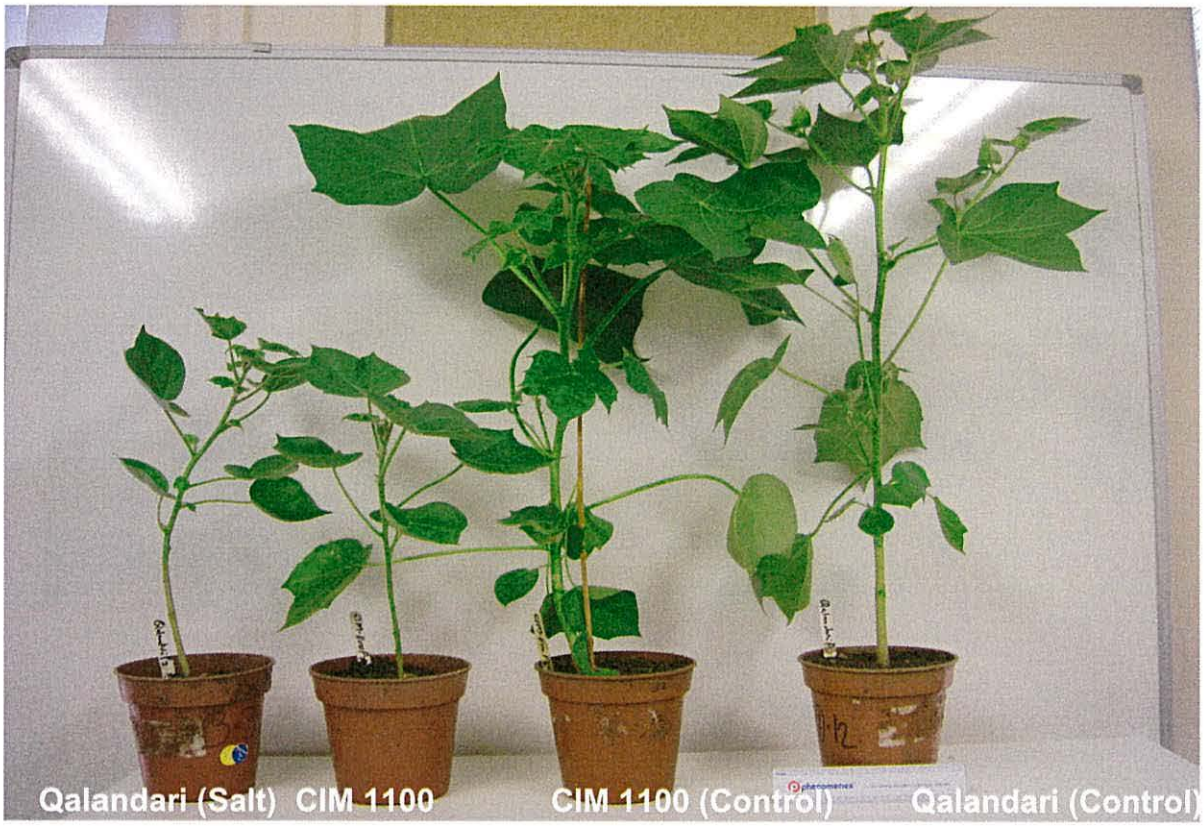

Fig. 3.3.1. Reduced plant growth of Qalandari and CIM 1100 under 150mM NaCl ((constant $CaCl_2$ treatment)

3.3.3. Results

3.3.3.1. Plant height, nodes per plant, fresh weight and Dry weight.

The effect of salt reduced plant height, nodes per plant, fresh weight and dry weight significantly ($P < 0.000$) (Table 3.3.10). Significant differences ($P < 0.000$) were observed between genotypes for nodes per plant, fresh weight and dry weight; however, for plant height genotypes was not significant. The interaction between treatments and genotypes was only detected for nodes per plant.

The variety Rehmani had tallest plant height (45 ± 22.3 (4) cm) in salt treatment followed by B 756 (36 ± 4.6 (4) cm), and in control treatment tallest plant height was observed in variety Qalandari (66 ± 4.2 (4) cm), followed by Karishma (60 ± 8.1 (4) cm). The lowest plant height was observed in S 12 (28 ± 9.4 (4) and 40 ± 4.3 (4) cm), in salt and control treatment respectively (Table 3.3.1). It was observed from the data presented from Table 3.3.7 that the plant height showed no significant correlation with any traits.

The variety MNH 93 had maximum number of nodes per plant (9.3 ± 0.5 (4)) in salt treatment followed by B 756 (9 ± 0.5 (4)), and in control treatment maximum number of nodes per plant was observed in variety B 756 (11.3 ± 0.5 (4)), followed by Karishma (10.5 ± 1 (4)). The minimum number of nodes per plant was observed in NIAB 78 (7 ± 0 (4) and 7 ± 1.6 (4)), in salt and control treatment respectively (Table 3.3.1). MNH 93 and Rehmani increased and NIAB 78 had no difference in nodes per plant by salt. The varieties MNH 93 and Rehmani increased (approximately 1 node per plant). It was observed from the data presented from Table 3.3.7 that the nodes per plant showed highly significant correlation with Na^+ in old petiole.

The variety B 756 had highest fresh weight (30.7 ± 7.1 (4) g) in salt treatment followed by Karishma (27.4 ± 8.4 (4) g), and in control treatment the highest fresh weight was observed in variety CIM 1100 (97.4 ± 26 (4) g), followed by Karishma (88.9 ± 17.9 (4) g). The lowest fresh weight was observed in NIAB 78 (11.7 ± 5.2 (4) and 17.6 ± 14.9 (4) g), in salt and control

treatment respectively (Table 3.3.1). The variety B 756 was significantly different and also had a maximum absolute reduction in fresh weight (56 g) from NIAB 78, which had minimum absolute reduction of 5 g. However, B 756 was not significantly different to Karishma, CIM 1100, Qalandari, Sarmast, Mc Nair 220 and S 12. It was observed from the data presented from Table 3.3.7 that the fresh weight showed highly significant correlation with dry weight, CO_2 uptake, Na^+ in young leaf.

The variety B 756 had highest dry weight (6.4 ± 1.5 (4) and 19 ± 8.5 (4) g) in salt and control treatment respectively, followed by Mc. Nair 220 (5.5 ± 2.1 (4) g) in salt treatment and CIM 1100 (18.5 ± 4.9 (4) g) in control treatment. The lowest dry weight was observed in NIAB 78 (1.1 ± 0.4 (4) and 2.7 ± 3 (4) g), in salt and control treatment respectively (Table 3.3.1). The variety B 756 was significantly different and also had a maximum absolute reduction in dry weight (13 g) from NIAB 78, which had minimum reduction (1.6 g). B 756 was not significantly different from Mc Nair 220, CIM 1100, Karishma, Qalandari, S 12 and Sarmast. NIAB 78 was not significantly different from MNH 93. It was observed from the data presented from Table 3.3.7 that the dry weight showed highly significant correlation with chlorophyll content, CO_2 uptake, Na^+ in young leaf.

Table 3.3.1. Means and standard deviation of plant height, fresh weight and dry weight of cotton genotypes.

	Plant height (mm)		Nodes per plant		FW (g)		DW (g)	
Genotypes	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**
B 756	59.5±9.6	36.1±4.6	11.3±0.5	9.0±0.5	86.7±28.1	30.7±7.1	18.9±8.5	6.4±1.5
CIM 1100	57.4±8.8	30.9±4.1	9.8±1.0	8.0±0.1	97.4±26.0	23.4±10.4	18.5±4.9	5.0±2.2
Karishma	59.7±8.1	32.1±4.0	10.5±1.0	8.8±1.0	88.9±18.0	27.4±8.5	17.2±6.8	5.1±3.3
Mc. Nair 220	59.2±5.9	31.4±4.5	8.5±0.6	7.3±1.0	64.9±31.6	22.5±9.1	13.4±7.8	5.5±2.1
MNH 93	49.8±19	34.8±3.0	8.0±2.4	9.3±0.5	43.3±47.7	19.6±7.1	9.1±11.1	4.5±1.3
NIAB 78	49.7±13	28.9±3.6	7.0±1.6	7.0±0.2	17.7±14.9	11.8±5.2	2.8±3.0	1.1±0.4
Qalandari	65.9±4.3	35.4±3.3	9.3±1.3	8.3±0.5	71.6±9.6	23.1±8.7	14.4±6.2	5.0±1.8
Rehmani	51.0±26	45.4±22	7.3±1.7	8.0±0.8	34.1±23.8	21.6±5.3	6.6±4.4	4.2±1.5
S 12	40.4±4.3	27.9±9.4	10.3±1.5	7.0±1.4	57.4±14.8	20.7±12.9	13.4±5.6	4.9±3.2
Sarmast	48.1±4.3	28.9±4.4	9.3±2.4	8.3±0.5	71.1±56.3	22.8±12.5	14.7±11.8	4.2±2.9

3.3.3.5. Chlorophyll content, transpiration rate, stomatal conductance and CO_2 uptake.

The effect of salt reduced chlorophyll content significantly ($P < 0.000$) in genotypes (Table 3.3.10). Significant differences ($P < 0.000$) were observed in between genotypes for chlorophyll content and transpiration rate; however, the genotypes were not significant different for stomatal conductance and CO_2 uptake. The interaction between treatments and genotypes was not significant.

The variety Mc. Nair 220 had highest chlorophyll florescence (76.5 ± 11.2 (4) and 89.8 ± 14.5 (4)) in salt and control treatment respectively, followed by Qalandari (71.5 ± 10.9 (4)) in salt treatment and Karishma (87.7 ± 13.5 (4)) in control treatment. The lowest chlorophyll florescence was observed in NIAB 78 (37.6 ± 13.6 (4) and 55.6 ± 12.5 (4)), in salt and control treatment respectively (Table 3.3.2). The varieties Mc. Nair 220, Karishma and Qalandari were significantly different from NIAB 78. They had 7% - 20% reduction in chlorophyll content, whilst NIAB 78 had 32% reduction. However, Mc. Nair 220, Karishma, Qalandari, CIM 1100, S 12 and B 756 were not significantly different from each other. It was observed from the data presented from Table 3.3.7 that the chlorophyll content showed highly significant correlation with CO_2 uptake, Na^+ in young petiole.

The variety Karishma had highest transpiration rate (4.2 ± 0.7 (4) $mmol/m^2/s$) in salt treatment followed by Qalandari (4.1 ± 0.6 (4) $mmol/m^2/s$), and in control treatment Qalandari had highest evaporation rate (6.27 ± 0.68 (4) $mmol/m^2/s$), followed by Sarmast (5.94 ± 1.89 (4) $mmol/m^2/s$). The lowest evaporation rate was observed in NIAB 78 (2.8 ± 0.56 (4) $mmol/m^2/s$) in salt treatment, and control treatment MNH 93 had a lowest evaporation rate (4.1 ± 0.4 (4) $mmol/m^2/s$) (Table 3.3.2). The varieties Karishma and Qalandari reduced (29% and 34% respectively) transpiration rate was significantly different from MNH 93 (23%). It was observed

from the data presented from Table 3.3.7 that the transpiration rate showed highly significant correlation with Na^+ in young petiole.

The variety S12 had highest stomatal conductance (322.3 ± 95.1 (4) $molm^2$/ s) in salt treatment followed by NIAB 78 (248.7 ± 146.5 (4) $molm^2$/ s), and in control treatment B 756 had highest stomatal conductance (565.5 ± 152.7 (4) $molm^2$/ s), followed by Mc.Nair 220 (553.3 ± 138.7 (4) $molm^2$/ s). The lowest stomatal conductance was observed in B 756 (80.5 ± 30.3 (4) $molm^2$/ s) in salt treatment, and control treatment Sarmast had the lowest stomatal conductance (406 ± 227.5 (4) $molm^2$/ s) (Table 3.3.2). The variety S12 had a lower absolute reduction (197 $molm^2$/ s) in stomatal conductance and B 756 had higher absolute reduction (485 $molm^2$/ s). It was observed from the data presented from Table 3.3.7 that stomatal conductance showed no significant correlation with any traits.

The variety Mc. Nair 220 had highest CO_2 uptake (10.15 ± 1.4 (4) and 16.9 ± 6.3 (4) $\mu molm^{-2}s^{-1}$) in salt and control treatment respectively, followed by B 756 (9.75 ± 1.8 (4) $\mu molm^{-2}s^{-1}$) in salt treatment and Qalandari (16.67 ± 4.8 (4) $\mu molm^{-2}s^{-1}$) in control treatment. The lowest CO_2 uptake was observed in NIAB 78 (5.3 ± 1.0 (4) $\mu molm^{-2}s^{-1}$) in salt treatment, whilst in control treatment MNH 93 had the lowest Co_2 (13.2 ± 7.4 (4) $\mu molm^{-2}s^{-1}$) (Table 3.3.2). However, a lower reduction in CO_2 uptake was observed in Karishma (4 $\mu molm^{-2}s^{-1}$) and higher reduction was observed in NIAB 78 (19 $\mu molm^{-2}s^{-1}$). It was observed from the data presented from Table 3.3.7 that the CO_2 uptake showed highly significant correlation with Na^+ in old stem.

Table. 3.3.2. Means and standard deviation of chlorophyll content, transpiration rate, stomatal conductance and CO_2 uptake of cotton genotypes.

	Chlorophyll content		Transpiration rate		Stomatal conductance		CO_2 uptake	
Genotypes	Control	Salt	Control	Salt	Control	Salt	Control	Salt
B 756	78.7±8.7	56.5±11.5	5.0±1.1	3.2±0.6	565.5±152.7	80.5±30.3	16.3±2.9	9.8±1.9
CIM 1100	75.7±3.8	63.6±9.8	5.2±1.0	3.0±0.9	547.3±213.6	220.8±116.5	15.1±6.7	7.7±3.6
Karishma	87.8±13.6	70.6±15.7	5.9±1.5	4.2±0.7	549.8±160.9	235.8±157.5	12.9±4.6	8.9±2.3
Mc. Nair 220	89.9±14.6	76.5±11.2	5.9±0.7	3.2±0.9	553.3±137.7	210.3±135.9	16.9±6.3	10.2±1.4
MNH 93	57.3±5.6	56.5±7.6	4.1±0.4	3.2±0.5	463.0±131.5	190.0±73	13.3±7.4	7.1±3.0
NIAB 78	55.7±12.6	37.6±13.6	4.8±1.0	2.8±0.6	426.5±263.5	248.8±146.5	14.4±5.7	5.3±1.0
Qalandari	76.9±17.2	71.6±11.0	6.3±0.7	4.1±0.6	525.5±223.2	231.3±131.6	16.7±4.9	7.9±2.9
Rehmani	58.5±5.4	55.7±21.3	5.3±1.5	3.0±0.7	512.3±374.5	247.8±244.4	13.3±5.2	8.1±2.7
S 12	81.2±33.5	59.6±15.9	4.6±0.6	3.4±1.8	519.3±304.6	322.3±95.2	16.1±4.8	7.3±2.1
Sarmast	62.5±9.8	46.8±19.1	5.9±1.9	3.4±0.7	406.0±227.5	113.5±57.5	13.8±4.6	8.0±3.3

3.3.3.9. Na^+ concentration in young and old leaves, petiole and stem.

The effect of salt increased Na^+ concentration in all young and old tissues significantly ($P < 0.000$) (Table 3.3.10). The genotypes were not significantly different from each other for all young and old tissues. The interaction between treatments and genotypes was not significant for all young and old tissues.

The variety B 756 had highest Na^+ concentration (164.5 ± 32.7 (4) mM) in salt treatment followed by S 12 (146.3 ± 83 (4) mM) in young leaf, and in control treatment Rehmani had highest Na^+ concentration (6.6 ± 11.3 (4) mM) followed by Qalandari (3.4 ± 3.3 (4) mM). Whereas, the lowest Na^+ concentration was observed in NIAB 78 (50.1 ± 19.4 (4) mM) in salt treatment, and control treatment CIM 1100 had a lowest Na^+ concentration (0.96 ± 0.5(4) mM) in young leaf. (Table 3.3.3). It was observed from the data presented from Table 3.3.7 showed that the Na^+ concentration in young leaf showed no significant correlation with any trait.

The variety Karishma had highest Na^+ concentration (168.4 ± 131.1 (4) and 7.9 ± 10 (4) mM) in salt and control treatment respectively, followed by S 12 (120.3 ± 104.7 (4) mM) in salt treatment and Rehmani (6.9 ± 12 (4) mM) in control treatment in young petiole. Whereas, the lowest Na^+ concentration was observed in NIAB 78 (38.4 ± 16.4 (4) mM) in salt treatment, and control treatment B 756 had a lowest Na^+ concentration (0.95 ± 0.8 (4) mM) in young petiole

(Table 3.3.3). It was observed from the data presented from Table 3.3.7 that the Na^+ concentration in young petiole showed no significant correlation with any trait.

The variety Karishma had highest Na^+ concentration (195.7 ± 142.9 (4) mM) in salt treatment, followed by S 12 (187.9 ± 132 (4) mM) in young stem, whilst in control treatment Rehmani had highest Na^+ concentration (17.7 ± 30.8 (4) mM) followed by S 12 (5 ± 3.9 (4) mM). The lowest Na^+ concentration was observed in Sarmast (103.4 ± 35.5 (4) mM) in salt treatment, and control treatment Qalandari had a lowest Na^+ concentration (0.49 ± 0.3 (4) mM) in young stem (Table 3.3.3). It was observed from the data presented from Table 3.3.7 that the Na^+ concentration in young stem showed no significant correlation with any traits.

The variety B 756 and MNH 93 had highest Na^+ concentration (239.9 ± 63.1 (4) and 239.9 ± 77.1 (4) mM) in salt treatment, followed by Rehmani (238.6 ± 46.3 (4) mM) in old leaf, whilst in control treatment MNH 93 had highest Na^+ concentration (27.8 ± 25.4 (4) mM) followed by Rehmani (17.1 ± 15.3(4) mM). The lowest Na^+ concentration was observed in Sarmast (210 ± 83.2 (4) mM) in salt treatment, and in control treatment Mc Nair 220 had a lowest Na^+ concentration (6.4 ± 4.2 (4) mM) in old leaf (Table 3.3.3). It was observed from the data presented from Table 3.3.7 that the Na^+ concentration in old leaf showed highly significant correlation with Na^+ in old petiole.

The variety MNH 93 had highest Na^+ concentration (224.3 ± 88.8 (4) mM) in salt treatment, followed by B 756 (189.2 ± 74.3 (4) mM) in old petiole, whilst in control treatment S 12 had highest Na^+ concentration (14.1 ± 8.1 (4) mM) followed by MNH 93 (9.8 ± 1.8 (4) mM).The lowest Na^+ concentration was observed in Mc Nair 220 (115.1 ± 38.4 (4) mM) in salt treatment, whilst in control treatment CIM 1100 had a lowest Na^+ concentration (2.2 ± 1.4 (4) mM) in old petiole (Table 3.3.3). Table 3.3.7 showed that the Na^+ concentration in old petiole showed no significant correlation with any trait.

The variety MNH 93 had highest Na^+ concentration (147.6 ± 61.7 (4) and 18.9 ± 15.1 (4) mM) in salt and control treatment respectively, followed by S 12 (141.1 ± 94.9 (4) mM) in salt treatment and Rehmani (16.7 ± 23.7 (4) mM) in control treatment. Whereas, the lowest Na^+ concentration was observed in Mc Nair 220 (51.4 ± 16.1 (4) mM) in salt treatment, whilst in control treatment CIM 1100 had a lowest Na^+ concentration (2.8 ± 1.35 (4) mM) in old stem (Table 3.3.3). It was observed from the data presented from Table 3.3.7 that the Na^+ concentration in old stem showed no significant correlation with any traits.

3.3.3.10. K^+ concentration in young and old leaves, petiole and stem

The effect of salt on K^+ concentration in young leaf was significant ($P < 0.000$) (Table 3.3.10). The genotypes were not significantly different from each other. The interaction between treatments and genotypes was not significant.

The variety Mc. Nair 220 had highest K^+ concentration (165 ± 27.6 (4) mM) in salt treatment followed by Karishma (143.4 ± 44.9 (4) mM) in young leaf, whilst in control treatment S 12 had highest K^+ concentration (167.1 ± 27.4 (4) mM) followed by Rehmani (166.7 ± 21.2 (4) mM). The lowest K^+ concentration was observed in NIAB 78 (110.1 ± 14.9 (4) mM) in salt treatment, whilst in control treatment MNH 93 had a lowest K^+ concentration (133.2 ± 20.7 (4) mM) in young leaf (Table 3.3.4). However, the highest absolute reduction was observed in S12 (-40 mM) and lowest absolute reduction was observed in Mc.Nair 220 (-1 mM). Karishma showed an increase (6 mM) in K^+ concentration in salt treatment. It was observed from the data presented from Table 3.3.8 that the K^+ concentration in young leaf showed highly significant correlation with K^+/Na^+ ratio in old leaf.

The variety CIM 1100 had highest K^+ concentration (282.9 ± 20.1 (4) mM) in salt treatment followed by Mc. Nair 220 (261.1 ± 28.9 (4) mM) in young petiole, whilst in control treatment Mc. Nair 220 had highest K^+ concentration (249.6 ± 9.2 (4) mM) followed by S 12 (243.5 ± 27.4

(4) mM).The lowest K^+ concentration was observed in S 12 (224.7 ± 45.3 (4) mM) in salt treatment, whilst in control treatment Rehmani had a lowest K^+ concentration (201.6 ± 21.8 (4) mM) in young petiole (Table 3.3.4). Most of genotypes showed increase in K^+ concentration except for S 12 and Qalandari. However, the highest absolute increase was observed in CIM 1100 (80 mM) from control and the lowest absolute increase was observed in Sarmast (5 mM). It was observed from the data presented from Table 3.3.8 that the K^+ concentration in young petiole showed no significant correlation with any trait.

The variety Qalandari had highest K^+ concentration (257.3 ± 22.8 (4) mM) in salt treatment followed by CIM 1100 (245.6 ± 13.5 (4) mM) in young stem, whilst in control treatment S 12 had highest K^+ concentration (227.7 ± 25.3 (4) mM) followed by Mc. Nair 220 (221.5 ± 13.5 (4) mM).The lowest K^+ concentration was observed in S 12 (196 ± 96.4 (4) mM) in salt treatment, whilst in control treatment MNH 93 had a lowest K^+ concentration (201.2 ± 18.4 (4) mM) in young stem (Table 3.3.4). Most of genotypes showed increase in K^+ concentration except for S 12 and Karishma. However, the highest absolute increase was observed in Rehmani (62 mM) from control and the lowest absolute increase was observed in Mc. Nair 220 (11 mM). It was observed from the data presented from Table 3.3.8 that the K^+ concentration in young stem showed highly significant correlation with K^+/Na^+ ratio in young stem.

The variety Karishma had highest K^+ concentration (90.3 ± 37.5 (4) mM) in salt treatment followed by Mc Nair 220 (82.3 ± 45.8 (4) mM) in old leaf, whilst in control treatment Mc Nair 220 had highest K^+ concentration (149.5 ± 38.6 (4) mM) followed by S 12 (137.6 ± 28.4 (4) mM).The lowest K^+ concentration was observed in NIAB 78 (9.4 ± 1.7 (4) and 68.35 ± 25.8 (4) mM) in salt and control treatment in old leaf (Table 3.3.4). However, Karishma, Mc Nair 220 and Qalandari were not significantly different, which decreased (44.5 mM – 67 mM) K^+ concentration from control, but they were significantly different from NIAB 78 and CIM 1100, which decreased (59 – 67 mM respectively) K^+ concentration in salt treatment. It was observed

from the data presented from Table 3.3.8 that the K^+ concentration in old leaf showed highly significant correlation with K^+ in young leaf and K^+/Na^+ ratio in old leaf and young petiole.

The variety Mc Nair 220 had highest K^+ concentration (260.4 ± 53.5 (4) mM) in salt treatment followed by Karishma (228.1 ± 85.1 (4) mM) in old petiole, whilst in control treatment S 12 had highest K^+ concentration (261.8 ± 9.4 (4) mM) followed by Mc Nair 220 (252.6 ± 18.2 (4) mM). The lowest K^+ concentration was observed in MNH 93 (156.6 ± 30.9 (4) mM) in salt treatment, whilst in control treatment NIAB 78 had a lowest K^+ concentration (189 ± 39.2 (4) in old petiole (Table 3.3.4). Most of genotypes showed decrease in K^+ concentration except for Mc Nair 220 and CIM 1100. However, Mc Nair 220 absolute increased (7.7 mM) K^+ concentration from control and was significantly different from NIAB 78 and MNH 93 which decreased (-3.4 – 49 mM respectively) K^+ concentration in salt treatment. It was observed from the data presented from Table 3.3.8 that the K^+ concentration in old petiole showed significant correlation with K^+ in young leaf and K^+/Na^+ ratio in old leaf

The variety Sarmast had highest K^+ concentration (198.6 ± 43.7 (4) mM) in salt treatment followed by CIM 1100 (190.6 ± 36.8 (4) mM) in old stem, whilst in control treatment B 756 had highest K^+ concentration (212.3 ± 10.7 (4) mM) followed by S 12 (200.8 ± 25.7 (4) mM).The lowest K^+ concentration was observed in NIAB 78 (118.3 ± 19.2 (4) mM) in salt treatment, whilst in control treatment MNH 93 had a lowest K^+ concentration (173 ± 21.1 (4) in old stem (Table 3.3.4). Most of genotypes showed decrease in K^+ concentration except for Sarmast. However, the significant differences were observed in NIAB 78, which had the highest absolute reduction (-66.3 mM), and Sarmast which showed the lowest absolute increase (7 mM) in K^+ concentration in salt treatment. It was observed from the data presented from Table 3.3.8 that the K^+ concentration in old stem showed significant correlation with K^+/Na^+ ratio in old stem.

3.3.3.11. K^+/Na^+ ratio in young and old leaves, petiole and stem

The effect of salt decreased K^+/Na^+ ratio in all young and old plant tissues significantly ($P < 0.000$) (Table 3.3.10). The genotypes were not significantly different from each other, except for old stem (P<000). The interaction between treatments and genotypes was not significant except for young and old stem.

The variety Rehmani had highest K^+/Na^+ ratio (3.3± 3.0 (4)) in salt treatment followed by Mc. Nair 220 (3.0 ± 2.0 (4)) in young leaf, whilst in control treatment S 12 had highest K^+/Na^+ ratio (245.3 ± 88.7 (4)) followed by NIAB 78 (210.7 ± 84.6 (4)). The lowest K^+/Na^+ ratio was observed in B 756 (0.8 ± 0.3 (4) and 57 ± 35.4) in salt and control treatments respectively, followed by MNH 93 (68.2 ± 25.5 (4)) in control treatment and Sarmast (1.0 ± 0.4 (4)) in salt treatment in young leaf (Table 3.3.5). It was observed from the data presented from Table 3.3.8 that the K^+/Na^+ ratio in young leaf showed no significant correlation with any trait.

The variety CIM 1100 had highest K^+/Na^+ ratio (7.9 ± 5.9 (4)) in salt treatment followed by NIAB 78 (7.4 ± 4.6 (4)) in young petiole, whilst in control treatment B 756 had highest K^+/Na^+ ratio (370.5 ± 279 (4)) followed by CIM 1100 (356 ± 290 (4)). The lowest K^+/Na^+ ratio was observed in Qalandari (2.2 ± 1.0 (4)) followed by Sarmast (2.8 ± 1.3 (4)) in salt treatment, whilst in control treatment Sarmast had the lowest K^+/Na^+ ratio (55.6 ± 17.5 (4)) followed by Mc. Nair 220 (158.6± 121.9 (4)) in young petiole (Table 3.3.5). It was observed from the data presented from Table 3.3.8 that the K^+/Na^+ ratio in young petiole showed no significant correlation with any trait.

The variety B 756 had highest K^+/Na^+ ratio (5.8 ± 3.1 (4)) in salt treatment followed by CIM 1100 (3.6 ± 3.0 (4)) in young stem, whilst in control treatment Rehmani had highest K^+/Na^+ ratio (499.9 ± 220.1 (4)) followed by Karishma (377.3 ± 263.3 (4)). The lowest K^+/Na^+ ratio was observed in Sarmast (1.4 ± 0.5 (4)) followed by S 12 (1.7 ± 1.4 (4)) in salt treatment, whilst in control treatment CIM 1100 had the lowest K^+/Na^+ ratio (64 ± 43.4 (4)) followed by S 12 (65.7

± 39.4 (4)) in young stem (Table 3.3.5). It was observed from the data presented from Table 3.3.8 that the K^+/Na^+ ratio in young stem showed no significant correlation with any trait.

The variety Karishma had highest K^+/Na^+ ratio (0.56 ± 0.4 (4) and 24 ± 10 (4)) in salt and control treatment respectively followed by Mc Nair 220 (0.55 ± 0.3 (4) and 0.55 ± 0.3 (4)) in old leaf. The lowest K^+/Na^+ ratio was observed in NIAB 78 (0.05 ± 0.5 (4)) followed by CIM 1100 (0.08 ± 0.5 (4)) in salt treatment, whilst in control treatment Rehmani had the lowest K^+/Na^+ ratio (9 ± 4 (4)) followed by MNH 93 (6 ± 4 (4)) in old leaf (Table 3.3.5). It was observed from the data presented from Table 3.3.8 that the K^+/Na^+ ratio in old leaf showed no significant correlation with any trait.

The variety Mc Nair 220 had highest K^+/Na^+ ratio (2.44 ± 0.8 (4)) in salt treatment followed by Sarmast (2.41 ± 1.9 (4)), whilst in control treatment Qalandari had highest K^+/Na^+ ratio (74.4 ± 17.7(4)) followed by CIM 1100 (69.5 ± 14.6 (4)) in old petiole. The lowest K^+/Na^+ ratio was observed in MNH 93 (0.8 ± 0.5 (4)) followed by B 756 (1.1± 0.5 (4)) in salt treatment, whilst in control treatment MNH 93 had the lowest K^+/Na^+ ratio (21 ± 4.5 (4)) followed by S 12 (23.7 ± 13.1 (4)) in old petiole (Table 3.3.5). It was observed from the data presented from Table 3.3.8 that the K^+/Na^+ ratio in old petiole showed no significant correlation with any trait.

The variety Mc Nair 220 had highest K^+/Na^+ ratio (3.6 ± 1.0 (4)) in salt treatment followed by B 756 (3.2 ± 1.3 (4)) whilst in control treatment CIM 1100 had highest K^+/Na^+ ratio (71 ± 8.5 (4)) followed by Mc Nair 220 (67.5 ± 13.9 (4)) in old stem. The lowest K^+/Na^+ ratio was observed in MNH 93 and NIAB 78 (1.2 ± 0.8 (4) and 1.2 ±0.7(4)) followed by B 756 (1.1± 0.5 (4)) in salt treatment, whilst in control treatment MNH 93 had the lowest K^+/Na^+ ratio (19.3 ± 20.4 (4)) followed by S 12 (20.4 ± 9.6 (4)) in old stem (Table 3.3.5). It was observed from the data presented from Table 3.3.8 that the K^+/Na^+ ratio in old stem showed no significant correlation with any trait.

3.3.3.12. Ca^{++} concentration in young and old leaves, petiole and stem

The effect of salt on Ca^{++} concentration in all young petiole and old leaf and petiole was significant ($P < 0.00$) Table 3.3.10). Most of genotypes showed decrease in Ca^{++} concentration, except for MNH 93, CIM 1100 and Rehmani. The genotypes were not significantly different ($P = 0.538$) from each other except for old petiole ($P < 0.000$). The interaction between treatments and genotypes was not significant in all young and old tissues.

The variety MNH 93 had highest Ca^{++} concentration (125.9 ± 23.5 (4) mM) in salt treatment followed by Mc. Nair 220 (123.1 ± 36.2 (4) mM) in young leaf, whilst in control treatment Mc. Nair 220 had highest Ca^{++} concentration (155.9 ± 36 (4) mM) followed by Qalandari (135.9 ± 19.7 (4) mM).The lowest Ca^{++} concentration was observed in Sarmast (98.8 ± 7.8 (4) mM) in salt treatment, whilst in control treatment CIM 1100 had a lowest Ca^{++} concentration (103.1 ± 31.8 (4) mM) in young leaf (Table 3.3.6). It was observed from the data presented from Table 3.3.9 that the Ca^{++} concentration in young leaf showed significant correlation with Ca^{++} concentration in young stem.

The variety CIM 1100 had highest Ca^{++} concentration (173.2 ± 17.3 (4) mM) in salt treatment followed by Sarmast (163.8 ± 21.3 (4) mM) in young petiole, whilst in control treatment Sarmast had highest Ca^{++} concentration (148.7 ± 29.4 (4) mM) followed by Karishma (148.4 ± 18.4 (4) mM).The lowest Ca^{++} concentration was observed in S 12 (137.9 ± 25.4 (4) mM) in salt treatment, whilst in control treatment Rehmani had a lowest Ca^{++} concentration (117.5 ± 26.6 (4) mM) in young petiole (Table 3.3.6). It was observed from the data presented from Table 3.3.9 that the Ca^{++} concentration in young petiole showed no significant correlation with any trait.

The variety Qalandari had highest Ca^{++} concentration (135.7 ± 10.5 (4) mM) in salt treatment followed by MNH 93 (133.4 ± 32.3 (4) mM) in young stem, whilst in control treatment B 756 had highest Ca^{++} concentration (133.3 ± 27.2 (4) mM) followed by Karishma (126.9 ± 15.9 (4) mM).The lowest Ca^{++} concentration was observed in S 12 (97.1 ± 40.2 (4) mM) in salt treatment,

whilst in control treatment Rehmani had a lowest Ca^{++} concentration (103 ± 10 (4) mM) in young stem (Table 3.3.6). Most of genotypes showed increase in Ca^{++} concentration except for Mc Nair 220, Karishma, B 756 and S 12. However, Qalandari had the highest absolute increase (20 mM) and S 12 had the lowest absolute decrease (-20 mM). It was observed from the data presented from Table 3.3.9 that the Ca^{++} concentration in young stem showed no significant correlation with any trait.

The variety Karishma had highest Ca^{++} concentration (158 ± 28.2 (4) mM) in salt treatment followed by Mc Nair 220 (144.5 ± 43.6 (4) mM) in old leaf, whilst in control treatment Sarmast had highest Ca^{++} concentration (186.2 ± 27.5 (4) mM) followed by B 756 (184.5 ± 12.2 (4) mM).The lowest Ca^{++} concentration was observed in NIAB 78 (76.7 ± 37.6 (4) and 133.1 ± 13.5 (4) mM) in salt and control treatment respectively in old leaf (Table 3.3.6). However, Qalandari had the highest absolute decrease (20 mM) and NIAB 78 had an absolute decrease (56 mM) from control. It was observed from the data presented from Table 3.3.9 that the Ca^{++} concentration in old leaf showed highly significant correlation with Ca^{++} concentration in old petiole.

The variety Karishma had highest Ca^{++} concentration (185.1 ± 28.3 (4) and 173.7 ± 20.6 (4) mM) in salt and control treatment respectively, followed by Sarmast (170.4 ± 35.8 (4) mM) in salt treatment, and B 756 (162.8 ± 7.5 (4) mM) in control treatment of old petiole. The lowest Ca^{++} concentration was observed in NIAB 78 (137.3 ± 26.2 (4) and 117.1 ± 34.4 (4) mM) in salt and control treatment respectively in old petiole (Table 3.3.6). However, the significant difference was observed in between Karishma which had the highest Ca^{++} concentration in both treatments and NIAB 78 which had the lowest Ca^{++} concentration in both treatments. It was observed from the data presented from Table 3.3.9 that the Ca^{++} concentration in old petiole showed no significant correlation with any trait.

The variety CIM 1100 had highest Ca^{++} concentration (95.9 ± 16.3 (4) mM) in salt treatment followed by Qalandari (90.8 ± 11.1 (4) mM), whilst in control treatment B 756 had highest Ca^{++} concentration (109.4 ± 33.4 (4) mM) followed by Sarmast (99.4 ± 14.3 (4) The lowest Ca^{++} concentration was observed in NIAB 78 (64.3 ± 27.3(4) mM) in salt treatment and Rehmani (65.9 ± 27.1 (4) mM) in control treatment in old stem (Table 3.3.6). However, the highest absolute decrease was observed in NIAB 78 (-24.4 mM) from control and the lowest absolute increase was observed in Mc Nair 220 (2.2 mM). It was observed from the data presented from Table 3.3.9 that the Ca^{++} concentration in old stem showed no significant correlation with any trait.

Table. 3.3.3. Means and standard deviation of Na^+ concentration of all young and old plant tissues of cotton genotypes. (young leaf = Y.L, young petiole = Y.P, young stem = Y.S, old leaf = O.L, old petiole = O.P and old stem = O.S).

	Na^+ (Y.L)		Na^+ (Y.P)		Na^+ (Y.S)		Na^+ (O.L)		Na^+ (O.P)		Na^+ (O.S)	
Genotypes	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**
B 756	3±2	165±33	1±1	97±40	3±4	196±143	11±4	240±63	6±1	189±74	7±5	67±26
CIM 1100	1±1	114±89	2±2	57±46	5±4	188±132	7±4	218±39	2±1	139±28	3±1	102±19
Karishma	3±2	108±97	8±10	168±131	3±4	174±49	9±3	187±12	5±2	161±31	5±2	105±19
Mc. Nair 220	2±1	84±61	3±2	118±75	4±1	141±87	6±2	162±39	7±4	115±39	3±2	51±16
MNH 93	2±1	126±84	2±1	90±38	1±1	136±120	28±25	240±77	10±2	224±89	19±15	148±62
NIAB 78	2±2	50±20	3±3	38±16	1±1	135±111	9±6	219±70	5±3	133±36	13±8	129±60
Qalandari	3±3	132±64	2±2	116±51	1±0	131±56	8±4	231±72	4±3	176±79	6±3	96±86
Rehmani	7±11	79±60	7±12	103±74	18±31	131±119	17±15	239±46	9±7	172±63	17±24	77±7
S 12	2±2	146±83	5±5	12±105	2±2	103±36	12±3	223±73	14±8	174±65	11±4	141±95
Sarmast	3±2	133±50	5±2	100±40	4±5	57±47	10±5	210±94	8±5	131±77	9±5	88±44

Table. 3.3.4. Means and standard deviation of K^+ concentration of all young and old plant tissues of cotton genotypes. (young leaf = Y.L, young petiole = Y.P, young stem = Y.S, old leaf = O.L, old petiole = O.P and old stem = O.S).

	K^+ (Y.L)		K^+ (Y.P)		K^+ (Y.S)		K^+ (O.L)		K^+ (O.P)		K^+ (O.S)	
Genotypes	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**
B 756	139±26	129±26	206±55	256±46	204±36	257±23	111±35	29±17	242±28	189±45	212±11	190±34
CIM 1100	139±20	129±9	203±32	283±20	208±14	246±14	83±31	16±13	211±44	220±29	200±12	191±37
Karishma	137±32	143±45	221±14	249±29	201±19	245±44	135±24	90±38	242±48	228±85	184±36	168±44
Mc. Nair 220	166±12	165±28	250±9	261±29	178±36	240±26	150±39	82±46	253±18	261±54	179±4	175±13
MNH 93	133±21	127±30	204±33	257±15	219±15	238±64	108±24	33±24	206±45	157±31	173±21	145±21
NIAB 78	143±36	110±15	212±27	230±17	214±41	234±18	68±26	9±2	189±39	186±12	185±11	118±19
Qalandari	138±13	134±32	232±45	229±58	222±14	233±38	119±41	64±36	221±49	189±25	190±18	178±12
Rehmani	167±21	133±19	202±22	230±27	208±20	225±23	112±40	45±15	210±19	200±35	179±22	177±18
S 12	167±28	127±5	244±28	225±45	212±14	212±53	138±28	39±30	262±9	228±37	201±26	167±42
Sarmast	147±16	122±25	243±22	247±42	228±25	196±96	110±35	37±25	237±13	216±34	191±14	199±44

Table. 3.3.5. Means and standard deviation of K^+/Na^+ ratio of all young and old plant tissues of cotton genotypes. (young leaf = Y.L, young petiole = Y.P, young stem = Y.S, old leaf = O.L, old petiole = O.P and old stem = O.S).

	K^+/Na^+ (Y.L)		K^+/Na^+ (Y.P)		K^+/Na^+ (Y.S)		K^+/Na^+ (O.L)		K^+/Na^+ (O.P)		K^+/Na^+ (O.S)	
Genotypes	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**
B 756	57±35	0.8±0.3	371±279	3.2±1.8	214±235	1.5±0.5	11±7	0.14±0.1	43±11	1.1±0.5	50±32	3.2±1.3
CIM 1100	208±181	1.6±1.0	356±290	7.4±4.6	64±43	3.6±3.4	13±2	0.08±0.1	70±15	1.6±0.4	71±9	1.9±0.3
Karishma	129±171	2.1±1.7	180±278	3.4±4.0	377±263	2.7±3.4	24±10	0.56±0.4	53±23	1.5±0.7	41±11	1.6±0.4
Mc. Nair 220	198±65	3.0±2	159±122	3.2±2.1	444±233	2.6±2.4	20±9	0.55±0.3	49±30	2.4±0.8	68±14	3.6±1.0
MNH 93	68±26	2.0±2.2	197±176	3.4±1.8	240±271	3.2±2.9	6±4	0.14±0.1	21±5	0.8±0.5	19±20	1.2±0.8
NIAB 78	211±85	2.4±0.7	206±226	7.9±5.9	339±323	5.8±3.1	10±4	0.05±0.5	67±13	1.5±0.5	29±15	1.2±0.7
Qalandari	122±162	1.3±0.8	248±219	2.2±1.0	57±17	2.5±1.6	17±8	0.30±0.1	74±18	1.6±1.5	34±13	3.0±1.9
Rehmani	177±178	3.3±3	304±296	4.4±4.8	500±220	3.6±3.0	9±4	0.18±0.0	37±23	1.3±0.4	32±23	2.3±0.3
S 12	245±89	1.1±0.7	190±281	3.7±3.5	66±39	1.7±1.4	12±3	0.19±0.1	24±13	1.5±0.5	20±10	1.7±1.1
Sarmast	140±100	1.0±0.4	56±18	2.8±1.3	117±61	2.6±1.3	12±5	0.22±0.2	36±14	2.4±1.9	29±18	2.9±2.0

Table. 3.3.6. Means and standard deviation of Ca^{++} concentration of all young and old plant tissues of cotton genotypes. (young leaf = Y.L, young petiole = Y.P, young stem = Y.S, old leaf = O.L, old petiole = O.P and old stem = O.S).

	Ca^+ (Y.L)		Ca^+ (Y.P)		Ca^+ (Y.S)		Ca^+ (O.L)		Ca^+ (O.P)		Ca^+ (O.S)	
Genotypes	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**
B 756	124±37	115±26	129±22	159±27	116±33	136±11	185±12	115±29	163±8	156±40	109±33	88±19
CIM 1100	103±32	120±17	119±12	173±17	114±10	133±32	148±22	124±27	130±31	162±22	84±15	96±16
Karishma	128±34	116±29	148±18	154±17	119±26	130±22	177±6	158±28	174±21	185±28	96±8	83±19
Mc. Nair 220	156±36	123±36	149±19	147±39	108±9	130±10	157±53	145±44	146±7	166±30	82±12	78±17
MNH 93	109±17	126±24	130±13	160±12	126±8	127±18	169±24	127±7	130±22	158±8	84±13	78±10
NIAB 78	124±44	108±15	132±15	159±22	104±17	121±8	133±14	77±38	117±34	137±26	89±7	64±27
Qalandari	136±20	119±18	146±34	160±15	124±13	121±28	173±56	132±24	133±34	164±10	89±17	91±11
Rehmani	109±34	119±24	118±27	157±6	127±16	114±19	164±22	109±18	122±9	149±23	66±27	84±19
S 12	133±34	103±12	134±47	138±25	133±27	113±20	176±70	124±16	149±56	154±22	90±20	74±11
Sarmast	127±34	99±8	149±29	164±21	118±28	97±40	186±28	110±38	151±30	170±36	99±14	91±27

Table.3.3.7. Correlation of plant height, nodes per plant, fresh and dry weight, chlorophyll content, transpiration rate, CO2 uptake and Na^+ concentration in young and old leaf, petiole and stem. (young leaf = Y.L, young petiole = Y.P, young stem = Y.S, old leaf = O.L, old petiole = O.P and old stem = O.S).

	Plant height	Nodes /plant	FW	DW	Chlorophyll content	Transpiration rate	Stomatal conductance	CO2 uptake	Na^+ (O.L)	Na^+ (O.P)	Na^+ (O.S)	Na^+ (Y.L)	Na^+ (Y.P)	Na^+ (Y.S)
Plant height	1	0.382	0.245	0.204	0.115	-0.06	-0.093	0.244	0.44	0.443	-0.354	-0.09	0.124	0.236
Nodes/plant		1	0.591	0.46	0.136	0.352	-0.611	0.309	0.332	.640(*)	-0.04	0.497	0.277	0.308
FW			1	.897(**)	0.541	0.471	-0.498	.830(**)	-0.066	0.199	-0.54	.669(*)	0.586	0.428
DW				1	.718(*)	0.393	-0.328	.849(**)	-0.1	0.266	-0.465	.713(*)	0.574	0.351
Chlorophyll content					1	0.578	0.175	.681(*)	-0.477	-0.005	-0.377	0.204	.651(*)	0.353
Transpiration rate						1	0.079	0.302	-0.221	0.166	-0.013	0.377	.802(**)	-0.017
Stomatal conductance							1	-0.431	-0.106	-0.057	0.479	-0.4	0.104	-0.101
CO2 uptake								1	-0.431	-0.086	-.795(**)	0.354	0.595	0.317
Na^+ (O.L)									1	.713(*)	0.378	0.34	-0.38	0.017
Na^+ (O.P)										1	0.432	0.494	0.162	0.178
Na^+ (O.S)											1	0.025	-0.208	-0.191
Na^+ (Y.L)												1	0.322	0.018
Na^+ (Y.P)													1	-0.033
Na^+ (Y.S)														1

Table 3.3.8 Correlation of plant K^+ concentration and K^+/Na^+ ratio in young and old leaf, petiole and stem. (young leaf = Y.L, young petiole = Y.P, young stem = Y.S, old leaf = O.L, old petiole = O.P and old stem = O.S).

	K^+ (O.L)	K^+ (O.P)	K^+ (O.S)	K^+ (Y.L)	K^+ (Y.P)	K^+ (Y.S)	K^+/Na^+ (O.L)	$K+/Na^+$ (O.P)	K^+/Na^+ (O.S)	K^+/Na^+ (Y.L)	K^+/Na^+ (Y.P)	K^+/Na^+ (Y.S)
Plant height	0.111	-0.34	0.169	0.147	-0.156	0.237	-0.021	-0.437	0.175	0.433	-0.202	0.242
Nodes/plant	0.12	-0.501	0.271	0.009	0.349	0.346	-0.051	-0.466	0.021	-0.272	-0.439	0.435
Fresh weight	0.42	0.233	.767(**)	0.436	0.374	0.343	0.369	-0.025	0.524	-0.381	-0.554	0.618
Dry weight	0.452	0.3	.737(*)	0.597	0.408	0.301	0.443	-0.005	0.564	-0.324	-0.631	0.489
Chlorophyll content	.785(**)	0.503	0.396	.863(**)	0.29	0.357	.795(**)	0.179	0.415	0.116	-0.468	0.239
Transpiration rate	.731(*)	0.147	0.243	0.317	-0.196	-0.016	0.569	0.089	0.142	-0.294	-.636(*)	-0.031
Stomatal cond.	0.143	0.222	-0.447	0.044	-0.44	-0.171	0.136	-0.08	-0.498	0.374	0.288	-0.483
CO2 uptake	0.629	0.517	.681(*)	.797(**)	0.385	0.319	.678(*)	0.286	.760(*)	0.004	-0.593	0.526
Na^+ (O.L)	-.642(*)	-.830(**)	-0.072	-.708(*)	-0.282	-0.02	-.809(**)	-.731(*)	-0.283	-0.337	0.073	0.053
Na^+ (O.P)	-0.109	-.723(*)	-0.147	-0.234	-0.165	0.216	-0.332	-.860(**)	-0.31	-0.232	-0.351	0.19
Na^+ (O.S)	-0.389	-0.473	-.636(*)	-0.598	-0.259	-0.202	-0.487	-0.495	-.879(**)	-0.198	0.285	-0.416
Na^+ (Y.L)	-0.006	-0.12	0.568	-0.061	0.13	-0.04	-0.091	-0.185	0.271	-.846(**)	-0.582	0.24
Na^+ (Y.P)	.883(**)	0.406	0.323	0.607	-0.182	-0.052	.774(**)	0.121	0.242	-0.01	-.759(*)	0.01
Na^+ (Y.S)	0.021	-0.059	0.002	0.231	0.509	.958(**)	0.015	-0.47	-0.049	0.082	0.288	.883(**)
K^+ (O.L)	1	0.529	0.222	.825(**)	-0.067	0.074	.957(**)	0.338	0.374	0.278	-.640(*)	0.006
K^+ (O.P)		1	0.401	.662(*)	0.192	-0.15	.719(*)	.752(*)	0.407	0.178	-0.079	-0.086
K^+ (O.S)			1	0.333	0.358	-0.127	0.267	0.381	.689(*)	-0.374	-0.438	0.185
K^+ (Y.L)				1	0.312	0.283	.910(**)	0.389	0.539	0.412	-0.443	0.213
K^+ (Y.P)					1	0.481	0.079	0.099	0.132	-0.056	0.201	0.48
K^+ (Y.S)						1	0.061	-0.463	0.007	0.146	0.202	.881(**)
K^+/Na^+ (O.L)							1	0.525	0.456	0.332	-0.521	0
K^+/Na^+ (O.P)								1	0.554	0.054	-0.125	-0.366
K^+/Na^+ (O.S)									1	-0.11	-0.536	0.253
K^+/Na^+ (Y.L)										1	0.255	-0.121
K^+/Na^+ (Y.P)											1	0.037
K^+/Na^+ (Y.S)												1

Table 3.3.9 Correlation of plant Ca^{++} concentration in young and old leaf, petiole and stem. (young leaf = Y.L, young petiole = Y.P, young stem = Y.S, old leaf = O.L, old petiole = O.P and old stem = O.S).

	Ca++ (O.L)	Ca++ (O.P)	Ca++ (O.S)	Ca++ (Y.L)	Ca++ (Y.P)	Ca++ (Y.S)
Plant height	-0.002	-0.173	0.252	0.521	0.13	0.102
Nodes/plant	0.343	0.441	0.515	0.393	0.434	0.272
Fresh weight	0.617	.641(*)	.692(*)	0.178	0.052	0.323
Dry weight	.726(*)	0.569	0.613	0.334	-0.14	0.352
Chlorophyll content	.907(**)	.634(*)	0.365	0.561	-0.257	0.439
Transpiration rate	.701(*)	.754(*)	0.309	0.002	-0.179	-0.02
Stomatal conductance	0.08	-0.2	-0.469	-0.009	-0.483	-0.08
CO2 uptake	.662(*)	0.578	0.462	0.319	-0.186	0.322
Na^+ (O.L)	-0.505	-0.54	0.126	-0.02	0.293	-0.104
Na^+ (O.P)	0.103	-0.113	0.024	0.328	-0.052	0.188
Na^+ (O.S)	-0.21	-0.256	-0.451	-0.197	-0.079	-0.128
Na^+ (Y.L)	0.322	0.341	0.512	-0.155	-0.046	0.002
Na^+ (Y.P)	.810(**)	.740(*)	0.156	0.051	-0.524	-0.035
Na^+ (Y.S)	0.261	0.059	0.179	0.61	0.251	.943(**)
K^+ (O.L)	.829(**)	.722(*)	0.099	0.273	-0.444	0.091
K^+ (O.P)	0.462	0.453	0.069	-0.173	-0.403	-0.047
K^+ (O.S)	0.378	0.52	.867(**)	-0.06	0.201	-0.125
K^+ (Y.L)	.787(**)	0.553	0.159	0.522	-0.362	0.346
K^+ (Y.P)	0.326	0.372	0.481	0.443	0.527	0.555
K^+ (Y.S)	0.253	0.021	0.068	.709(*)	0.218	.975(**)
K^+/Na^+ (O.L)	.801(**)	.697(*)	0.08	0.248	-0.447	0.11
K^+/Na^+ (O.P)	0.129	0.356	0.141	-0.364	-0.09	-0.417
K^+/Na^+ (O.S)	0.237	0.255	0.467	0.07	-0.063	-0.029
K^+/Na^+ (Y.L)	0.006	-0.189	-0.387	0.472	-0.137	0.116
K^+/Na^+ (Y.P)	-0.571	-0.53	-0.28	-0.047	0.39	0.212
K^+/Na^+ (Y.S)	0.18	0.076	0.248	0.443	0.218	.827(**)
Ca++ (O.L)	1	.841(**)	0.347	0.424	-0.264	0.342
Ca++ (O.P)		1	0.523	0.086	0.033	0.066
Ca++ (O.S)			1	0.169	0.578	0.041
Ca++ (Y.L)				1	0.191	.711(*)
Ca++ (Y.P)					1	0.138
Ca++ (Y.S)						1

Table 3.3.10. Mean square, degree of freedom and P value of cotton genotypes.

Variables	Genotypes	DF	Treatment	DF	Genotypes x Treatments	DF
Plant height	205.680^{NS}	9	8727.842**	1	125.520^{NS}	9
Nodes/plant	6.696**	9	21.013**	1	3.763**	9
FW	1772.807**	9	33526.176**	1	934.895^{NS}	9
DW	82.931**	9	1377.551**	1	35.005^{NS}	9
Chlorophyll content	1102.011**	9	3333.362**	1	115.835^{NS}	9
Transpiration rate	2.160*	9	76.421**	1	.636^{NS}	9
Stomatal conductance	17394.400^{NS}	9	1761178.608**	1	14332.956^{NS}	9
CO_2 uptake	11.484^{NS}	9	936.396**	1	5.810^{NS}	9
Na^+ (y.l)	2376.528^{NS}	9	245593.744**	1	2385.782^{NS}	9
K^+ (y.l)	1060.299^{NS}	9	5042.694**	1	512.972^{NS}	9
K^+/Na^+ (y.l)	7946.335^{NS}	9	472028.650**	1	7815.801^{NS}	9
Ca^{++} (y. l)	554.763^{NS}	9	2075.194^{NS}	1	702.645^{NS}	9
Na^+ (y.p)	2750.782^{NS}	9	188587.621**	1	2364.891^{NS}	9
K^+ (y.p)	1062.337^{NS}	9	12672.466**	1	1711.928^{NS}	9
K^+/Na^+ (y.p)	18609.199^{NS}	9	988506.265**	1	18108.758^{NS}	9
Ca^{++} (y. p)	332.053^{NS}	9	9520.066**	1	622.717^{NS}	9
Na^+ (y.s)	3389.356^{NS}	9	363784.851**	1	3391.563^{NS}	9
K^+(y.s)	509.381^{NS}	9	10798.466**	1	1497.795^{NS}	9
K^+/Na^+ (y.s)	55328.159**	9	1139587.461**	1	55106.066**	9
Ca^{++} (y. s)	302.683	9	211.738	1	567.551	9
Na^+ (o.l)	1710.971^{NS}	9	841520.184**	1	1013.264^{NS}	9
K^+ (o.l)	4788.139**	9	94464.231**	1	444.375^{NS}	9
K^+/Na^+ (o.l)	3828.776^{NS}	9	13665.685*	1	3784.818^{NS}	9
Ca^{++} (o. l)	2159.946^{NS}	9	40259.608**	1	898.464^{NS}	9
Na^+ (o.p)	2329.035	9	476852.822	1	1991.422	9
K^+ (o.p)	4632.694**	9	7879.954**	1	949.399^{NS}	9
K^+/Na^+ (o.p)	3702.434^{NS}	9	62119.904**	1	3680.618^{NS}	9
Ca^{++} (o. p)	1676.027**	9	6980.716**	1	306.071^{NS}	9
Na^+ (o.s)	2422.035NS	9	165904.497**	1	1687.966^{NS}	9
K^+ (o.s)	1982.203**	9	7052.829**	1	869.398^{NS}	9
K^+/Na^+ (o.s)	787.445**	9	27363.850**	1	737.794**	9
Ca^{++} (o. s)	512.359^{NS}	9	750.925^{NS}	1	380.682^{NS}	9

3.3.5. Principal component analysis of phenotypes.

Application of PCA to all the mean values of five physiological parameters (fresh weight, nodes per plant, K^+/Na^+ ratio in young and old stem and old petiole) generated (Fig. 3.3.4.1). The clearest distinguishing traits are generated by PC1 and PC2 (which accounts for 71% and 30% of the total variance respectively). Here we see varieties B 756, Qalandari, Karishma, CIM 1100, S 12, Sarmast and Mc Nair 220 (group I) grouping apart from Rehmani, NIAB 78 and MNH 93 (group II). Principal component 1 (PC1) is a function of fresh weight, nodes per plant and K^+/Na^+ ratio in young stem. Principal component 2 (PC2) is a function of K^+/Na^+ ratio in old stem and petiole. The Ward linkage correlation coefficient distance for these individual parameters is shown in Fig. 3.3.4.2. It is, perhaps, an expected division between the two PCs – for example fresh weight and nodes per plant being dependent of each other, and K^+/Na^+ being independent.

Comparison of the PCA groups I and II with the genetic dendrogram is interesting. Members of the Rehmani and NIAB 78 clade are found in same groups, as are members of the S12, Karishma and Mc.Nair 220 clade. Generating a relationship map based on the physiology confirms a relationship seen in the SSR dendrogram (Fig. 4.3.3.1) between Rehmani and NIAB 78, but not between these and MNH 93. There was a relationship between Qalandari, B756 and Sarmast, but not between these and S12, Karishma and Mc. Nair 220.

In conclusion, the physiological markers have to some extent confirmed the difference of varietal behaviour. They did not, however, lend themselves to being predictable from the deeper genetic information provided by the SSR dendrogram (Fig. 4.3.3.1).

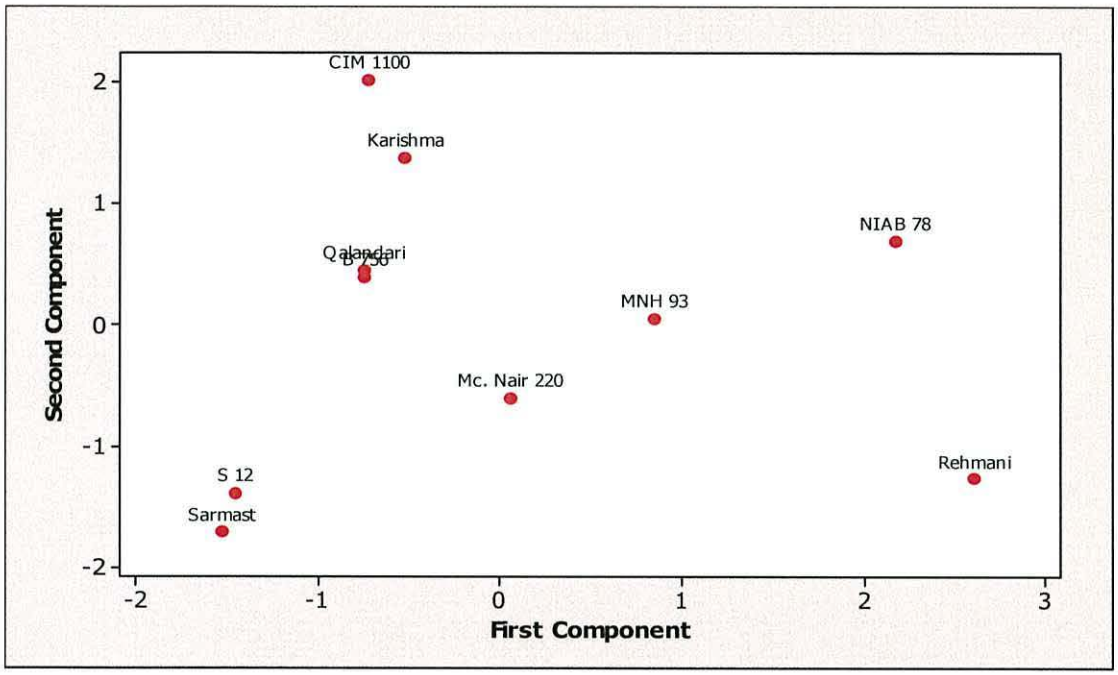

Fig.3.3.4.1. Scatter plot of sixteen cotton varieties based on principal component analysis generated by significant physiological traits.

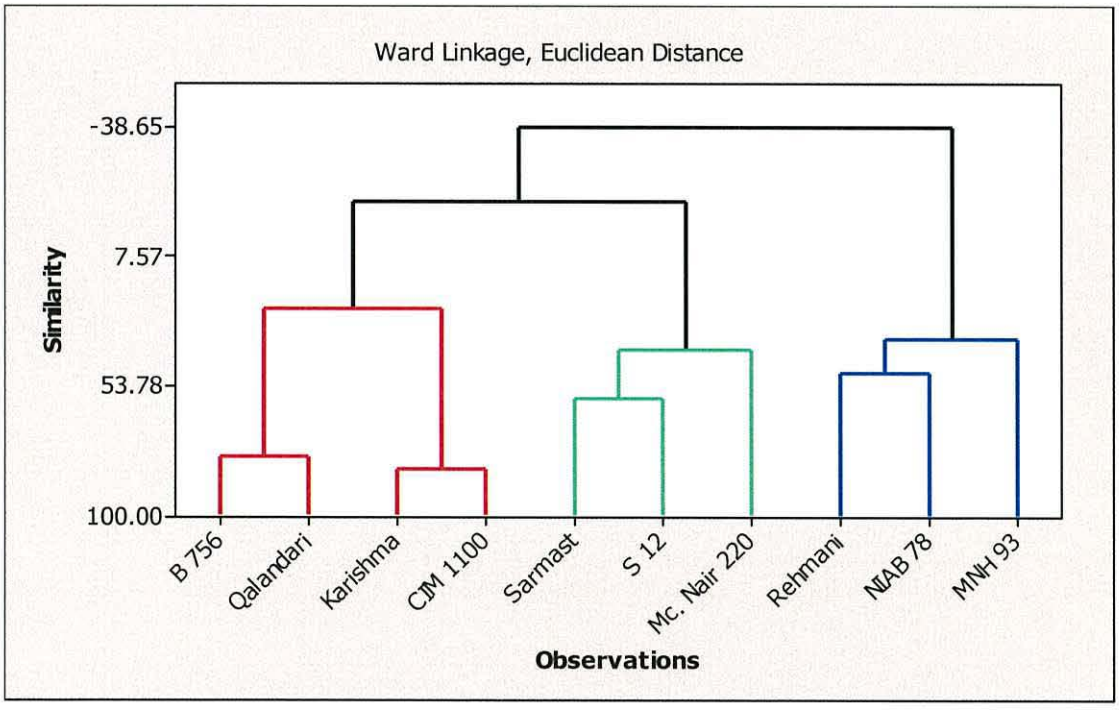

Fig.3.3.4.2. Dendrogram of sixteen cotton varieties based on principal component analysis generated by significant physiological traits.

3.4.1. A comparison of the response of three varieties of cotton stressed by direct addition of salt with responses obtained using the flood-bench system.

The flood – bench system described in all other sections of this thesis appeared to give satisfactory results. However, in this experiment we investigated the effect of sequential addition of sodium solution (top watering) to the plant soil. Unlike the situation on the flood benches, this treatment would be expected to result in a raised NaCl concentration as evapotranspiration remove water from the soil leaving salt behind. This was done as such a situation resembles that observed in the field in Pakistan where evaporation from the soil surface and transpiration from the crop create permanent contamination that is difficult to remove (see introduction).

3.4.2. Material and methods

The data from the flood bench experiment is taken from section 3.2.1 for varieties CIM 448, MNH 147 and their cross. These three varieties were again germinated on 27- 12 – 2007 in seed plug trays and after five days of germination ten seedlings (same size) of each variety were transplanted into 15 cm plastic pots, containing John Innes No. 1 compost. Growth room temperature was set at 30^0C and 16 hours day light. There were two treatments with two replications. Five plants of each variety were in each replication. Each pot was held in its individual pot saucer to prevent solution moving from plant to plant. Every day the plants were treated with a quantity of 150 mM NaCl and 7.5 mM $CaCl_2$ solution (Na: Ca ratio 20: 1) containing Phostrogen 1g l^{-1} (pbi Home & Garden Ltd, Middlesex) Table 2.1, micronutrients 0.5 ml l^{-1} of stock solution in chapter 2 (Table 2.2) and sodium silicate solution (BHD) 25% silicate 0.1 ml l^{-1} solution, that was not sufficient to wash the solution out of the soil. Any small quantity that did drain into the saucer was reabsorbed into the soil. As a result all the salt that was added accumulated in the soil and in the plant. The control plants were treated with the same solution lacking the 150 mM NaCl and 7.5 mM $CaCl_2$ [in Phostrogen, which contributed 6 mM K^+ and

0.37 mM Ca^{2+}]. After fifteen days the plants were harvested. For methods used in further analysis see chapter 2 (sections 2.4, 2.5 and 2.6).

Fig.3.4.1 Cotton genotypes with control and salt treatment.

3.4.3. Results

3.4.3.1. Plant height.

The tallest plant height was observed in 150 mM salt top watered treatment (WOF) as compare to 150 mM salt treatment used in WF with a range of 17% - 20% (Fig 3.4.3.1). We know that the Na^+ concentration of the WOF soil is high as compared to the concentration of the WF soil and that this would be expected to shorten the plants. However, the opposite were seen. This was probably due to the fact that the plants were much closer to each other in the WOF and low light intensity. There was less space for spreading the plants in this experiment. In the field the space in between plants is approximately 2.5 feet. It was observed from the data presented from Table 13 (see in Appendix) showed that the plant height showed significant negative correlation with Na^+ in old leaf and petiole and K^+ in old leaf at 15 day x 150 mM (without flood benches).

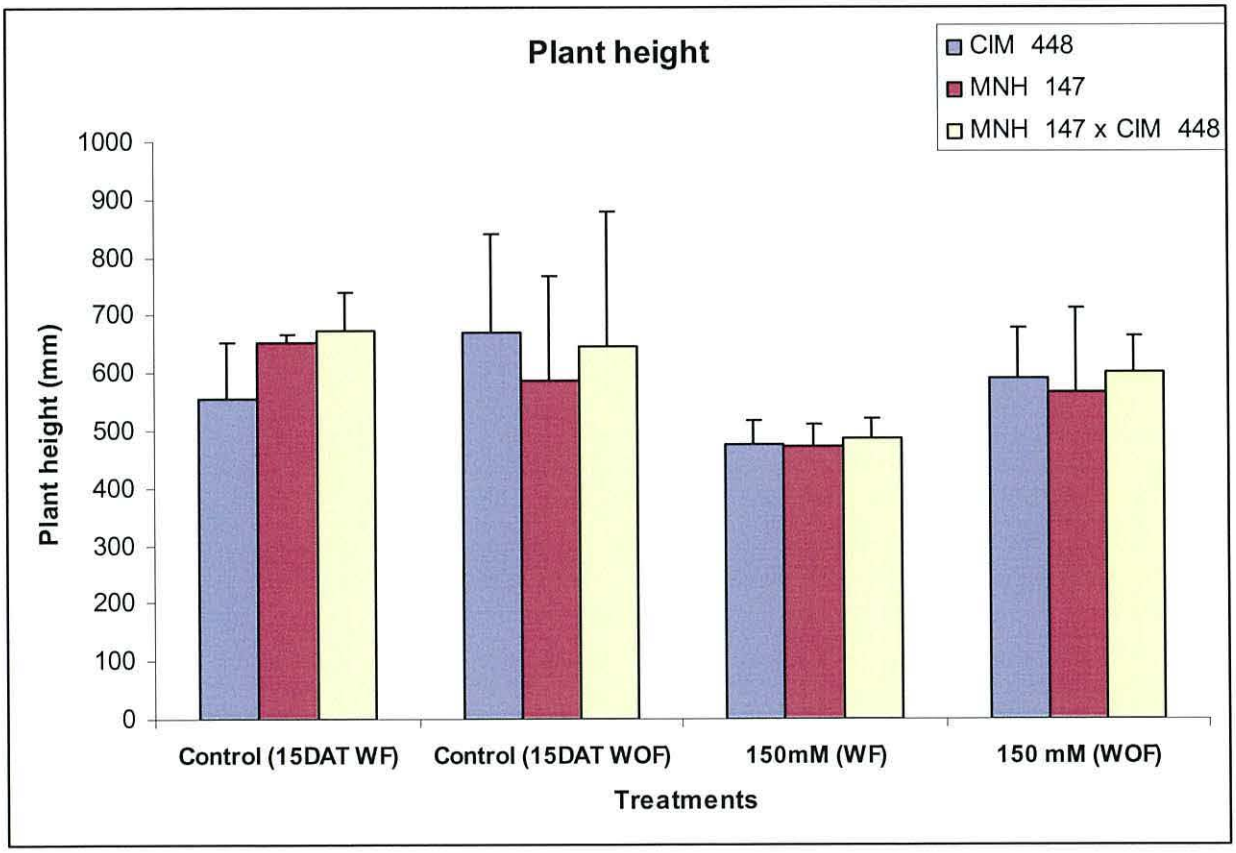

Fig.3.4.3.1. Effect of 150 mM NaCl with flood benches (WF) and 150 mM NaCl with out flood benches (WOF) on plant shoots height of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd (see in Appendix Table 15).

3.4.3.2. Nodes per plant.

The control top watered plants WOF which were also showed increase in number of nodes per plants at after 15 days to start treatment as compared to WF (Fig. 3.4.3.2). This result suggests that increase should be due to phostrogen which are absorbed in the soil of WOF control treatment. No differences were observed in 150 mM WF and WOF salt treatments. It was observed from the data presented from Table 13 (see in Appendix) showed that the nodes per plant showed no correlation with any trait at 15 day x 150 mM (without flood benches).

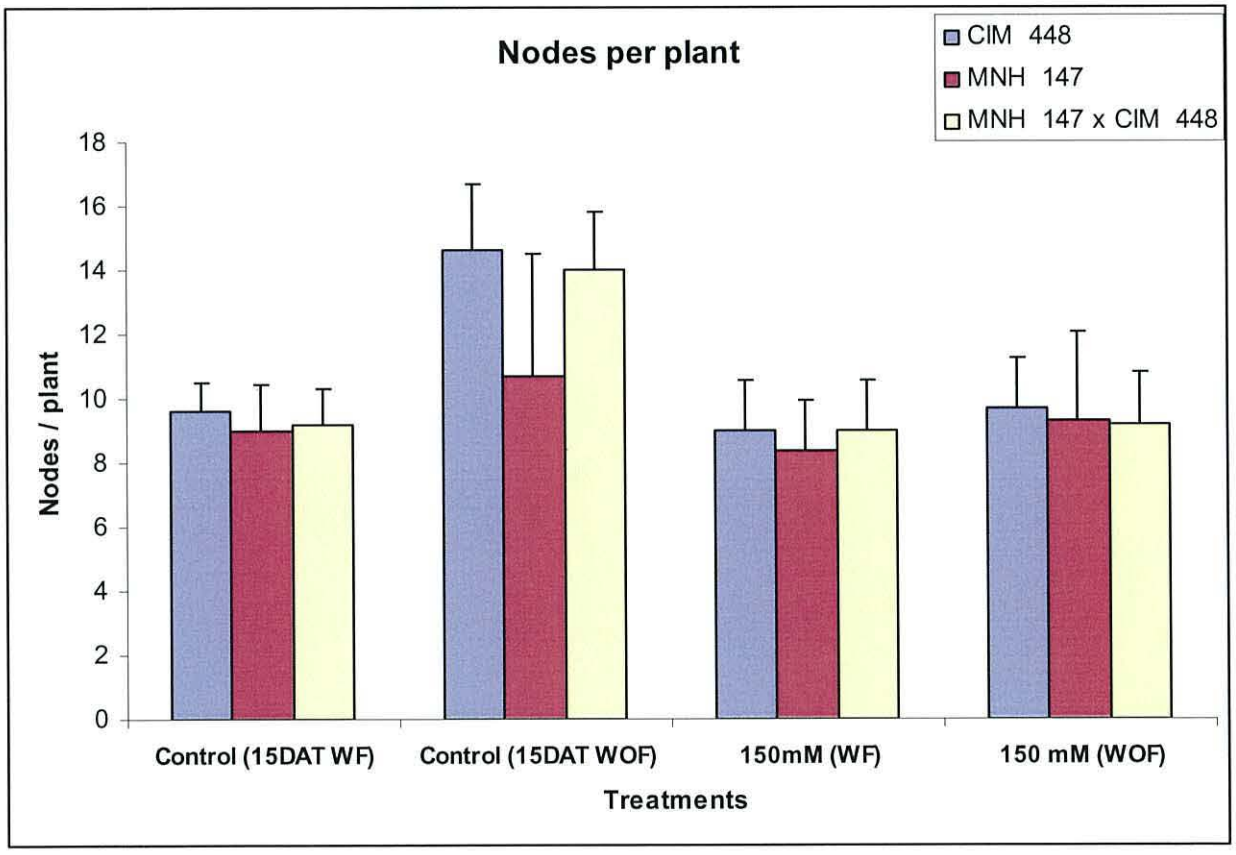

Fig.3.4.3.2. Effect of 150 mM NaCl with flood benches (WF) and 150 mM NaCl with out flood benches (WOF) on nodes per plant of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd (see in Appendix Table 15).

3.4.3.3. Fresh weight.

The control top watered plants WOF which were showed more reduction in fresh weight as compare to the control plants which was in WF after 15 days from to started treatment. (Fig. 3.4.3.3). This was probably due to low light intensity in growth room as compare to green house. In salt treatment (150 mM WOF) the plants reduced fresh weight as compare to150 mM WF. In this treatment plants reduced higher fresh weight than 150 mM WF treatment with a range of 74% -80%. This was probably due the fact that the plants were much closer to each other and less space for spreading the plants. It was observed from the data presented from Table 13 (see in Appendix) showed that the fresh weight showed no correlation with any trait at 15 day x 150 mM (without flood benches).

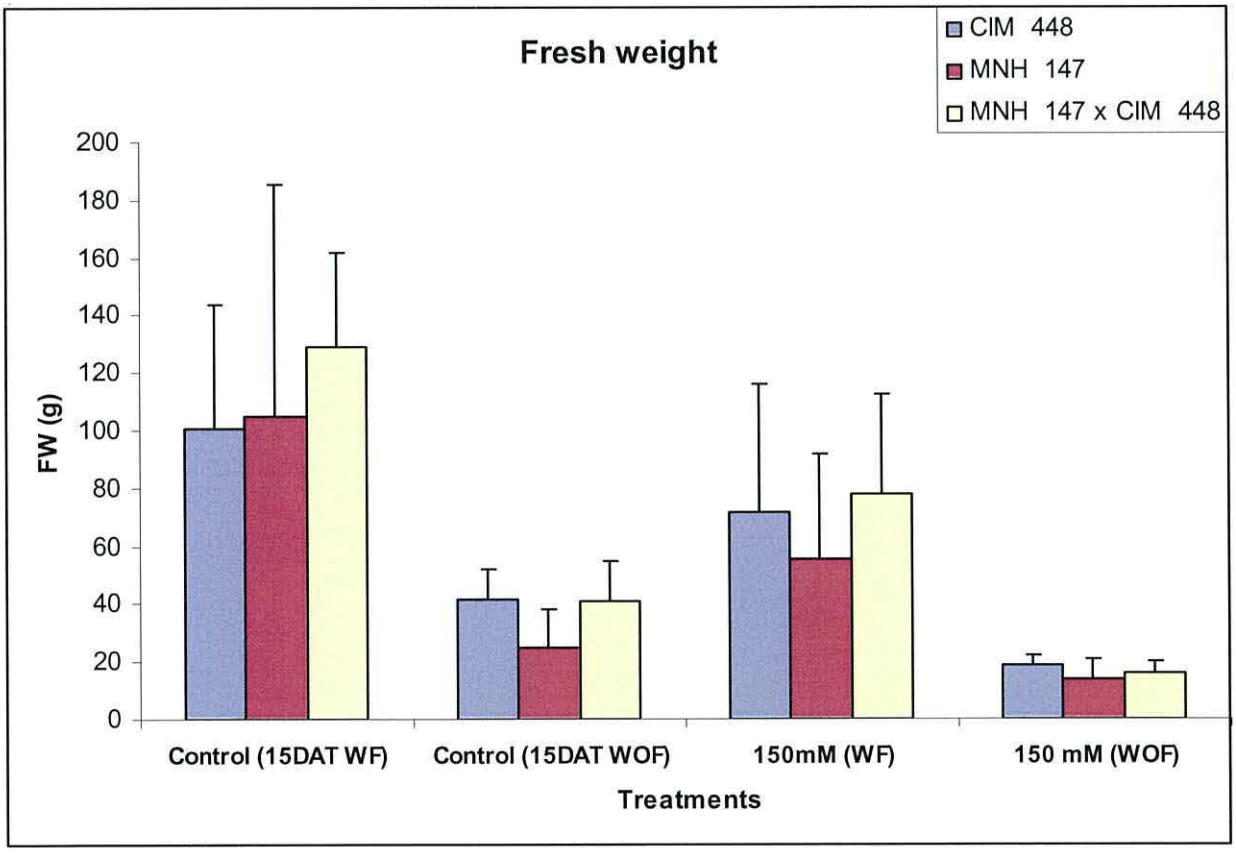

Fig.3.4.3.3. Effect of 150 mM NaCl with flood benches (WF) and 150 mM NaCl with out flood benches (WOF) on fresh weight of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd (see in Appendix Table 15).

3.4.3.4. Dry weight.

The control top watered plants WOF which were showed more reduction in dry weight as compare to the control plants which was in WF after 15 days from to started treatment (Fig. 3.4.3.4). This was probably due to low light intensity in growth room as compare to green house. It was observed that in salt treatment 150 mM WOF plants was reduced more dry weight as compare to 150 mM WF salt treatments. It was due to the less fresh weight of WOF salt treated plants. It was observed from the data presented from Table 13 and 14 (see in Appendix) showed that the dry weight showed significant positive correlation with K^+ in young petiole and stem and highly significant negative correlation with K^+/Na^+ ratio in young stem at 15 day x 150 mM (without flood benches).

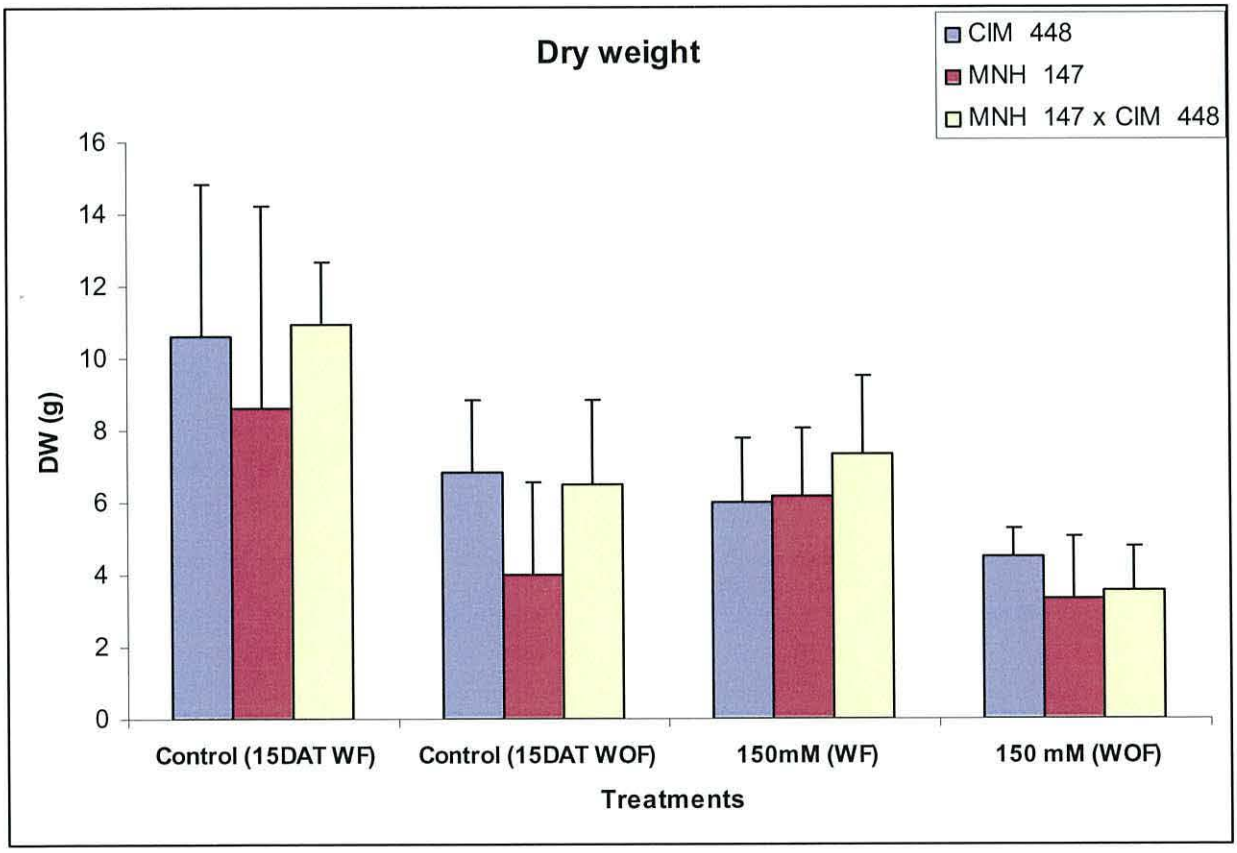

Fig.3.4.3.4. Effect of 150 mM NaCl with flood benches (WF) and 150 mM NaCl with out flood benches (WOF) on dry weight of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd (see in Appendix Table 15).

3.4.3.5. Na^+ concentration in the young leaf.

No difference was observed between both control treatments (Fig. 3.4.3.5). Na^+ concentration in young leaf of salt treated plants in treatments 150 mM WF was less as compare to150 mM WOF treatment. The plants used in treatment 150 mM WOF increases young leaf Na^+ concentration from treatment 150 mM WF with a range of 72% -87%. This was probably due to low light intensity in growth room as compare to green house. As we discussed above the salt concentration in top watered soil was high as compared to WF soil. It was observed from the data presented from Table 13 (see in Appendix) showed that the Na^+ concentration in young leaf showed high correlation with Na^+ in young petiole and stem, K^+ in young leaf, petiole and stem at 15 day x 150 mM (without flood benches).

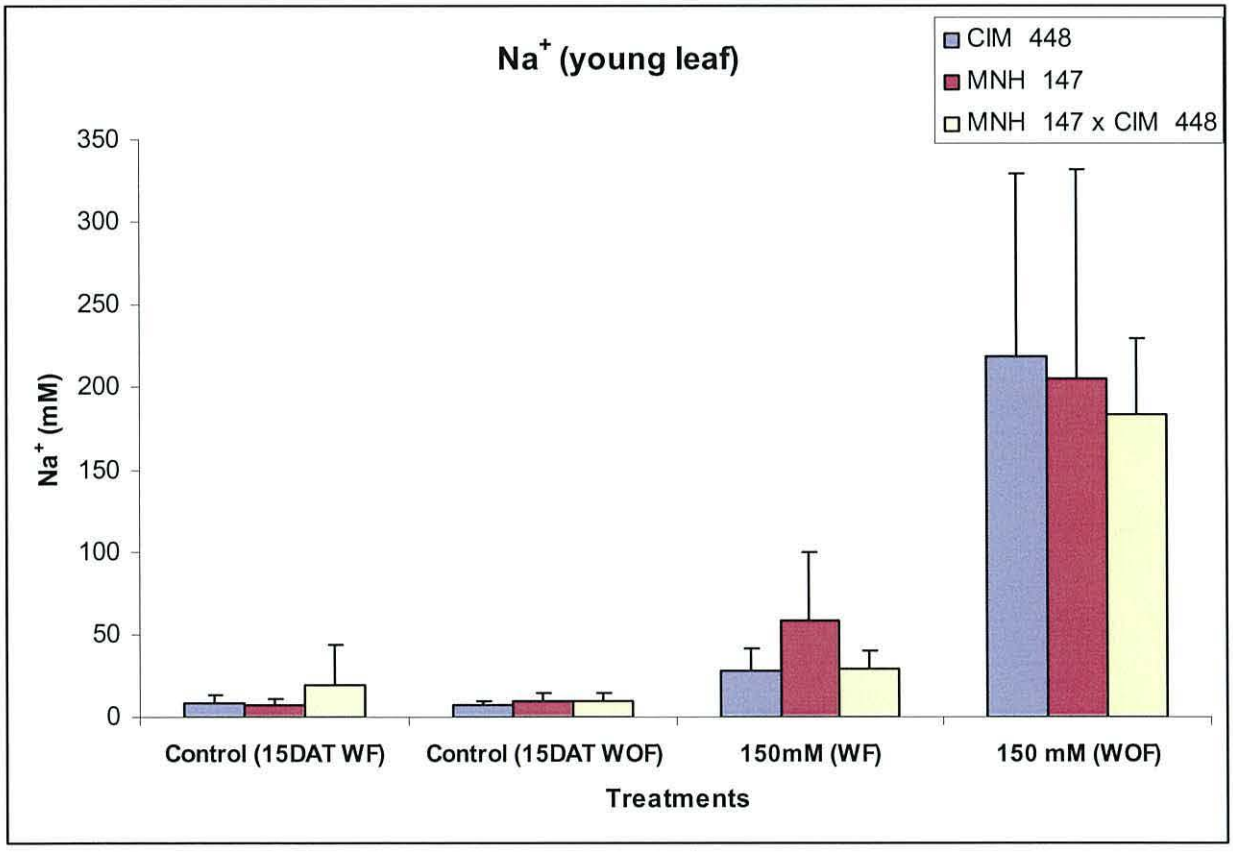

Fig.3.4.3.5. Effect of 150 mM NaCl with flood benches (WF) and 150 mM NaCl with out flood benches (WOF) on Na^+ concentration in young leaf of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd (see in Appendix Table 16).

3.4.3.6. K^+ concentration in the young leaf.

In control plants K^+ uptake was less at at 15 DAT WF as compare to 15 DAT WOF (Fig. 3.4.3.6). This was due to K^+ present in phostrogen, which increase the uptake of K^+ in control plants of WOF. K^+ concentration in young leaf of salt treated plants in treatments 150 mM WF was less as compare to150 mM WOF treatment. The plants used in treatment 150 mM WOF increases young leaf K^+ concentration from 150 mM WF with a range of 10% - 43%. It was observed from the data presented from Table 13 and 14 (see in Appendix) showed that the K^+ concentration in young leaf showed highly correlation with K^+/Na^+ ratio in young leaf and petiole at 15 day x 150 mM (without flood benches).

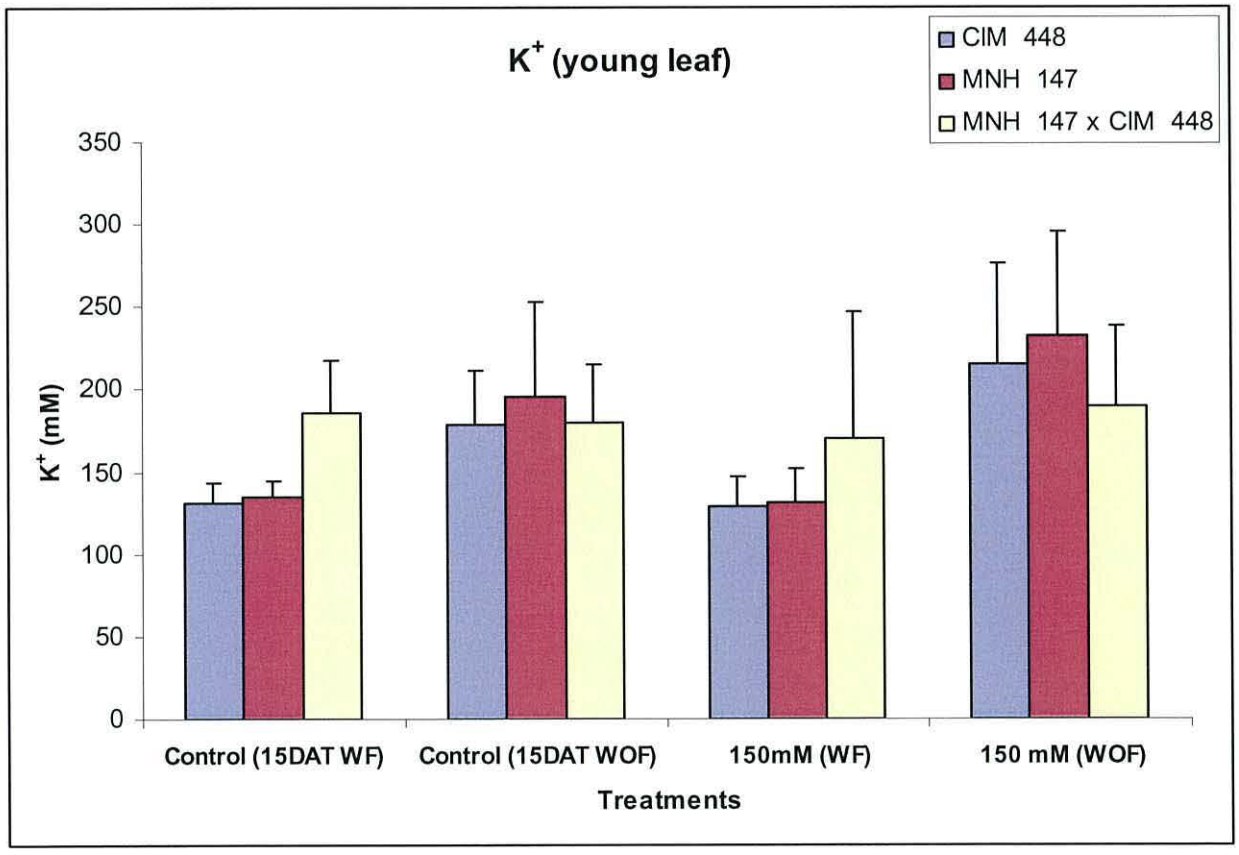

Fig.3.4.3.6. Effect of 150 mM NaCl with flood benches (WF) and 150 mM NaCl with out flood benches (WOF) on K^+ concentration in young leaf of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd (see in Appendix Table 17).

3.4.3.7. K^+/Na^+ ratio in the young leaf.

No difference was observed in both control treatment WF and WOF (Fig. 3.4.3.7). No differences were observed in young leaf K^+/Na^+ ratio in 150 mM WF and 150 mM WF salt treatments. The plants used in treatment 150 mM WOF decreases young leaf K^+/Na^+ ratio as compare to 150 mM WF with a range of 51% -83%. This was due to higher uptake of Na^+. It was observed from the data presented from Table 14 (see in Appendix) showed that the K^+/Na^+ ratio in young leaf showed highly positive correlation with K^+/Na^+ ratio in young petiole and stem at 15 day x 150 mM (without flood benches).

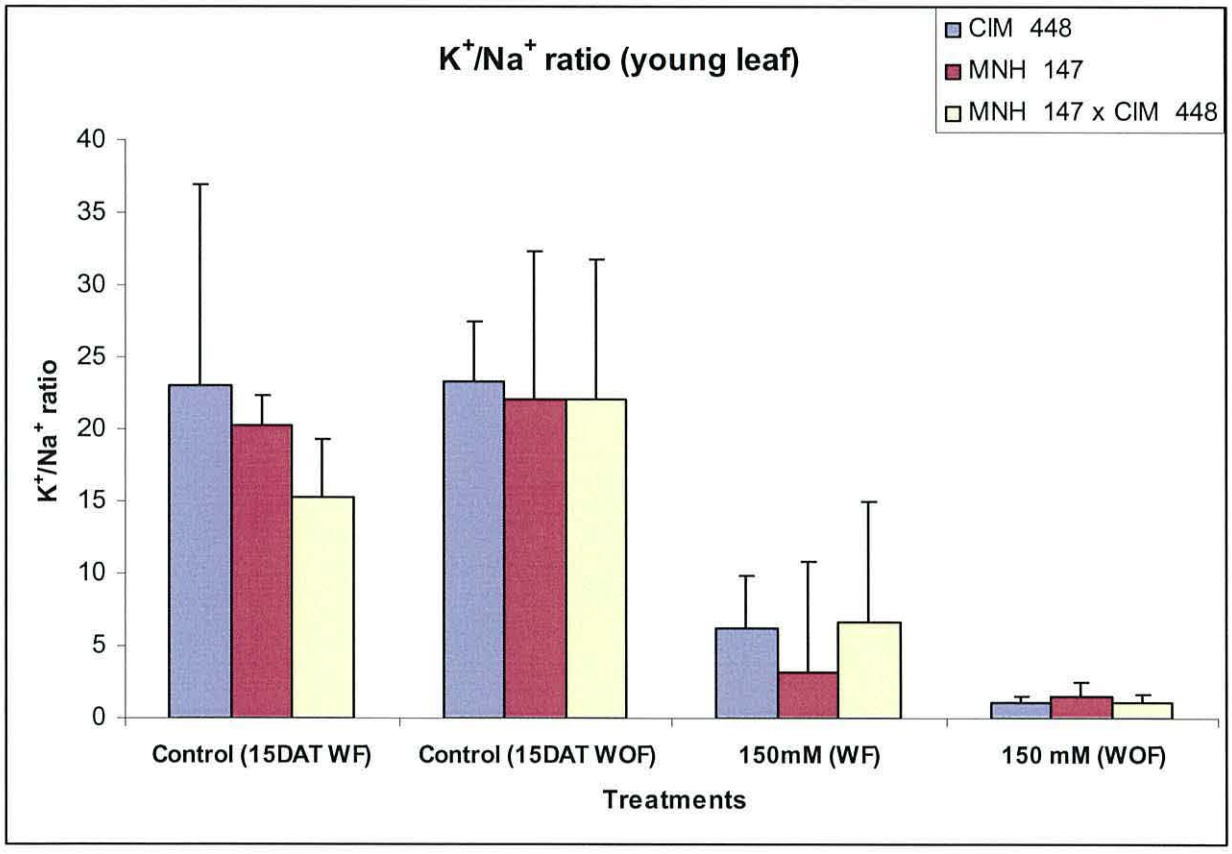

Fig. 3.4.3.7. Effect of 150 mM NaCl with flood benches (WF) and 150 mM NaCl with out flood benches (WOF) on K^+/Na^+ ratio in young leaf of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd (see in Appendix Table 18).

3.4.3.8. Na^+ concentration in the young petiole.

No difference was observed between both control treatments (Fig. 3.4.3.8). The plants showed difference to uptake Na^+ concentration in 150 mM WOF and 150 mM WF in salt treatments. The plants used in treatment 150 mM WOF increases young petiole Na^+ concentration from treatment 150 mM WF with a range of 72% -87%. As we discussed above the salt concentration in top watered soil was high as compared to WF soil. It was observed from the data presented from Table 13 (see in Appendix) showed that the Na^+ concentration in young petiole showed highly significant positive correlation with K^+ in young leaf at 15 day x 150 mM (without flood benches). It was observed from the data presented from Table 13 and 14 (see in Appendix) showed that the Na^+ concentration in young petiole showed highly significant correlation with K^+ in young leaf and K^+/Na^+ ratio in young leaf at 15 day x 150 mM (without flood benches).

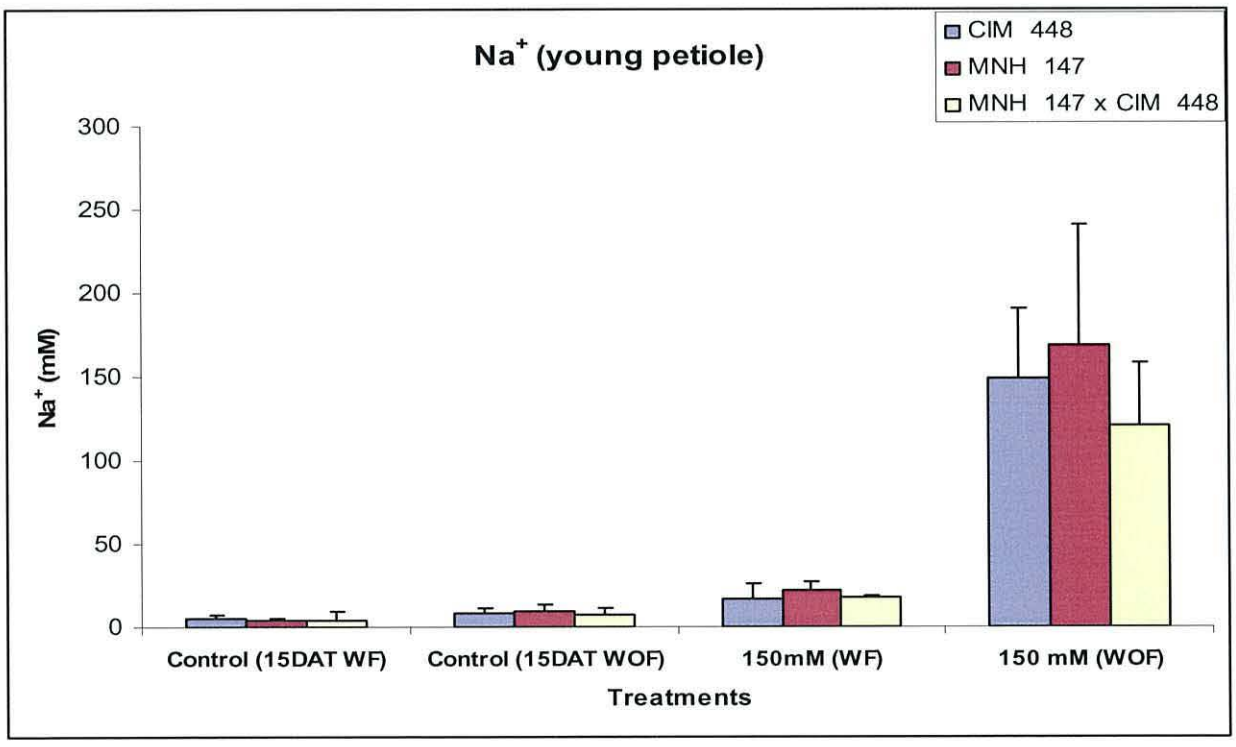

Fig.3.4.3.8. Effect of 150 mM NaCl with flood benches (WF) and 150 mM NaCl with out flood benches (WOF) on Na^+ concentration in young petiole of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd (see in Appendix Table 16).

3.4.3.9. K^+concentration in the young petiole.

It was observed that the K^+ content was less in15 DAT WF as compared to 15 DAT WOF in control treatment (Fig. 3.4.3.9). This was due to K^+ present in phostrogen, which increase the uptake of K^+ in control plants of WOF. In salt treatments (150 mM WF and 150 mM WOF) the plants were different from each other. The plants used in treatment 150 mM WOF increases young petiole K^+ concentration from 150 mM WF with a range of 26% - 36%. It was observed from the data presented from Table 13 and 14 (see in Appendix) showed that the K^+ concentration in young petiole showed significant correlation with K^+ in young stem and high correlation with K^+/Na^+ ratio in old leaf and petiole. However, significant negative correlation was observed in K^+/Na^+ ratio in young stem at 15 day x 150 mM (without flood benches).

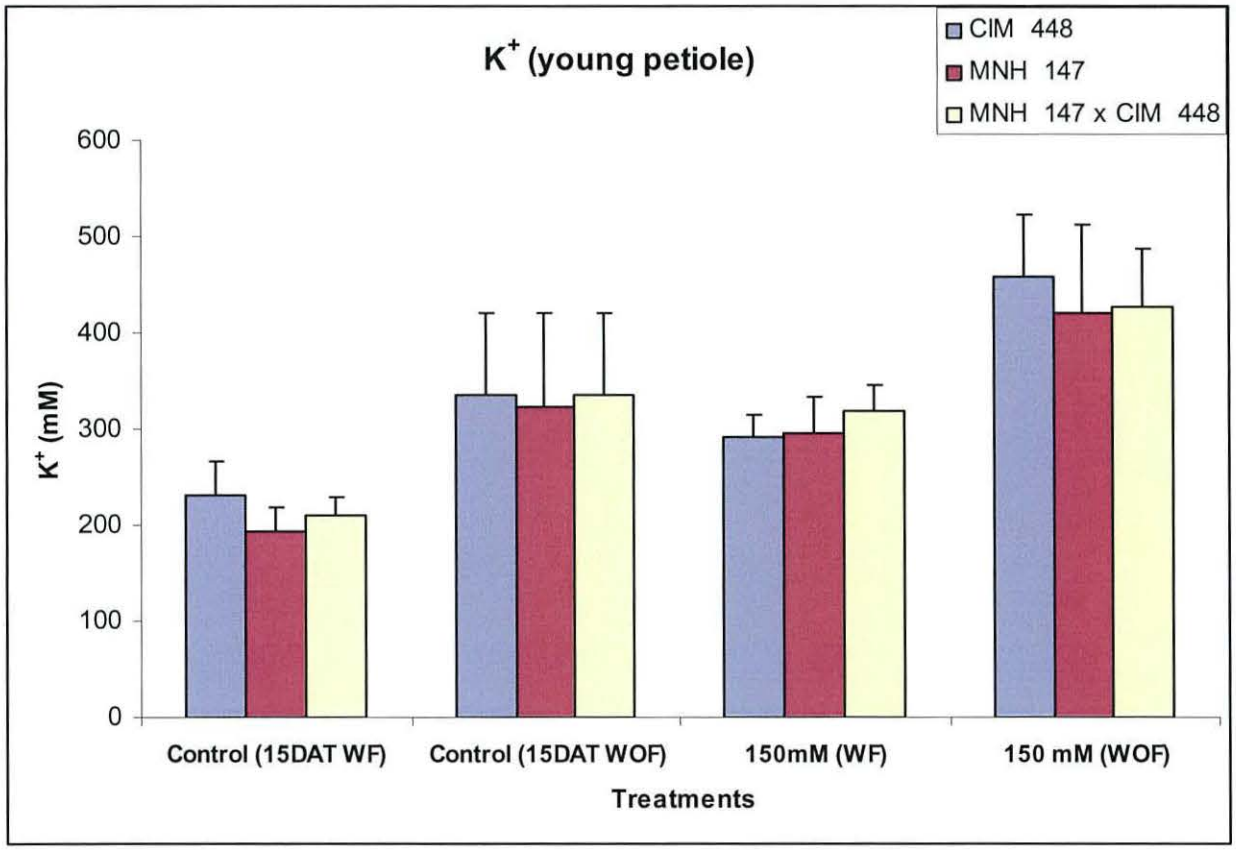

Fig.3.4.3.9. Effect of 150 mM NaCl with flood benches (WF) and 150 mM NaCl with out flood benches (WOF) on K^+ concentration in young petiole of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd (see in Appendix Table 17).

3.4.3.10. K^+/Na^+ ratio in the young petiole.

No difference was observed in both control treatment WF and WOF (Fig. 3.4.3.10). The young petiole K^+/Na^+ ratio was higher in 150 mM WF as compare to 150 mM WOF salt treatment. The plants used in treatment 150 mM WOF decreases young leaf K^+/Na^+ ratio from 150 mM WF with a range of 79% -85%. This was due to high concentration of Na^+ in WOF treatments. It was observed from the data presented from Table 14 (see in Appendix) showed that the K^+/Na^+ ratio in young petiole showed no correlation with any trait at 15 day x 150 mM (without flood benches).

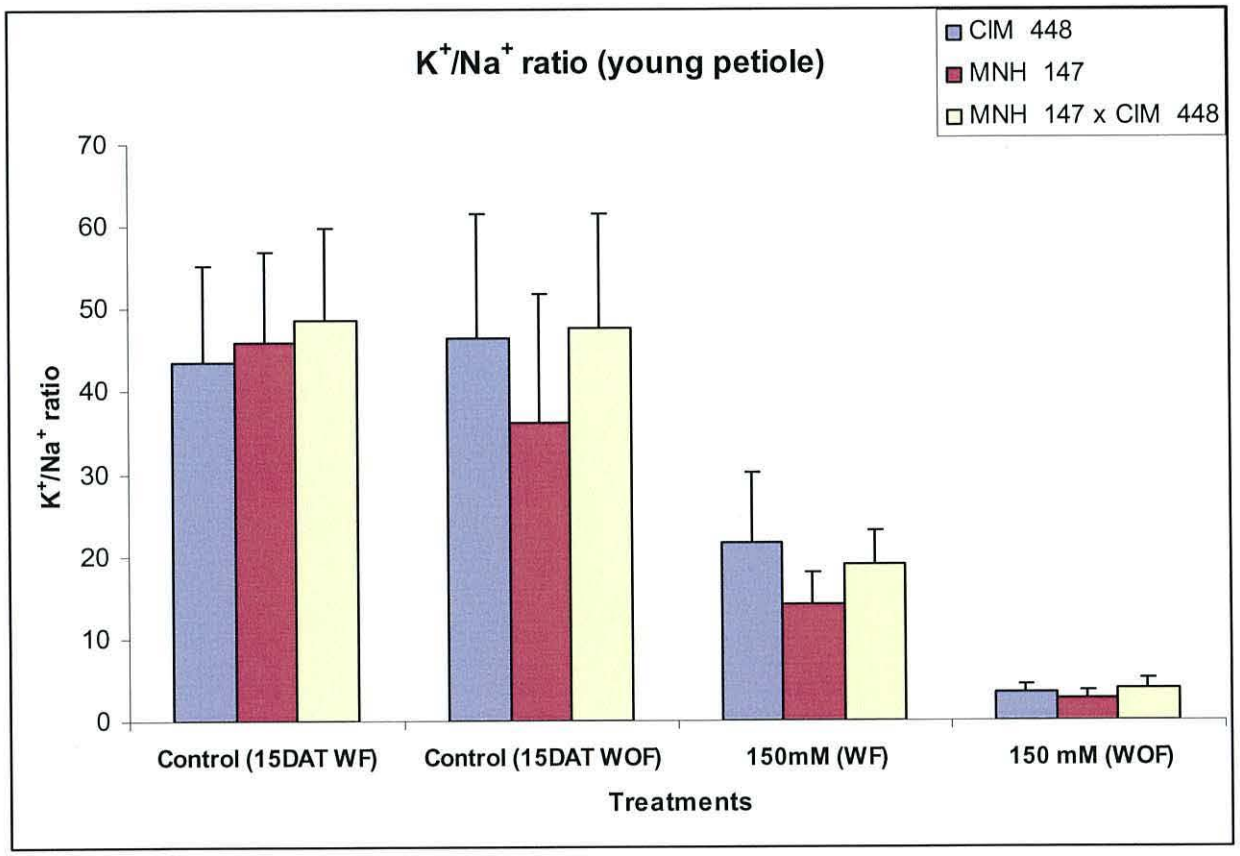

Fig.3.4.3.10. Effect of 150 mM NaCl with flood benches (WF) and 150 mM NaCl with out flood benches (WOF) on K^+/Na^+ ratio in young petiole of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd (see in Appendix Table 18).

3.4.3.11. Na^+ concentration in the young stem.

No difference was observed between both control treatments (Fig. 3.4.3.11). The plants showed difference to uptake Na^+ concentration in 150 mM WOF and 150 mM WF salt treatments. The plants used in treatment 150 mM WOF increases young stem Na^+ concentration from treatment 150 mM WF with a range of 85% -92%. As we discussed above the salt concentration in top watered soil was high as compared to WF soil. This was probably due to low light intensity in growth room as compare to green house. This result also suggests that the salt concentration in WOF pots were presume similar to WF 250 mM. It was observed from the data presented from Table 13 and 14 (see in Appendix) showed that the Na^+ concentration in young stem showed highly correlation with K^+ in old and young petiole and stem and K^+/Na^+ ratio in old leaf and petiole at 15 day x 150 mM (without flood benches).

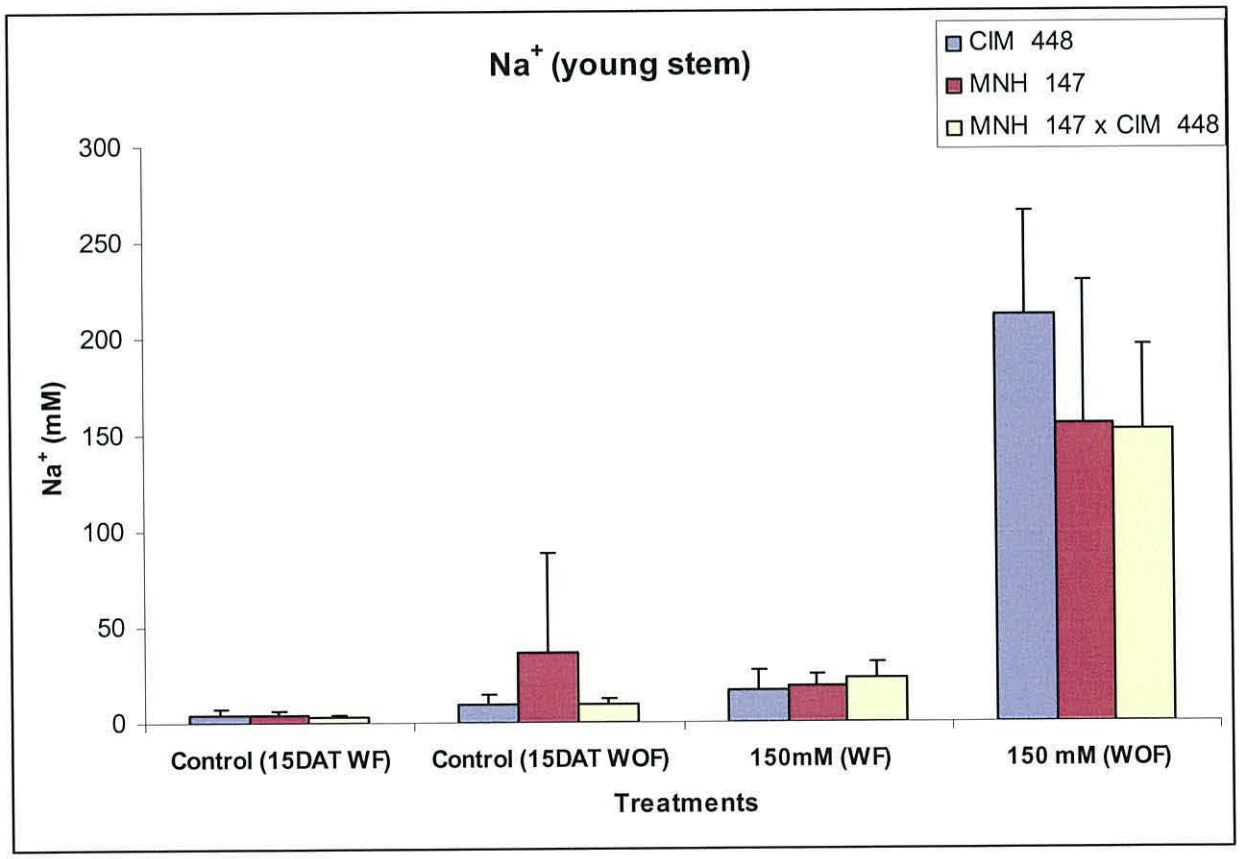

Fig.3.4.3.11. Effect of 150 mM NaCl with flood benches (WF) and 150 mM NaCl with out flood benches (WOF) on Na^+ concentration in young stem of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd (see in Appendix Table 16).

3.4.3.12. K^+ concentration in the young stem.

It was observed that the K^+ content was less in15 DAT WF as compared to 15 DAT WOF in control treatment (Fig. 3.4.3.12). This was due to K^+ present in phostrogen, which increase the uptake of K^+ in control plants of WOF. The plants used in treatment 150 mM WF and 150 mM WOF were different from each other. The plants used in treatment 150 mM WOF increases young stem K^+ concentration from 150 mM WF with a range of 25% - 40%. It was observed from the data presented from Table 13 and 14 (see in Appendix) showed that the K^+ concentration in young stem showed high correlation with K^+/Na^+ ratio in old leaf and petiole. However, significant negative correlation was observed in K^+/Na^+ ratio in young stem at 15 day x 150 mM (without flood benches).

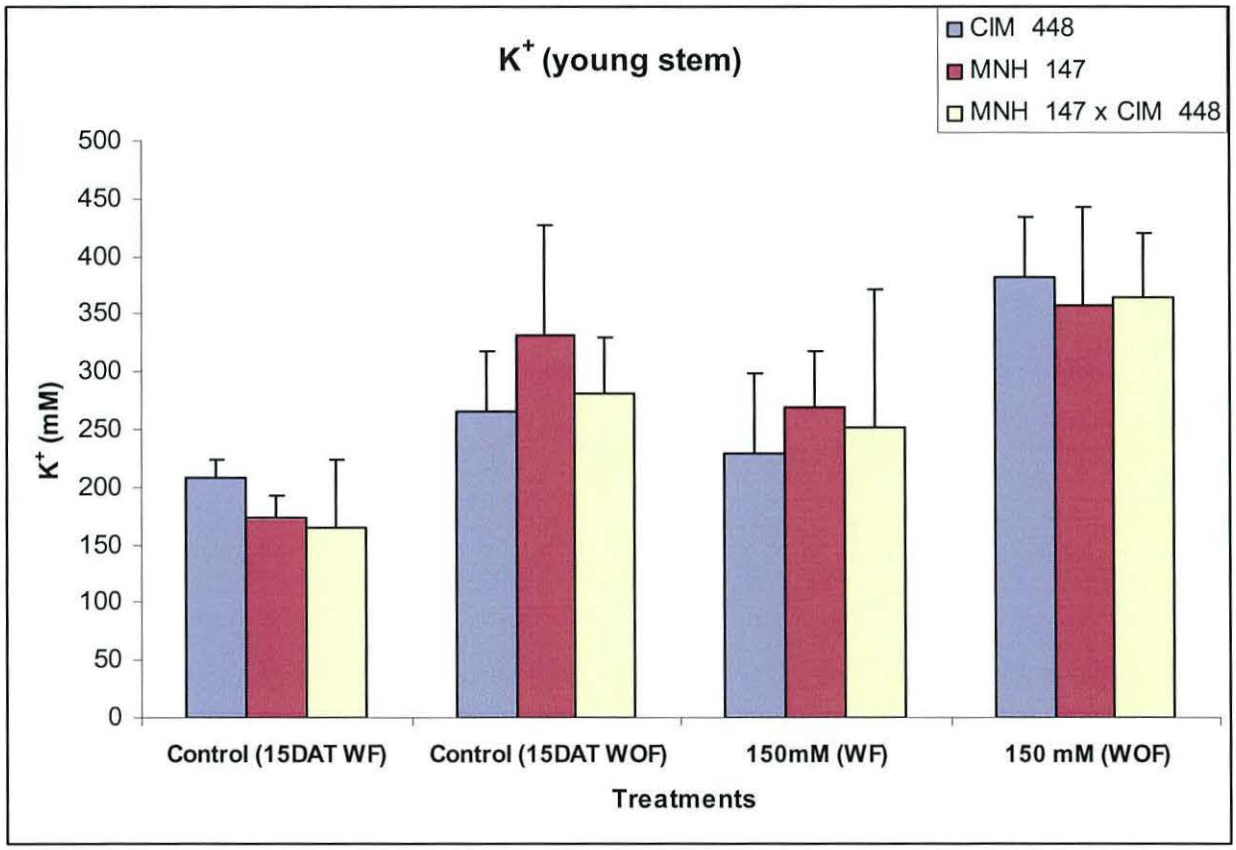

Fig.3.4.3.12. Effect of 150 mM NaCl with flood benches (WF) and 150 mM NaCl with out flood benches (WOF) on K^+ concentration in young stem of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd (see in Appendix Table 17).

3.4.3.13. K^+/Na^+ ratio in the young stem.

It was observed that the K^+/Na^+ ratio in young stem was less in control treatment of WOF as compare to WF control treatments (Fig. 3.4.3.13). The plants used in treatment 150 mM WF young petiole K^+/Na^+ ratio was higher as compare to 150 mM WOF salt treatment. The plants used in treatment 150 mM WOF decreases young leaf K^+/Na^+ ratio from 150 mM WF with a range of 80% -89%. This was due to high concentration of Na^+ in both WOF and 250 mM WF treatments. It was observed from the data presented from Table 14 (see in Appendix) showed that the K^+/Na^+ ratio in young stem showed no correlation with any trait at 15 day x 150 mM (without flood benches).

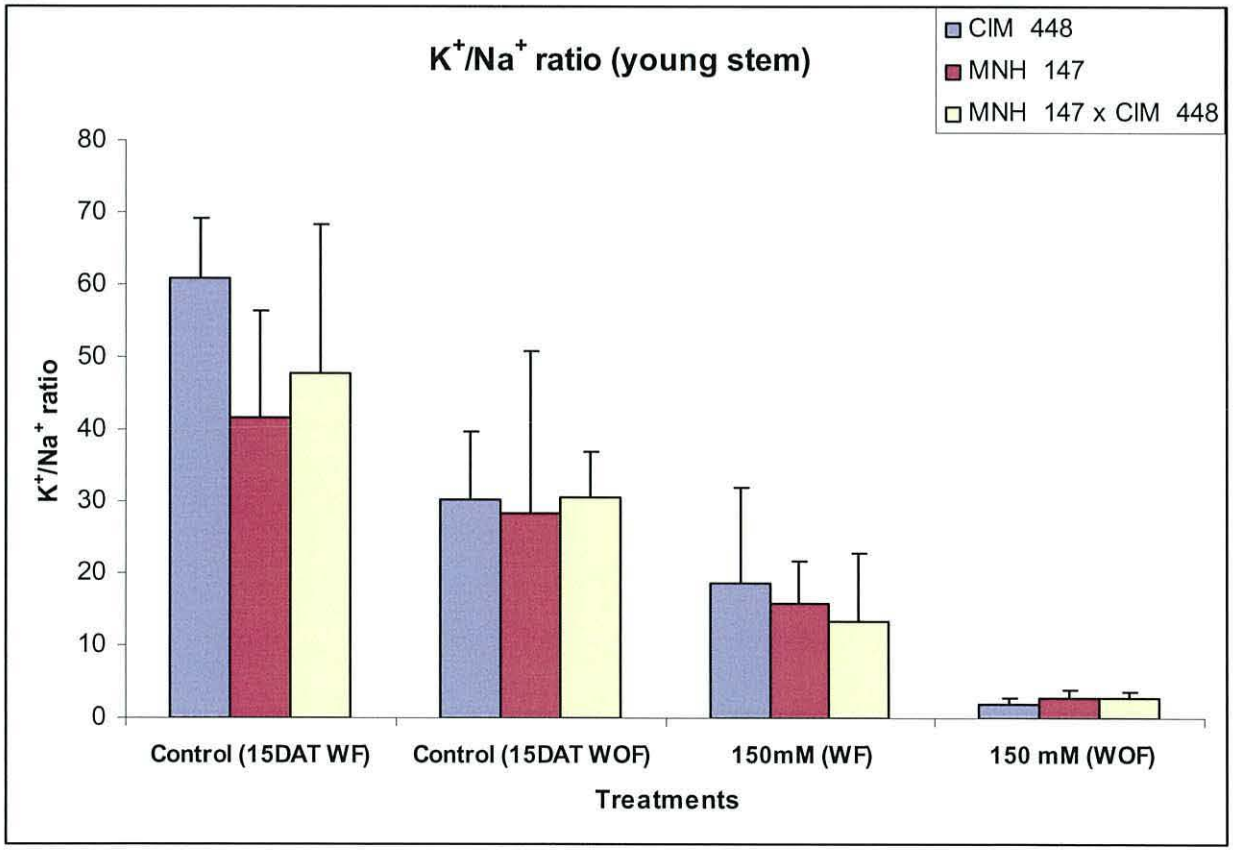

Fig.3.4.3.13. Effect of 150 mM NaCl with flood benches (WF) and 150 mM NaCl with out flood benches (WOF) on K^+/Na^+ ratio in young stem of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd (see in Appendix Table 18).

3.4.3.14. Na^+ concentration in the old leaf.

No difference was observed between both control treatments (Fig. 3.4.3.14). Treatments 150 mM WF and 150 mM WOF were different from each other. The plants used in treatment 150 mM WOF increases old leaf Na^+ concentration as compare to 150 mM WF salt treatment with a range of 28% -80%. As we discussed above the salt concentration in top watered soil was high as compared to WF soil. This was probably due to low light intensity in growth room as compare to green house. This result also suggests that the salt concentration in WOF pots were presume similar to WF 250 mM. It was observed from the data presented from Table 13 (see in Appendix) showed that the Na^+ concentration in old leaf showed highly significant positive correlation with K^+ in old leaf at 15 day x 150 mM (without flood benches).

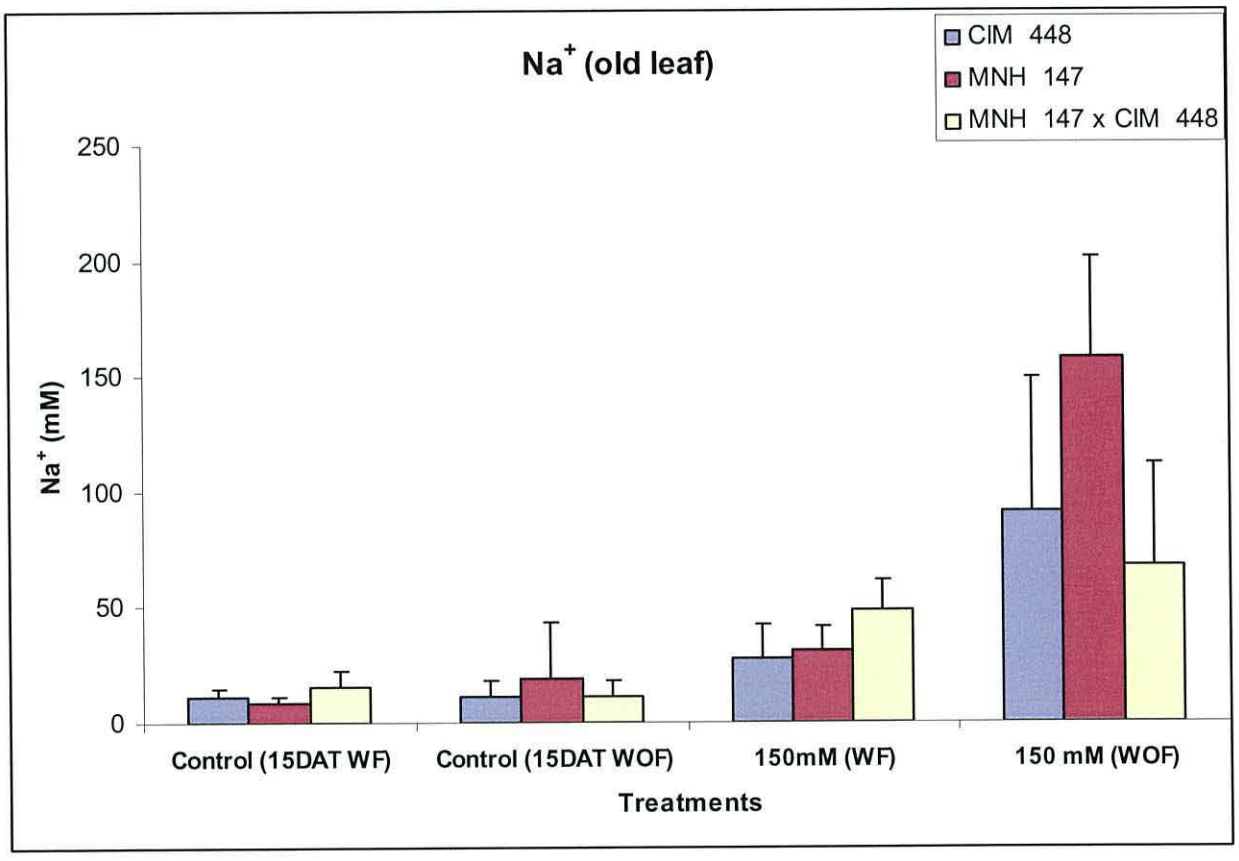

Fig.3.4.3.14. Effect of 150 NaCl with flood benches (WF) and 150 mM NaCl with out flood benches (WOF) on Na^+ concentration in old leaf of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd (see in Appendix Table 16).

3.4.3.15. K^+ concentration in the old leaf.

In control plants the K^+ uptake in old leaf was similar in both treatments. (Fig. 3.4.3.15). The plants used in treatment 150 mM WOF were different from 150 mM WF treatments. The plants used in treatment 150 mM WOF increases old leaf K^+ concentration as compare to 150 mM WF with a range of 52% - 61%. It was observed from the data presented from Table 13 and 14 (see in Appendix) showed that the K^+ concentration in old leaf showed highly correlation with K^+ in young leaf and K^+/Na^+ ratio in young leaf at 15 day x 150 mM (without flood benches).

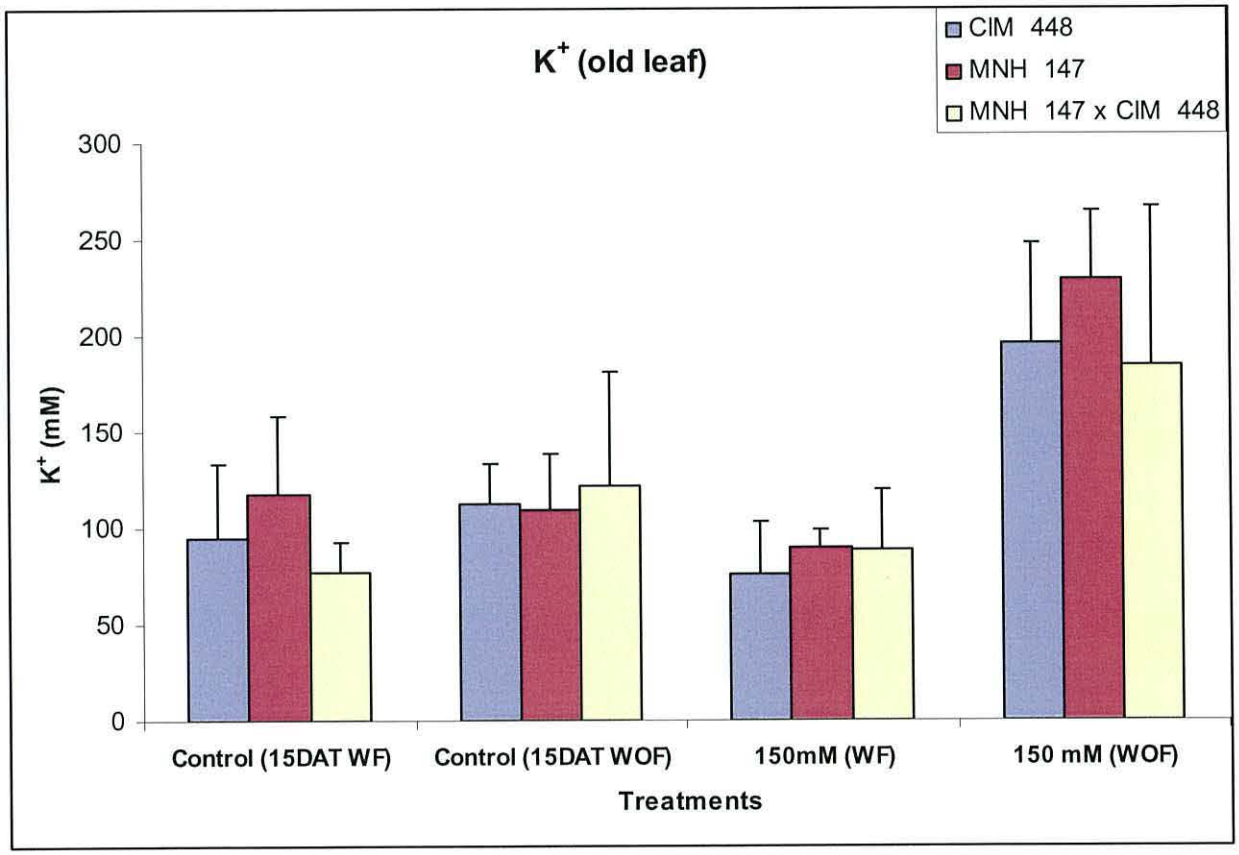

Fig.3.4.3.15. Effect of 150 mM NaCl with flood benches (WF) and 150 mM NaCl with out flood benches (WOF) on K^+ concentration in old leaf of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd (see in Appendix Table 17).

3.4.3.16. K^+/Na^+ ratio in the old leaf.

No difference was observed in old leaf K^+/Na^+ ratio in both control treatments (Fig. 3.4.3.16). No differences were observed in plants old leaf K^+/Na^+ ratio in 150 mM WF and WOF salt treatment. It was observed from the data presented from Table 14 (see in Appendix) showed that the K^+/Na^+ ratio in old leaf showed highly significant positive correlation with K^+/Na^+ ratio in old petiole highly significant negative correlation was observed in K^+/Na^+ ratio in young leaf at 15 day x 150 mM (without flood benches).

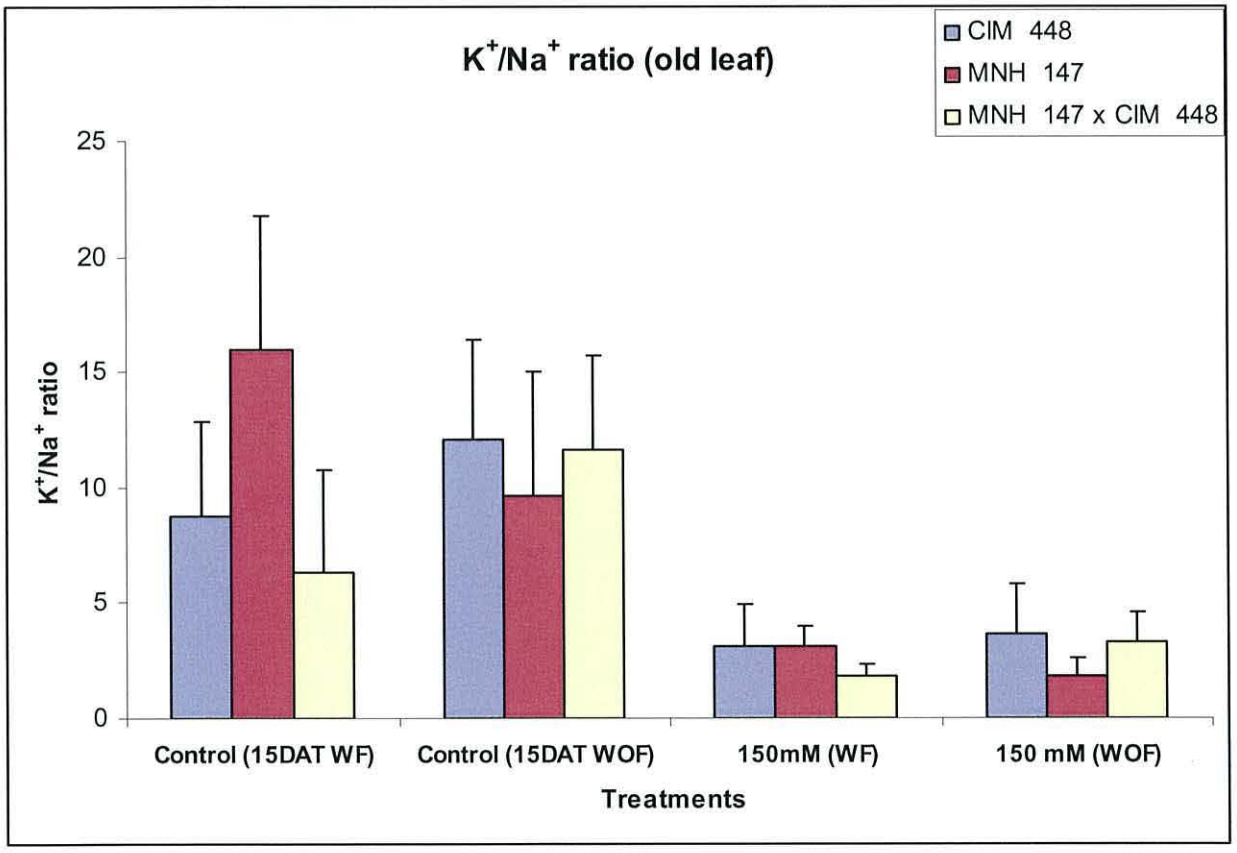

Fig.3.4.3.16. Effect of 150 mM NaCl with flood benches (WF) and 150 mM NaCl with out flood benches (WOF) on K^+/Na^+ ratio in old leaf of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd (see in Appendix Table 18).

3.4.3.17. Na^+ concentration in the old petiole.

No difference was observed between both control treatments (Fig. 3.4.3.17). The plants used in treatments 150 mM WF and 150 mM WOF were different from each other. The plants used in treatments 150 mM WOF increases old petiole Na^+ concentration from treatment 150 mM WF with a range of 64% -78%. As we discussed above the salt concentration in top watered soil was high as compared to WF soil. This result also suggests that the salt concentration in WOF pots were presume similar to WF 250 mM. It was observed from the data presented from Table 13 (see in Appendix) showed that the Na^+ concentration in old petiole showed positive correlation with Na^+ old stem and young petiole, K^+ in old and young leaf at 15 day x 150 mM (without flood benches).

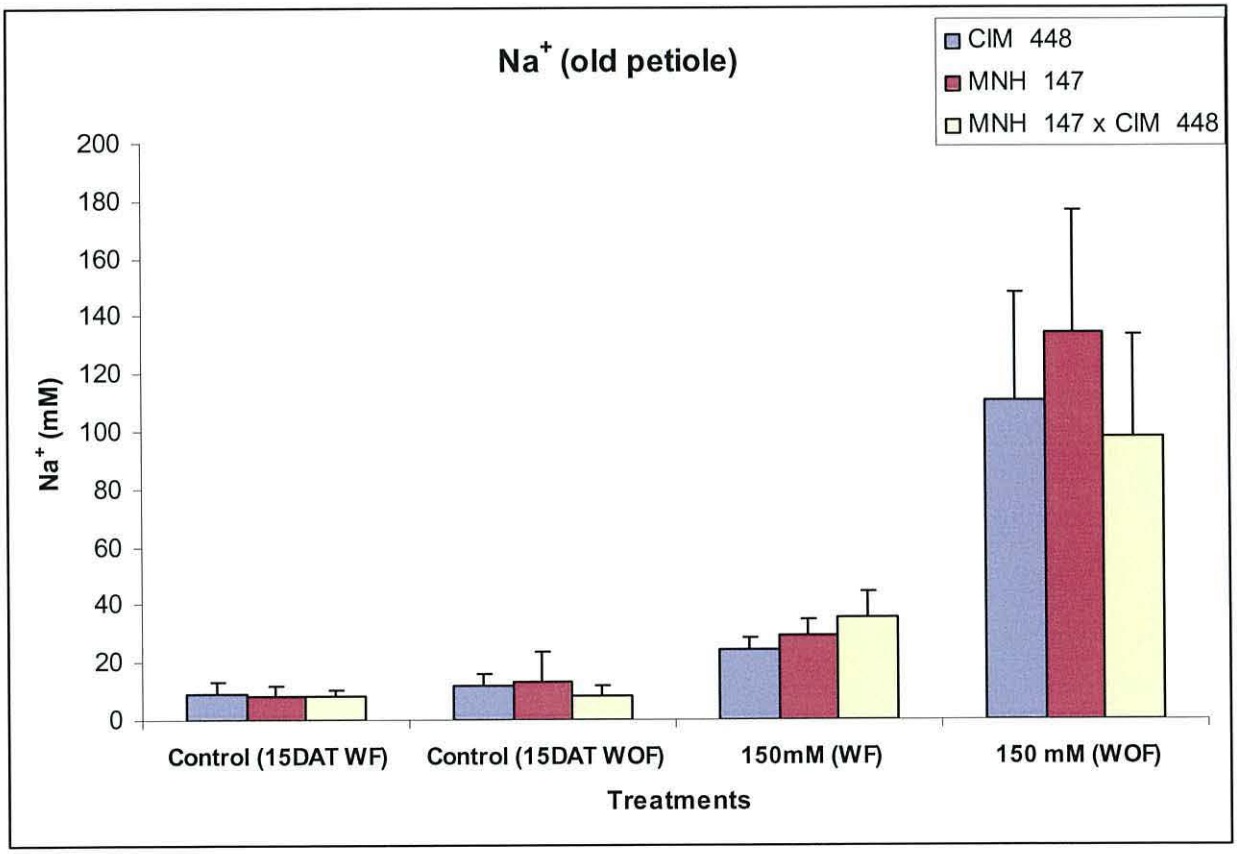

Fig.3.4.3.17. Effect of 150 mM NaCl with flood benches (WF) and 150 mM NaCl with out flood benches (WOF) on N^+ concentration in old petiole of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd (see in Appendix Table 16).

3.4.3.18. K^+ concentration in the old petiole.

In control plants K^+ uptake in old petiole in treatment 15 DAT WF was less as compare to 15 DAT WOF treatments. (Fig. 3.4.3.18). The plants used in treatment 150 mM WF were different from 150 mM WOF. The plants used in treatments 150 mM WOF without flood bench increases old petiole K^+ concentration as compare to 150 mM WF with a range of 24% -35%. It was observed from the data presented from Table 13 and 14 (see in Appendix) showed that the K^+ concentration in old petiole showed highly correlation with K^+ in old stem and young petiole and stem and significant correlation with K^+/Na^+ ratio in old leaf and petiole at 15 day x 150 mM (without flood benches).

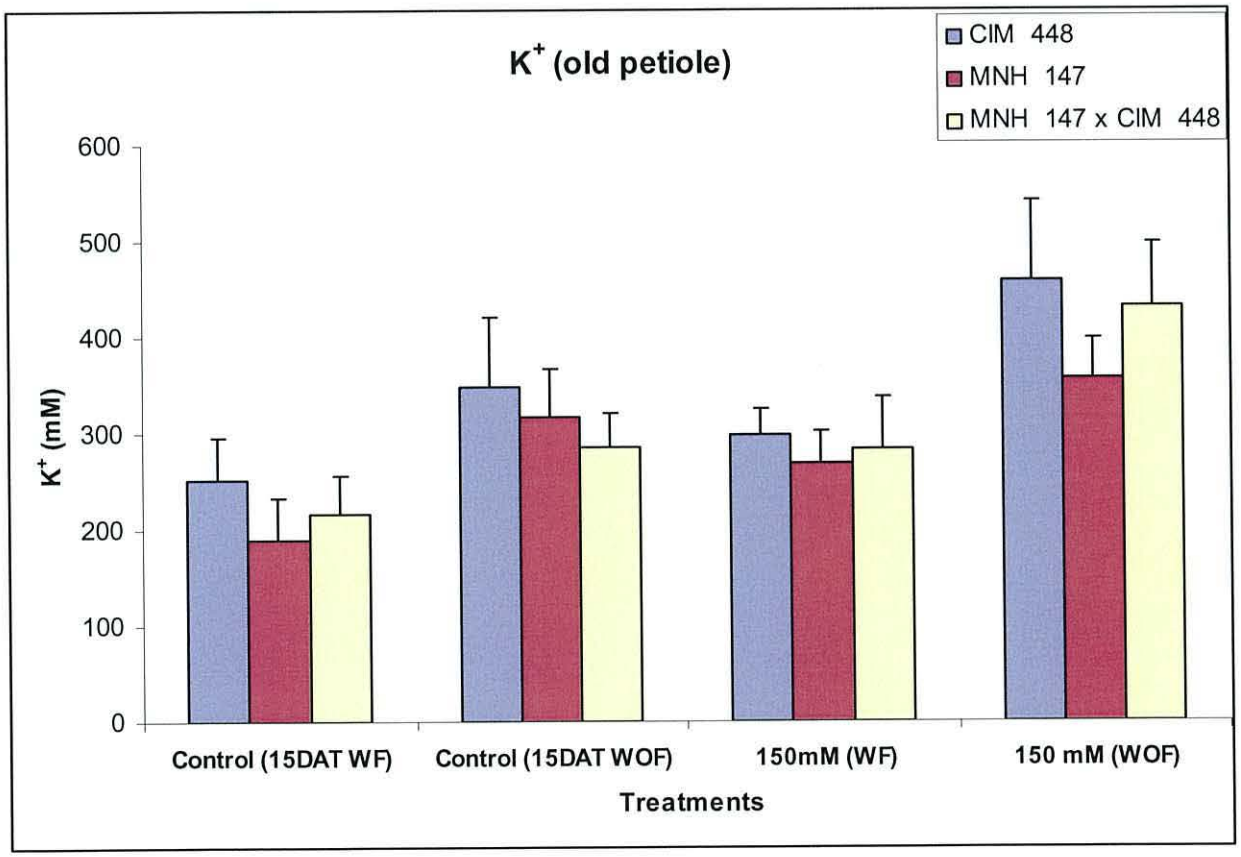

Fig.3.4.3.18. Effect of 150 mM NaCl with flood benches (WF) and 150 mM NaCl with out flood benches (WOF) on K^+ concentration in old petiole of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd (see in Appendix Table 17).

3.4.3.19. K^+/Na^+ ratio in the old petiole.

No difference was observed in old leaf K^+/Na^+ ratio in both control treatments (Fig. 3.4.3.19). The plants used in treatments 150 mM WF was different as compare to 150 mM WOF. The plants used in treatments 150 mM (WOF) decreases old petiole K^+/Na^+ ratio as compare to 150 mM WF with a range of 42% -70%. It was observed from the data presented from Table 14 (see in Appendix) showed that the K^+/Na^+ ratio in old petiole showed highly positive correlation with K^+/Na^+ ratio in young petiole and highly significant negative correlation was observed in K^+/Na^+ ratio in old petiole at 15 day x 150 mM (without flood benches).

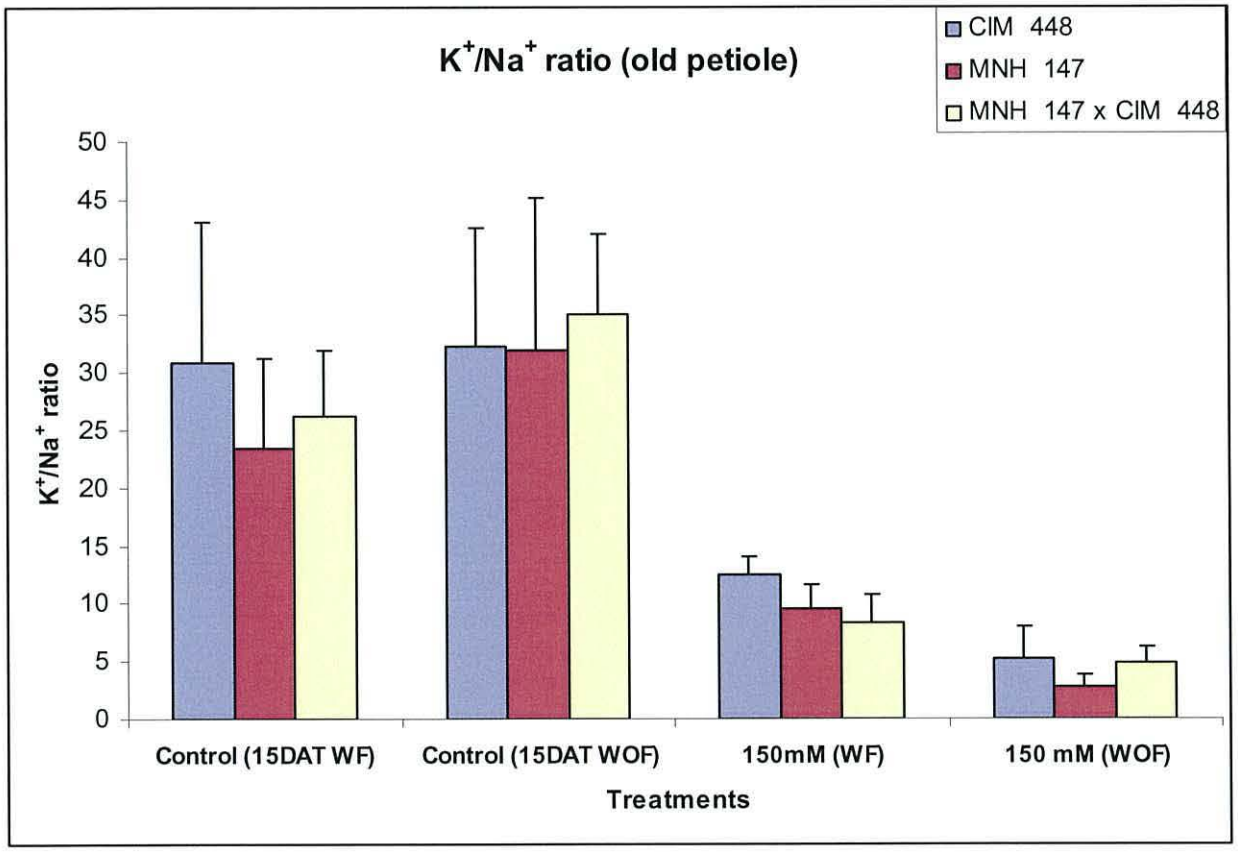

Fig.3.4.3.19. Effect of 150 mM NaCl with flood benches (WF) and 150 mM NaCl with out flood benches (WOF) on K^+/Na^+ ratio in old petiole of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd (see in Appendix Table 18).

3.4.3.20. Na^+ concentration in the old stem.

No difference was observed between both control treatments (Fig. 3.4.3.20). The plants used in treatments 150 mM WF and 150 mM WOF were different from each other. The plants used in treatment 150 mM WOF increases old stem Na^+ content from 150 mM WF with a range 30% - 63%. As we discussed above the salt concentration in top watered soil was high as compared to WF soil. This result also suggests that the salt concentration in WOF pots were presume similar to WF 250 mM. It was observed from the data presented from Table 13 (see in Appendix) showed that the Na^+ concentration in old stem showed high correlation with Na^+ in young leaf and petiole, K^+ in old and young leaf at 15 day x 150 mM (without flood benches).

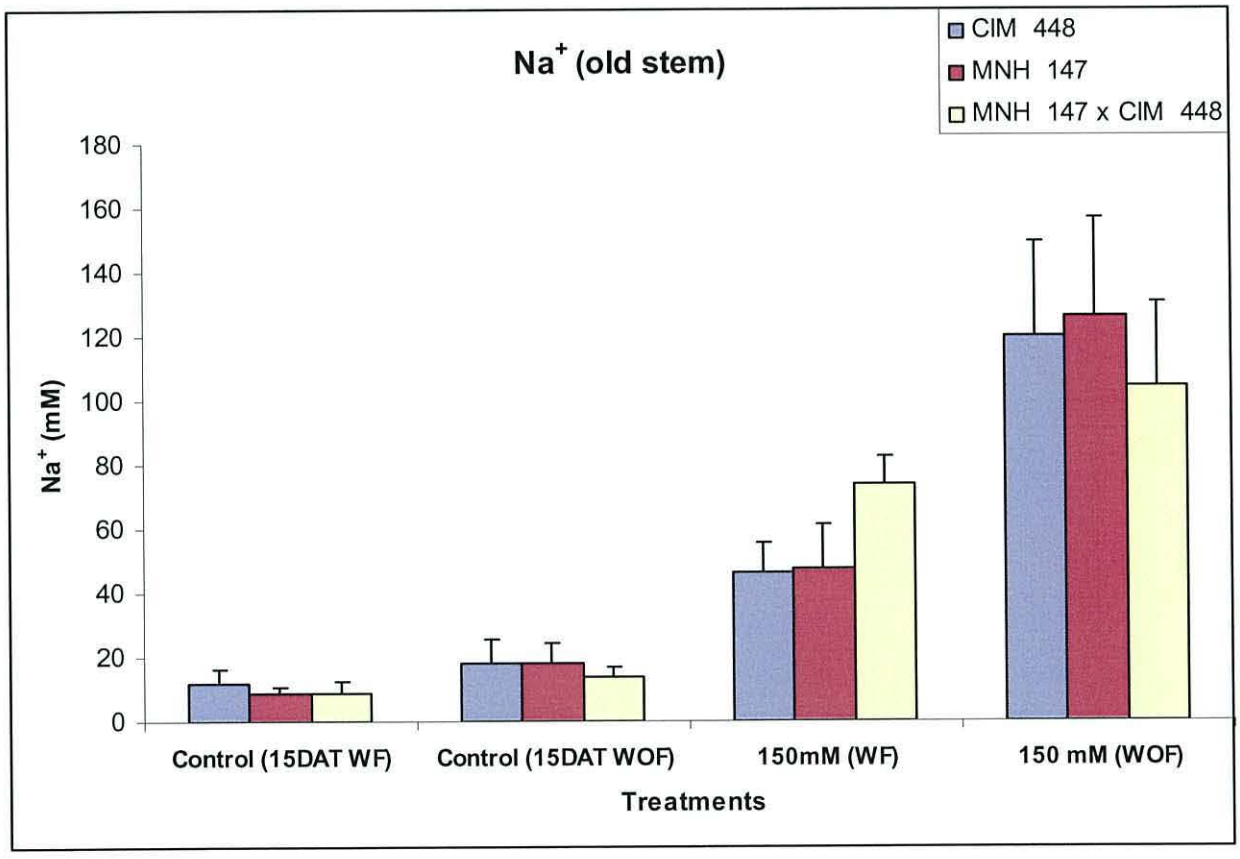

Fig.3.4.3.20. Effect of 150 mM NaCl with flood benches (WF) and 150 mM NaCl with out flood benches (WOF) on Na^+ concentration in old stem of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd (see in Appendix Table 16).

3.4.3.21. K^+ concentration in the old stem.

No differences were observed in between all salt and all control treatments. (Fig. 3.4.3.21).

The plants used in treatments 150 mM without flood bench increases old stem K^+ concentration from 150 mM WF with a range of 13% -31%. It was observed from the data presented from Table 13 and 14 (see in Appendix) showed that the K^+ concentration in old stem showed highly correlation with K^+ in young petiole and stem and K^+/Na^+ ratio in old leaf, petiole and stem at 15 day x 150 mM (without flood benches).

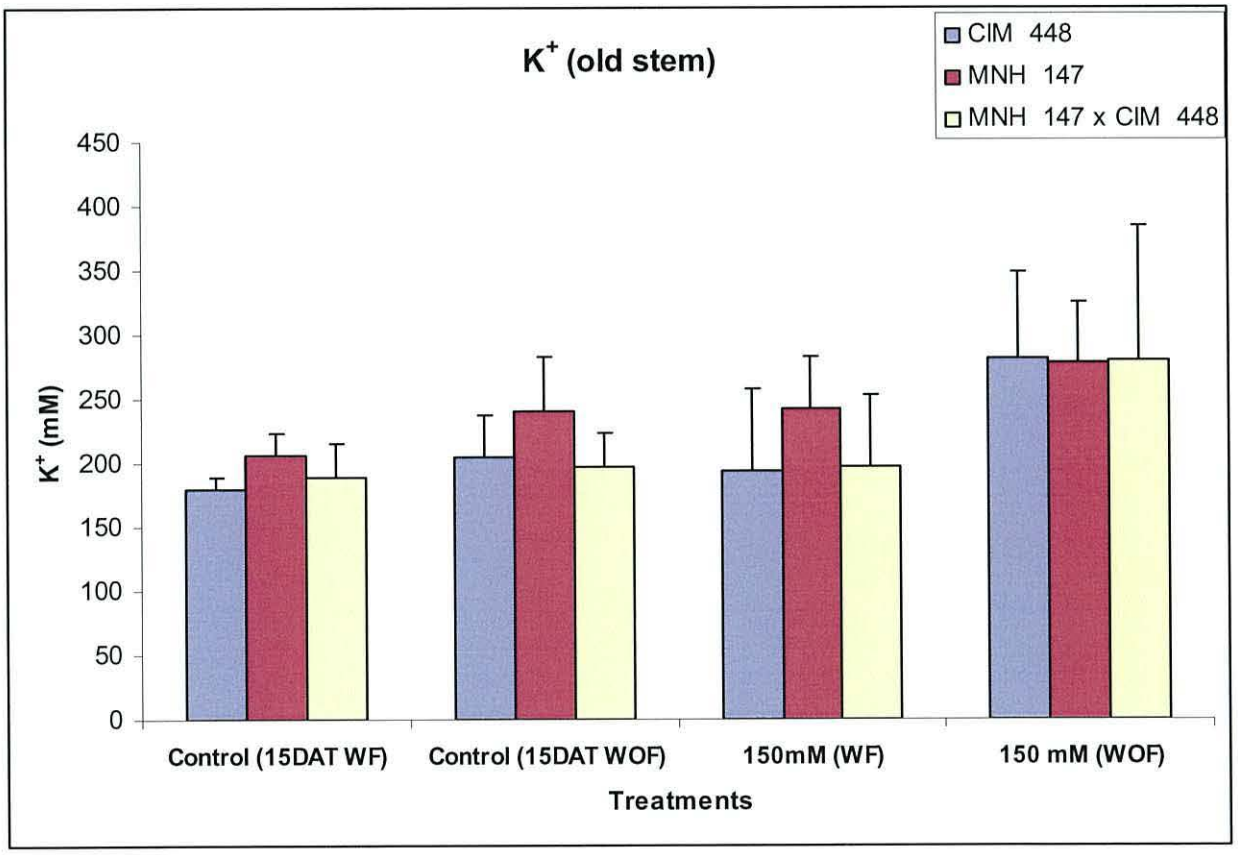

Fig.3.4.3.21. Effect of 150 mM NaCl with flood benches (WF) and 150 mM NaCl with out flood benches (WOF) on K^+ concentration in old stem of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd (see in Appendix Table 17).

3.4.3.22. K^+/Na^+ ratio in the old stem.

No differences were observed in between both salt treatments (Fig. 3.4.3.22). In control treatments the plants used in treatments 150 mM WF and 150 mM WOF were different to each other. The plants used in treatments 150 mM WF showed an increases old stem K^+/Na^+ ratio from 150 mM WOF with a range 35% -41%. It was observed from the data presented from Table 14 (see in Appendix) showed that the K^+/Na^+ ratio in old stem showed highly positive correlation with K^+/Na^+ ratio in young petiole at 15 day x 150 mM (without flood benches).

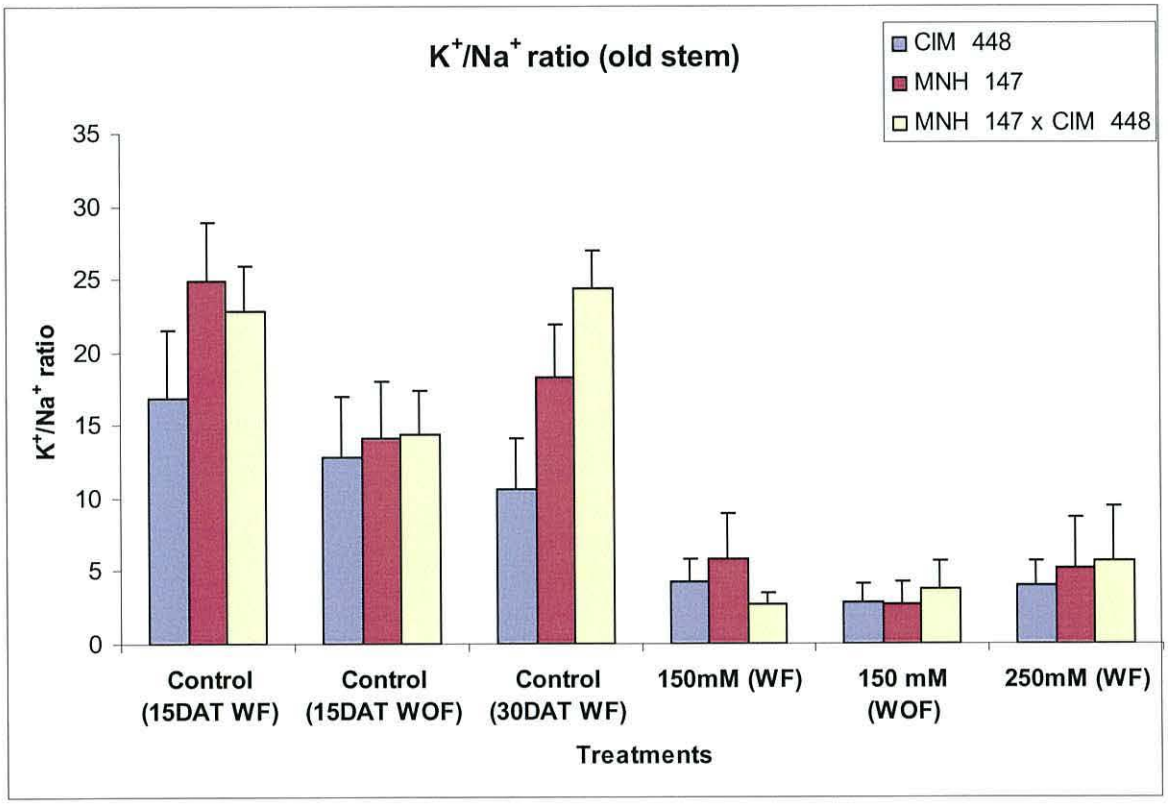

Fig.3.4.3.22. Effect of 150 mM NaCl with flood benches (WF) and 150 mM NaCl with out flood benches (WOF) on K^+/Na^+ ratio in old stem of cotton genotypes. Each mean value is average of 3 or more repeats, error bars as ± sd (see in Appendix Table 18).

Discussion

The objectives of the experiments described in this chapter were to study the effect of salt 150 mM and (150 – 250 mM) on the physiological responses of cotton genotypes and screen the cotton varieties as a salt tolerant or susceptible for salt.

Cotton is a salt tolerant plant with a salinity threshold level 7.7 dSm^{-1}. However, salinity reduced the yield of cotton plants by 5.2 % per dSm^{-1} over a threshold level (Ashraf, 2002). Ahmad *et al.*, (2002) reported that the yield of cotton decreased at 17 dSm^{-1}. In chapter 3 (section 3.1.1) the purpose was to study the genotypic variation of different cotton varieties, with regards to salt tolerance and sensitive. The result showed a significant variation of plant height, fresh weight and dry weight of genotypes following treatment with 150 mM salt (approximately 15 dSm^{-1}). Bhatti and Azhar (2002) reported that salt tolerance varies between genotypes. However, significant differences were observed between genotypes due to uptake of Na^+ into leaf and petiole but not stem. It was observed that the Na^+ content of the leaf was more affected by salt than petiole and stem. The variety Sarmast had a higher Na^+ concentration in leaf, petiole and stem sap and MNH 93 and Stonvelle 312 had a lower Na^+ concentration in leaf, petiole and stem sap. Akhtar, *et al* (2005) reported that the high concentration of Na^+ in leaves could be due to high uptake of Na^+ ion to build up osmotic pressure. Karl and Lauchli (2002) observed that the significantly higher concentration of Na^+ was found in the leaves of sensitive corn cultivars. The K^+ concentration in leaf, petiole and stem was significantly different between genotypes. The variety Stonvelle 312 had a higher K^+ concentration in leaf, petiole and stem sap and MNH 93 and Sarmast had a lower K^+ concentration in leaf sap. The K^+/Na^+ ratio was reduced in all part of cotton genotypes.

The high K^+/Na^+ ratio is a good indicator for salt tolerance in plants (Abd Ella and Shalaby, 1993 and Bagci, 2003). The variety Stonvelle 312 had a higher K^+/Na^+ ratio in leaf sap and Sarmast had a lower K^+/Na^+ ratio in leaf sap. This result is the agreement with above authers.

In section (3.2.1) the result showed in Table 3.2.4 that the effect of 150 – 250 mM NaCl reduced plant height, nodes per plant, fresh weight, and dry weight significantly from control. Similar findings were observed in literature (Basal, 2010). It was expected that the plant height would be reduced more by 250 mM than by 150 mM NaCl treatment. No further growth was observed in 250 mM in most of genotypes. Our data indicated that cotton genotypes *G.hirsutum* x *G. sturtianum*, *G. stocksii*, DNH 40, Dandara, CIM 448, DNH 40 x S6, S6 x DNH 40, NIAB 313 and NIAB 313 x DNH 40 showed the greatest salt /control ratio in plant height, nodes per plants, fresh and dry weight (Table 3.5.1) in 150 mM salt stress. However, Dandara x S6, MNH 147 x S6, CIM 448, DNH 40 x CIM 448, S6 x Dandara, DNH 40, *G. stocksii*, DNH 40 x S6, S6 x DNH 40, MNH 147 x CIM 448 and Dandara showed a greatest salt /control ratio in plant height, nodes per plants, fresh and dry weight (Table 3.5.2) in 250 mM salt stress. This result suggest that *G. stocksii*, DNH 40, Dandara, DNH 40 x S6, S6 x DNH 40 and CIM 448 were salt tolerant, which maintained the greatest height and biomass in both salt stresses

The effect of 150 – 250 mM NaCl showed significant increase in Na^+ concentration in young and old parts of plants from control. Our data indicated that those cotton genotypes DNH 40 x CIM 448, *G. stocksii* x *G. hirsutum*, DNH 40, MNH 147 x CIM 448, NIAB 313, S6 x DNH 40, CIM 448 and S6 x Dandara showed the lower Na^+ concentration in young and old plant parts in 150 mM salt stress. Rathert (1982) observed that the salt tolerant variety showed lower Na^+ content and higher K^+ content in its leaves in comparison with salt sensitive variety.

However, DNH 40 x CIM 448, *G. hirsutum* x *G. sturtianum*, MNH 147, MNH 147 x S6, S6 x DNH 40, DNH 40, *G. stocksii* x *G. hirsutum,* NIAB 313 and S6 x Dandara showed the lower Na^+ concentration in young and old plant parts in 250 mM salt stress. This result suggested that DNH 40 x CIM 448, *G. stocksii* x *G. hirsutum*, DNH 40, S6 x DNH 40, NIAB 313, CIM 448, MNH 147 x CIM 448 and S6 x Dandara were salt tolerant as they maintained the lower

Na^+ content in both salt stresses. The lower increases were noted in old parts than young plant parts. This increase of Na^+ in cotton plants could play an important role in osmotic adjustment (Ghoulam *et al.*, 2002).

The effect of 150 – 250 mM NaCl showed significant increase in K^+ concentration in most of young and old parts of plants from their controls. Our data indicated that those cotton genotypes Dandara, MNH 147, *G. stocksii*, DNH 40 x S6, DNH 40 x CIM 448 and DNH 40 showed a higher K^+ concentration in young and old plant parts in 150 mM salt stress. Leidi and Saiz, (1997) also observed the high concentration of K^+ in leaves of salt sensitive genotype.

However, *G. stocksii* x *G. hirsutum*, *G. hirsutum* x *G. sturtianum*, Dandara, DNH 40 x S6, MNH 147 x S6 and MNH 147 showed a greatest a higher K^+ concentration in young and old plant parts in 250 mM salt stress. This result indicate that Dandara, MNH 147, DNH 40 x S6 and DNH 40 maintained the higher K^+ content in both salt stresses, but *G. stocksii* and DNH 40 x CIM 448 did not maintained higher K^+ in high salt.

For cotton, that K^+/Na^+ ratio is a reliable index of salinity was also reported by (Munis *et al.*, 2010). The effect of 150 – 250 mM NaCl showed a significant decrease in K^+/Na^+ ratio in most young and old parts of plants significantly from control. Our data indicated that those cotton genotypes *G. stocksii* x *G.hirsutum*, *G. hirsutum* x *G. sturtianum*, NIAB 313 x DNH 40, DNH 40 x CIM 448, MNH 147 and *G. stocksii*, showed the greatest K^+/Na^+ ratio in young and old plant parts in 150 mM salt stress. However, DNH 40 x CIM 448, S6 x Dandara, MNH 147 s S6, MNH 147 x CIM 448, *G. stocksii* x *G. hirsutum*, NIAB 313 x DNH 40 and CIM 448 showed a greatest salt /control ratio in K^+/Na^+ ratio in young and old plant parts (Table 3.5.8) in 250 mM salt stress. This result indicate that DNH 40 x CIM 448, *G. stocksii* x *G. hirsutum* and NIAB 313 x DNH 40 maintained the higher K^+/Na^+ ratio in both salt stresses, but MNH

147, *G. hirsutum* x *G. sturtianum* and *G. stocksii* did not maintain a higher K^+/Na^+ ratio in high salt.

It was concluded that varieties DNH 40, Dandara and CIM 448 maintained greater ratios in plant height, nodes per plant fresh and dry weight and those varieties were close genotypically in SSR dendrogram. It was observed that the varieties DNH 40 and CIM 448 were salt tolerant varieties because they maintained the highest salt/control ratio in growth parameters, K^+ content and lower Na^+ content.

In section (3.3.1), as expected, the result shown in Table 3.3.1 indicate that the effect of 150 mM NaCl reduced plant height, nodes per plant, fresh and dry weight, chlorophyll content, evaporation rate, stomatal conductance and CO_2 uptake differ significantly from control plants. Zhang, *et al.*, (2009) reported that the plant growth and yield of cotton and photosynthesis can be reduced due to osmotic stress, nutritional imbalance and toxicity of Na^+ and Cl to the metabolism of plant. However, Rehmani, MNH 93, S12, Qalandari, Karishma and NIAB 78 had the greatest response to reduced lower plant height, nodes per plant, fresh and dry weight, chlorophyll content, evaporation rate, stomatal conductance and CO_2 uptake.

The effect of 150 mM NaCl, as expected, showed significant increase in Na^+ concentration in young and old leaves, petiole and stem from the control. Rehmani and NIAB 78 showed a lower increase and CIM 1100 and Qalandari showed a higher Na^+ content increase in their old and young parts. Glycophytes can exclude Na^+ from shoot tissues but the accumulation of sodium in the shoot appears to be one of the general characteristics of halophytes (Ashraf, 2004).

The effect of 150 mM NaCl showed a decrease in K^+ concentration in most of young and old plant parts. Rehmani, CIM 1100, Mc. Nair 220 and MNH 93 showed higher uptake of K^+ content in their most of young and old plant parts and S 12 and NIAB 78 showed lower uptake of K^+ content in their most of young and old plant parts. It was observed that K^+ concentration of young petiole and stem was increase in variety MNH 93. Khan, *et al* (2004)

also observed that the cotton variety MNH 93 showed increase in K^+ concentration in young leaf. The effect of 150 mM NaCl showed significant decreased in K^+/Na^+ ratio in all young and old plant parts significantly.

The effect of 150 mM NaCl showed a decrease in Ca^{++} concentration in most of young and old plant parts. Rehmani and CIM 1100 showed higher uptake of Ca^{++} in most of their young and old plant parts and S 12, B 756, Sarmast and NIAB 78 showed lower uptake of Ca^{++} content in most of their young and old plant parts. Boscaiu, *et al*, (2007) observed that the K^+, Ca^{++} and Mg^{++} were increase with higher NaCl concentration in halophytes. They also reported that this might be the cause of changes in the level of mono and divalent cations. In contrast Abd Ella and Shalaby (1993) reported that the Ca^{++} content in cotton leaves was not changed due to high salinity. In my study constant Calcium was used. It was observed that varieties Rehmani, NIAB 78 and MNH 93 were salt tolerant varieties due to lower reduction in growth and photosynthesis, higher K^+ and lower Na^+ uptake.

In section (3.4.1) the main objects was to investigate the effect of top watering to the plant soil. The solutions for salt were used in this study was approximately 15 Sd/m-^{2}. However, soil conductivity was not directly measured in this study. However, due to small area and size of pots, the plant height was increased in top watering 150 mM NaCl (WOF) compared to 150 mM NaCl (on flood benches) in salt treatment. No differences were observed between genotypes. Number of nodes per plant was similar as expected in both 150 mM (WF & WOF) treatments. It was noticed that in 150 mM (WOF), plant height was highest but fresh weight was low in both control and salt treatment. Because of small area and size of pots plants did not produce more branches and nodes. However, dry weight was similar in both 150 mM (WF & WOF) treatments. This result indicated that the highest plant height, low fresh weight might be due to low light intensity in growth room.

Na^+ concentration in all old and young parts as expected was increased significantly by salt treatments. Due to adding salt solution by hand, it was observed that Na^+ concentration of all parts in150 mM (WOF) was similar to 250 mM (WF). In cotton shoot, low Na^+ and high K^+ accumulation by salt was reported by (Ahmed, *et al.*, 2002).

The effect of 150 and 250 mM NaCl (WF) and 150 mM NaCl (WOF) showed decreased in K^+/Na^+ ratio in all young and old plant parts significantly. The K^+/Na^+ ratio in all young and old plant parts was decreased with increasing salt was investigated in this study. It was noticed that the K^+/Na^+ ratio of all parts in 150 mM (WOF) were also similar to 250 mM (WF). The genotypes used in this study showed similar response to salinity, however, that assay might be useful for screening the salt tolerance of cotton genotypes.

Highly significant correlation in the salt stress plants was observed between growth parameters which suggest that the growth parameters in salt stress conditions appear to be the most reliable selection criterion for salt tolerance. Chlorophyll content, transpiration rate and CO2 uptake were high significantly correlated with Na^+ accumulations. Na^+ concentration in leaf recorded in the stressed plants was high significantly correlated with Na^+ in petiole and stem, this result indicate that the salinity is an important concern for plant tissues.

The K^+/Na^+ ratio recorded in the stressed plants was high significantly negative correlated with growth parameter, suggesting that this physiological parameter in stress conditions appears to be the most reliable selection criterion for salt tolerance.

An attempt to bring all of the individuals "ranking" of the parameters together in the form of a "universal rank" was performed as described in chapter 2 (section 2.13). This involved normalization, the value of each parameter and adding these values.

I found S6 x Dandara, CIM 448, MNH 148 x CIM 448, *G. hirsutum* x *G. sturtainum*, Dandara x S6, S6 x DNH 40 and DNH 40 (Table 3.5.1) ranked highest for this "universal ranking" of the parameters likely to correlate with salt tolerance. Although the salt sensivity of yield of the most

varieties is unknown. Noor *et al*, (2001) observed that CIM 448 is a salt tolerant variety. Khan, *et al*. (2004) observed that MNH 93 as a salt tolerant variety based on cell sap Na^+ and K^+, and also observed that the tolerant cultivar had lower concentration of Na^+ in leaves. Munis *et al*, (2010) found Coker 312 as salt tolerant variety accumulated less of Na^+ and more K^+ in leaves. Above result are agreed with my results, in contrast Gossett *et al,* (1996) for Coker 312 and Lira and Hernandez (1988) for Acalla SJ 2 as a salt sensitive variety. No previous evidence were found about DNH 40, Rehmani,149 F, *G. hirsutum* x *G. sturtainum*, *G. stocksii*, *G. stocksii* x *G. hirsutum* and Mc Nair 220 varieties for their salt tolerance bases.

I found S 12, Sarmast, Precoce 1, Rehmani and B 756 is a salt sensitive genotypes. This result agrees with Ali *et al*, (2005) for S 12, Rathert (1982) for Dandara, In contrast Ganesan and Jayabalan (2005) for Acalla 1517, Noor *et al* (2001) for CIM 1100, is a salt tolerant variety. Jafri and Ahmed (1995) found NIAB 78 as a salt tolerant and Sarmast and Qalandari as a salt sensative varieties. I found above varieties as sesative to salt.

G. stocksii was found salt tolerant. That genotype accumulate less reduction in growth parameters, lower uptake of Na^+, high K^+/Na^+ ratio and high uptake of K^+ in 150 mM salt, but when increase salt up to 250 mM (Table 3.5.2) that genotype accumulate more Na^+ in their plant parts.

3.6. Conclusion

Plant height, nodes per plant, fresh and dry weight was reduced by salt treatment. Na^+ content of all young and old plant parts was increased by salt. K^+ content decreased in most cases. Ca^{++} content increased in most cases. K^+/Na^+ ratio in all young and old plant parts was decreased. Chlorophyll content decreased. Transpiration rate, stomal conductance and CO_2 uptake reduced by salt. Some differential behaviour between the genotypes was noted.

Table 3.5.1. Ranking of growth parameters, chlorophyll content, photosynthesis, Na^+, K^+, $^+/Na^+$ and Ca^{++} in 150 mM stress. Each entry represents {parameter value – mean}/s.d. (see 2.13 for explanation.

Genotypes	Growth parameters	Chlor-ophyll	Photo-synthesis	Na^+	K^+	K^+/Na^+	Ca^{++}	Average	Ranks
S6 x Dhandara	0.953	-	-	-0.769	2.636	1.495	-	1.079	1
CIM 448	0.528	-	-	-1.480	1.073	0.409	-	0.133	2
MNH 147 x CIM 448	-0.157	-	-	-2.190	1.572	0.434	-	-0.085	3
G.hirsutum x *G. sturtianum*	-0.294	-	-	-0.746	1.480	2.203	-	0.661	4
Dhandara x S6	0.816	-	-	-0.482	2.190	0.357	-	0.720	5
S6 x DNH 40	0.772	-	-	-1.073	0.769	1.907	-	0.594	6
DNH 40	0.121	-	-	-1.572	-0.014	1.026	-	-0.110	7
G. stocksii x *G. hirsutum*	0.210	-	-	-0.488	0.644	0.910	-	0.319	8
G. stocksii	-2.194	-	-	-0.644	0.311	2.422	-	-0.026	9
NIAB 313	-0.206	-	-	-0.470	0.158	1.052	-	0.133	10
MNH 147 x S6	0.477	-	-	-1.240	0.482	1.142	-	0.215	11
149 F	0.393	-	-	0.014	0.501	-0.794	-	0.028	12
Coker 201	0.821	-	-	-0.158	-0.295	-0.659	-	-0.073	13
MNH 147	-0.024	-	-	-0.311	1.240	-0.016	-	0.222	14
DNH 40 x S6	0.593	-	-	-0.229	0.488	0.711	-	0.391	15
CIM 443	0.608	-	-	0.205	-0.311	-0.813	-	-0.078	16
AC 134	0.551	-	-	-0.501	-0.018	-0.890	-	-0.215	17
Acalla SJ 2	1.791	-	-	0.050	0.470	-0.601	-	0.428	18
DNH 40 x CIM 448	0.318	-	-	0.242	0.746	1.090	-	0.599	19
Dhandara	-0.214	-	-	1.125	0.229	0.512	-	0.413	20
Qalandari	-0.682	1.027	0.317	0.367	-1.291	-0.865	0.569	-0.080	21
NIAB 313 x DNH 40	-0.883	-	-	0.295	-0.205	1.586	-	0.198	22
Krishma	-0.917	0.942	0.398	0.767	-1.125	-0.717	1.360	0.101	23
CIM 1100	-0.753	0.348	0.146	-0.378	-0.767	-0.273	1.012	-0.095	24
Mc. Nair 220	-1.298	1.443	0.032	-0.025	-0.242	-0.440	0.594	0.009	25
Acalla 1517	-0.324	-	-	0.018	-1.062	-0.833	-	-0.550	26
Stonvelle 213	0.326	-	-	1.291	-0.396	-0.485	-	0.184	27
B 496	0.759	-	-	0.311	-0.929	-1.006	-	-0.216	28
Allepo 45	0.114	-	-	0.396	0.378	-0.659	-	0.057	29
CIM 240	1.237	-	-	0.027	-0.367	-0.968	-	-0.018	30
BP 52-63	0.648	-	-	0.795	0.025	-0.852	-	0.154	31
Coker 312	0.660	-	-	0.929	-0.050	-0.562	-	0.244	32
CIM 109	0.078	-	-	1.353	-0.027	-0.833	-	0.143	33
S 12	-1.088	0.008	1.621	1.438	-1.438	-0.833	-1.089	-0.197	34
B 756	-0.516	-0.255	-1.861	1.470	-1.105	-0.556	0.210	-0.373	35
Sarmast	-0.252	-1.078	-1.404	1.105	-0.435	-0.784	-0.562	-0.487	36
MNH 93	-1.397	-0.255	-0.308	1.062	-1.470	-0.717	0.338	-0.392	37
Rehmani	-0.521	-0.323	0.544	0.435	-1.353	-0.569	-0.571	-0.337	38
Precoce 1	0.629	-	-	1.700	-0.795	-0.968	-	0.141	39
NIAB 78	-0.814	-1.859	0.515	0.000	-1.700	-0.562	-1.861	-0.897	40

Table 3.5.2. Ranking of growth parameters, Na^+, K^+ and K^+/Na^+ in 250 mM stress. Each entry represents {parameter value – mean}/s.d. (see 2.13 for explanation.

Genotypes	Growth	Na^+	K^+	K^+/Na^+	Average	Ranks
MNH 147 x CIM 448	0.828909	1.4366511	1.026957	1.965081	1.3144	1
S6 x DNH 40	0.470275	1.0001913	1.33912	1.378361	1.046987	2
G. stocksii x *G. hirsutum*	0.301156	0.9369464	0.438784	0.722615	0.599875	3
DNH 40 x CIM 448	0.669506	0.8069814	-0.08543	-0.00216	0.347226	4
MNH 147 x S6	0.548672	0.4726866	-0.06805	-0.00216	0.237789	5
MNH 147	-0.1084	0.5519166	0.17112	0.032356	0.161738	6
DNH 40	-0.14484	0.5303716	0.027201	0.170408	0.145786	7
S6 x Dhandara	0.301065	-0.625413	1.338425	-0.62339	0.097672	8
NIAB 313 x DNH 40	-0.02339	0.6811865	-0.3239	-0.3818	-0.01197	9
Dhandara	-0.29151	-1.688762	1.145148	-0.20923	-0.26109	10
G.hirsutum x *G. sturtianum*	-1.13745	0.5435766	-0.05206	-0.58888	-0.3087	11
Dhandara x S6	0.524291	-1.036158	-0.11949	-0.76144	-0.3482	12
NIAB 313	-0.22451	0.0424819	-0.62424	-1.00303	-0.45233	13
DNH 40 x S6	0.445265	-1.113303	-0.64301	-1.03755	-0.58715	14
CIM 448	0.384852	-1.436477	-0.91485	-1.41719	-0.84592	15
G. stocksii	-2.54385	-1.102878	-2.65573	1.758003	-1.13611	16

Chapter 4

Molecular marker diversity in cotton genotypes

4.1 Introduction.

Introduction of salt tolerant plants and the selection and breeding of varieties for salt tolerance can play a major role in increasing the agricultural productivity under these conditions. The genus *Gossypium* consists of 50 wild and cultivated species. Forty-five are diploid and six are allotetraploid (Fryxell, 1992). Only four species of *Gossypium* are grown on a commercial scale. The basic haploid number (n) of cotton is 13. Forty five of the species of genus *Gossypium* are diploid, having a 2n chromosome number equal to 26. *G. arboreum* and *G. herbaceum* are both diploid species having the A genome, while *G. hirsutum* and *G. barbadense* are allotetraploids (Zhang, *et al*, 2002) species (2n=4x=52) having (AADD) genomes.

The use of molecular markers is a powerful technique for measuring the genetic diversity within and between plant populations. Mondini, *et al*, (2009) describe simple sequence repeats (SSR) DNA as one group of such genetic molecular markers. They have been used since the 1990s. The motifs of SSRs are generally 2-6 tandem repeats of nucleotides. Polymorphism occurs at a specific locus due to the variation of microsatellite length, due to changes in the numbers of repeats. SSRs are informative due to their high degree of polymorphism. Each primer pair detected one or more than one different size of alleles. Three hundred and ninety two microsatellite alleles from tetraploid cotton have been developed and mapped (Nguyen, *et al*, 2004). In the present study, SSR markers were used to investigate cotton genotypes and to attempt to identify the allelic variability and genetic relationship between them.

4.2 Material and methods.

Seventeen SSR markers (Table 4.1) were used to test polymorphism in 43 cotton genotypes. The cotton genotypes used in this study are shown in Table 4.2. Microsatellite markers were selected randomly from the database www./cottonmarker.org. Some of them were randomly selected from previous studies (Lui, *et al.*, 2000, Adawy, 2007, Gutierrez, *et al.*, 2002 and Qureshi, *et al.*, 2004).

Molecular techniques were described in chapter 2 (2.10 for DNA extraction and Quantification, 2.11 for PCR (Polymerase chain reaction) amplification, 2.12 for Gel Electrophoresis, 2.13 for Capillary Electrophoresis and 2.14 for principle component analysis)

Table 4.1. Microsatellite (SSR) primers, their sequence (R & F), motif, frequency and size

S. No	SSR PRIMER	Sequence forward	Sequence reverse	Motif(s) and Frequency	Size (bp)
1	BNL1434	AAATTCAAGAAT CAAAAAACAACA	TTATGCCAAAGTA TATGGAGTAACG	(AG)13	246
2	BNL1679	AATTGAGTGATA CTAGCATTTCAGC	AAAGGGATTTGCT GGCAGTA	(AG)17	170
3	BNL1681	GTGTGTGGGTGT GCATGTTT	TGGGGAGACTTTA TCACGCT	(AG)11	111
4	BN1694	CGTTTGTTTTCGT GTAACAGG	TGGTGGATTCACA TCCAAAG	(AG)19, (TC)19	250
5	BNL1878	TGCTTCAACTGCT CTTGCAT	TCGATATCTGGAA CACCCAC	(AG)14	174
6	MGHES 06	TCGCTTGACTTTC CATTTCC	AACCCTCGGGATT ATCGTCT	(CCA)7	189
7	MGHES 13	CAGGGGAGCCAT TGTTAGAA	CAGGGGTCCTGTG TTTCAGT	(GA)3	235
8	MGHES 21	TTTTTCGGGCTAT GCTTTTG	GGGGTTGACATGT CCTATGC	(GA)14	200
9	MGHES 25	TGAGGAACCAAG CAAAAACC	CTTGGGCAACTTC CAAGGTA	(TTA)7, (TTC)3	200
10	MGHES 29	TTTCCATTTTCTC CTGCTTCA	TCAGCTCATGGGA GCAAATA	(TCA)6, (TCT)3	205
11	MGHES 31	AAGTTAGCGGCT TCTTGTGG	GGGTCAGAACTG GACAAGGA	(CAT)9	202
12	NAU 920	CATCCTAACCCA AAACAAGA	TTGGAGCATTGAA ATTACCC	(TCA)6	193
13	NAU 1195	TAAGGATTTCGG ATCTGCTC	CCCTGTTGATCTT TTGCTCT	(TGA)6	205
14	NAU 2748	ATTGTGGGATAG GAAAAGCA	TGACCTTCTAGAG ATCCTCCT	(ATAA)4	181
15	NAU 2777	CCCTGTTGATCTT TTGCTTT	TTTCAGGCCTAAG GATTTTG	(TCA)7	217
16	NAU 3232	GCAGGGGCATAG TACCTACA	CTTTTGAGGCCAG AACCTAA	(TTTTC)5	179
17	Gh 137	TTCCATTGAAACA TCATGTCTTTAC	GACTTAAACCCA ACAGCGAAGAC	TCT(9)	191

CMD- Cotton marker data base (www.cottonmarker.org/)

Table. 4.2. Track numbers of cotton genotypes used in gel photographs.

Track NO.	Genotypes	Track NO.	Genotypes	Track NO.	Genotypes
1	Sindh 1 x GMS 2	16	Precoce 1	31	S 6 x Dandara
2	GMS 7	17	Stoneville 213	32	Dandara x S 6
3	GMS 2	18	CIM448	33	NIAB 313 x DNH 40
4	GMS 7 x Sindh 1	19	MNH 147	34	Karishma
5	GMS 2 x Sindh 1	20	MNH 147 x CIM448	35	Rehmani
6	Allepo 45	21	MNH 147 x S 6	36	Qalandari
7	BP 52 65	22	DNH 40	37	Sarmast
8	Qalandari	23	S 6 x DNH 40	38	CIM 1100
9	CIM 109	24	NIAB 313	39	MNH 93
10	Coker 312	25	DNH 40 x CIM 448	40	NIAB 78
11	B 496	26	*G. stocksii x G. hirsutum*	41	B 765
12	Coker 201	27	DNH 40 x S 6	42	Mc Nair 220
13	CIM 443	28	*G. hirsutum x G. startanium*	43	S 12
14	Acalla SJ 2	29	*G. stocksii*		
15	Acalla 1517	30	Dandara		

4.2.1. Polymorphic information content (PIC)

Polymorphic information content (PIC) values were calculated for each microsatellite marker based on the allelic frequency detected in the studied genotypes. This gives a relative value to each marker on its diversity. It is calculated from the number of detectable alleles and their frequency (Ni, *et al.*, 2002). A marker with low information content has few alleles at low frequency. PIC values ranges from 0 to 1 and increase as the number of alleles increase and allele occur more or less equal frequency. The markers with highest PIC values are best used to distinguish variation. (Luce *et al.*, 2001).

It is calculated for each marker as follows.

$$PIC_i = 1 - \sum_{j=1}^{n} p^2 ij$$

Where *pij* is the frequency of the jth allele for marker i and the summation extends over n alleles.

Genetic diversity of the genotypes based on a set of measured molecular data, can also be estimated using different diversity parameters other than PIC (Sun *et al.*, 2001). These are calculated as follows:

4.2.1.1 Percentage of polymorphic loci (P)

$P = (k/n) \times 100\%$

Where k is the number of polymorphic loci and n is the total number of loci investigated.

4.2.1.2 Average number of alleles per locus (A)

$A = \Sigma A_i / n$

Where A_i is the number of alleles at the ith locus, n is the total number of loci investigated.

4.2.1.3 Average number of alleles per polymorphic locus (AP)

$AP = \Sigma Ap_i / n_p$

Where Ap_i is the number of alleles at a certain polymorphic locus, n_p is the total number of polymorphic loci investigated.

4.3 Results.

4.3.1 Fragment analysis using agarose gel electrophoresis

The results of PCR amplification of SSR loci in the 43 cotton genotypes using 17 microsatellite (SSR) primer pairs are summarized in Table 4.3. The experiment revealed a total of 19 alleles at 17 loci (an average of 1.1 alleles per locus). The number of polymorphic loci was 2 (BNL 1434 and BNL 1878), representing a level of polymorphism of 11 %. Fifteen primer pairs were found to be homogenous with a single allele (monomorphic) across all genotypes. Polymorphism between the genotypes was observed at each of 2 loci.

Table 4.3. Number of alleles of each SSR locus in different cotton genotypes.

S.NO	Locus	Total number of alleles	S.NO	Locus	Total number of alleles
1	BNL 1434	2	10	MGHES 29	1
2	BNL 1878	2	11	MGHES 31	1
3	BNL 1679	1	12	NAU 920	1
4	BNL 1681	1	13	NAU 1195	1
5	BNL 1694	1	14	NAU 2748	1
6	MGHES 06	1	15	NAU 2777	1
7	MGHES 13	1	16	NAU 3232	1
8	MGHES 21	1	17	Gh 137	1
9	MGHES 25	1			

4.3.1.1. BNL 1434

Two alleles were detected at the BNL 1434 locus (Fig.4.3.1.1a – d)). The polynucleotide sequences were observed to be approximately 240 bp size, except for one other approximately 248 bp (S6 x DNH 40) (track 23). The expected size of the marker BNL 1434 $(AG)_{13}$ is 246 bp (Table 4.1). This suggests the occurrence of a mutation of three repeats of AG the observed size of 240. The genotypes Coker 312 (Track 10), G. *stocksii* (track 29) and S6 x Dandara (track 31) were not amplified successfully.

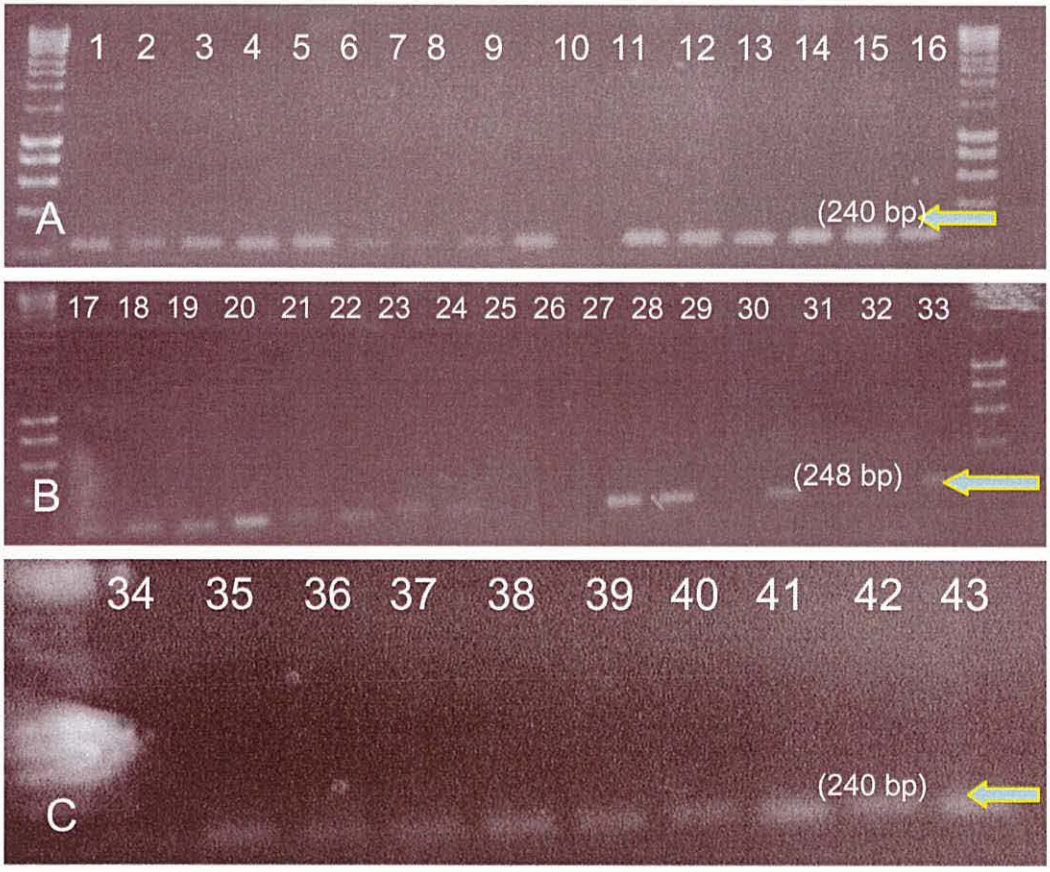

Fig. 4.3.1.1.a Microsatellite DNA profiles of 43 cotton genotypes amplified using by primer pair BNL 1434. The amplified microsatellite DNA was separated in 3% agarose gel and detected by ethidium bromide. Hyper ladder was used. The names of genotypes used in this gel are given in Table. 4.2.

For confirmation of polymorphism on same genotype, the S6 x DNH 40 was screened with BNL 1434 primer, twice and run next to one of the parent DNH 40 (Fig. 4.3.1.1b).

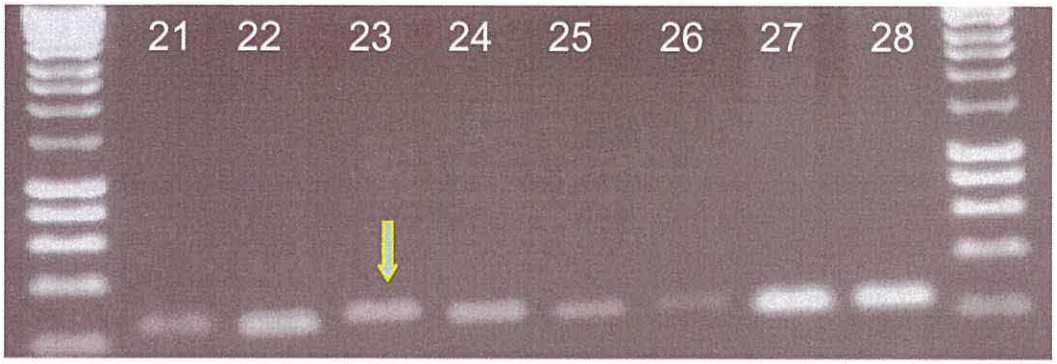

Fig. 4.3.1b. Microsatellite DNA profiles of 8 cotton genotypes amplified using by primer pair BNL 1434. The amplified microsatellite DNA was separated in 3% agarose gel and detected by ethidium bromide. Hyper ladder was used. The names of genotypes used in this gel are given in Table. 4.2.

The same primer amplified products was run on a capillary electrophoresis (CEQ 8000). This analysis confirmed the polymorphism in the S6 x DNH 40 genotype (Fig. 4.3.1.1c). Two peaks, corresponding to 258 bp size and 266 bp were observed. Since the tail forward primer used in the CE experiment to detect the fragment is 18 long, this corresponds to the 240 bp and 248 bp sequences observed on gel electrophoresis. Fig.4.3.1.2d shows the result of same primer (BNL 1434), which screen with variety DNH 40 with only one peak 258 bp size.

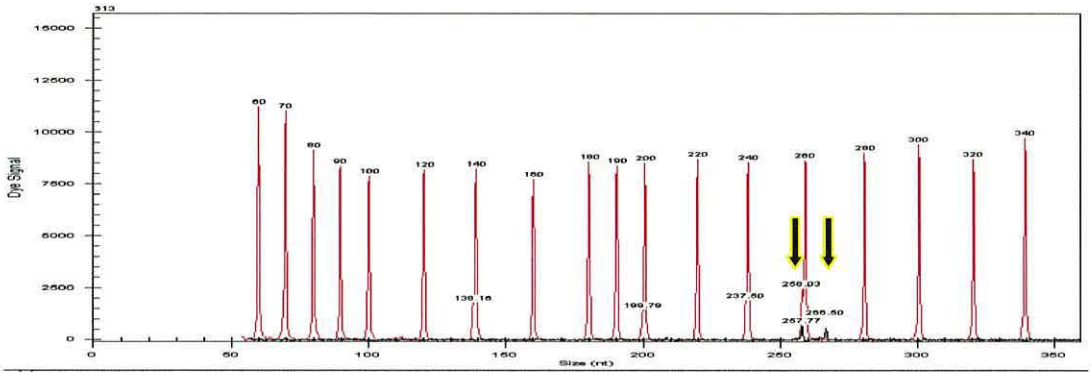

Fig. 4.3.1c. Microsatellite DNA profile of S6 x DNH 40 (track 23) genotype detected by primer pair BNL 1434. Red colour peaks shows the size standard using size fragments (PA 400).

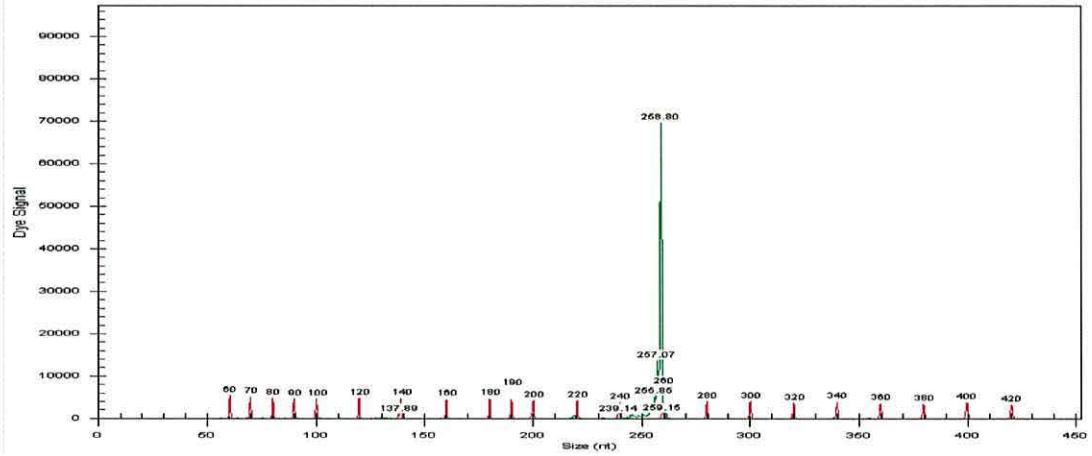

Fig. 4.3.1d. Microsatellite DNA profile of DNH 40 (track 22) genotype detected by primer pair BNL 1434. Red colour peaks shows the size standard using size fragments (PA 400).

4.3.1.2. BNL 1878

Two alleles were detected at the BNL 1878 locus (Fig. 4.3.1.2a - c). The locus showed little polymorphism across the 43 genotypes tested. Most genotypes were found a common allele approximately 200 bp. The exceptions are *G. stocksii x G. hirsutum* (track 26) and *G. stocksii* (track 29) which shows a second allele approximately 180 bp. The expected size of the primer BNL 1878 $(AG)_{14}$ is 174 bp (Table 4.1). This suggests the occurrence of a mutation of extra thirteen repeats of AG the observed size of 200.

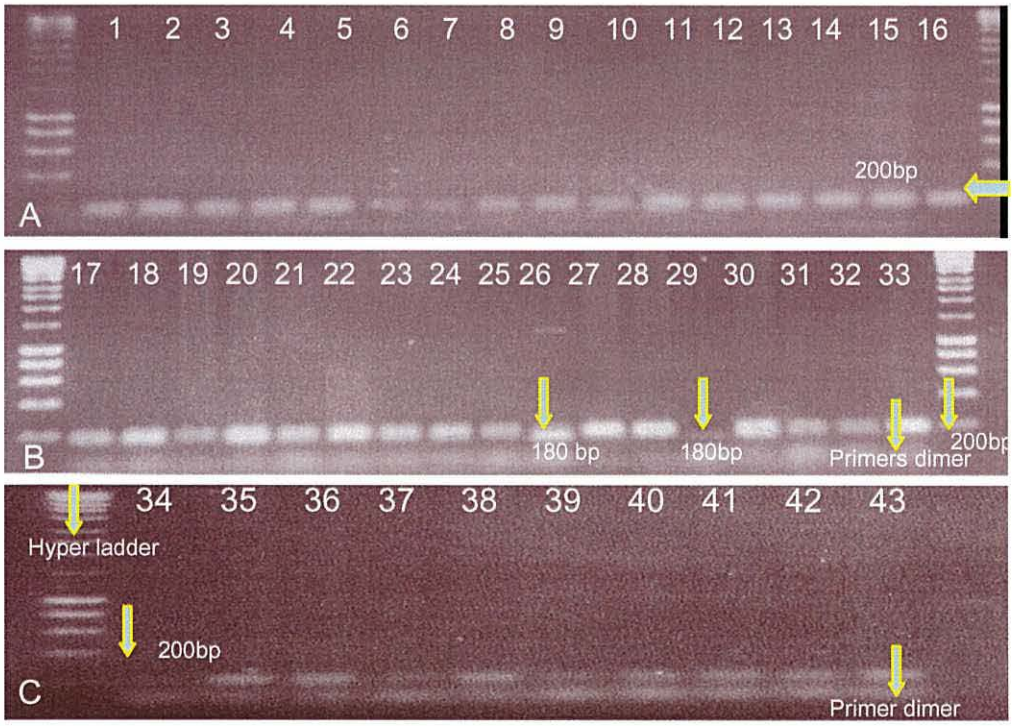

Fig. 4.3.1.2a. Microsatellite DNA profiles of 43 cotton genotypes amplified using by primer pair BNL 1878. The amplified microsatellite DNA were separated in 3% agarose gel and detected by ethidium bromide. The names of genotypes used in this gel are given in Table. 4.2.

For further conformation of polymorphism on same genotypes, the primer amplified product was run on a capillary electrophoresis (CEQ 8000). This analysis confirmed the polymorphism in *G.stocksii x G. hirsutum* genotype (Fig 4.3.1.2b). Two peaks, corresponding to 190 bp and 214 bp were observed. Since the tail forward primer used in the CE experiment to detect the fragment 18 long, this corresponds to the 172bp and 196 bp sequences observed on gel electrophoresis. Fig. 4.3.1.2c shows the result of same primer (BNL 1878), which screen with variety MNH 147 with only one peak 190 bp size.

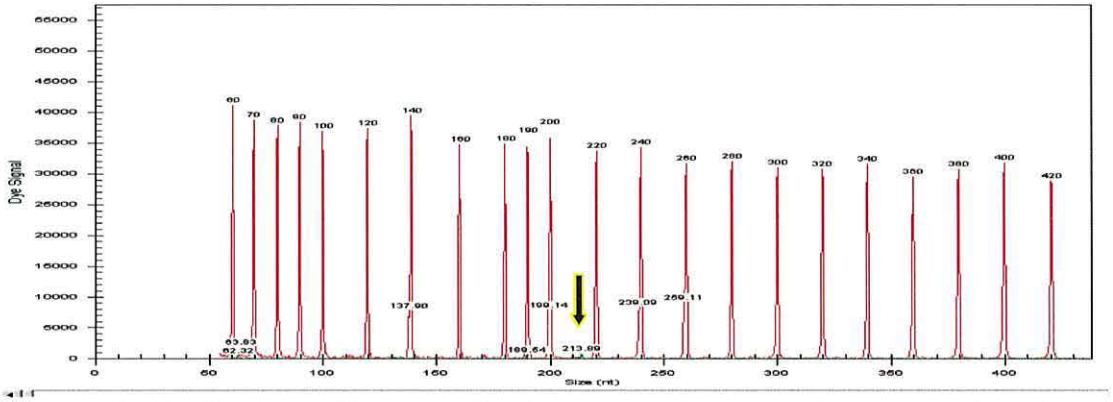

Fig. 4.3.1.2b. Microsatellite DNA profile of *G.stocksii x G. hirsutum* (track 26) genotype detected by primer pair BNL 1878. Red colour peaks shows the size standard using size fragments (PA 400).

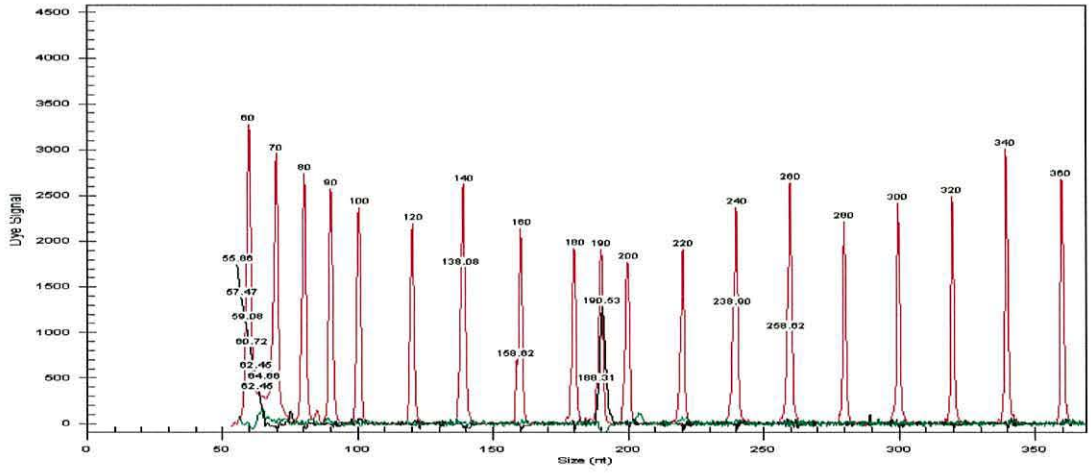

Fig. 4..3.1.2c. Microsatellite DNA profile of MNH 147 (track 19) genotype detected by primer pair BNL 1878. Red colour peaks shows the size standard using size fragments (PA 400).

4.3.2. Fragment analysis using CEQ 8000.

Only two polymorphic loci were found through the use of agarose gel electrophoresis. According to Tang *et al.,* (2004) capillary electrophoresis is better than conventional gel electrophoresis because of automated sample loading, faster separation, more sensitivity and higher resolution. For fragment analysis a CEQ 8000 instrument was used. Seventeen primer pairs were screened with twenty eight varieties. A total of 43 fragments were amplified, with an average of 2.5 SSR fragment per primer. Out of 43 amplified fragments 4 (11.6%) were found to be monomorphic, the remaining 39 (88.84%) fragments were polymorphic in one or other of the 28 varieties. The PIC value ranges from 0.00 to 0.70 (Table. 4.4). The size of amplified fragment also varied from different primers. Out of 28 varieties MNH 147 produced the maximum number (12) of DNA amplified fragment and S12 produced minimum numbers (4) of fragment. Other varieties produced common bands in the range of 6-11. A maximum of 5 fragments were amplified with primer BNL 1434 of and a minimum of 1 band was amplified with primer NAU 2748. To estimate the genetic similarities of the cotton varieties a similarity matrix was obtained using Jaccard's similarity coefficient. This similarity coefficient was used to generate a dendrogram (Fig. 4.3.3.1) by UPGMA analysis in order to determine the grouping of different varieties. The genotypes were grouped into seven main clusters. Each cluster distinguishes the genotypes clearly from the other. According to the dendrogram, cluster I had three varieties (CIM 448, CIM 1100 and DNH 40) genetically very similar (72%), CIM 448 and CIM 1100 showed closed relationship. Both these genotypes have the same geographical origin. Both varieties belong to CCRI, Multan (Table. 2.1), and both have the same parents (Fig.4.3.3.2). The molecular results fit to the known pedigree. The origin and pedigree of DNH 40 was not available. That cluster is similar to other sub cluster, had only one variety Dandara. That variety is a selection of G3 from Egypt. Cluster II has three sub clusters, similar to each other (84%), each cluster had three genotypes, in cluster IIA are MNH 147, DNH 40 x CIM 448 and CIM 443. In cluster IIB are

Karishma, CIM 109 and B 496, and in cluster IIC are S12, Coker 312 and Mc. Nair 220. In that cluster the origin of MNH 147, CIM 443, CIM 109 and one parent of the cross CIM 448 is the same (CRI and CCRI Multan) (Table. 2.1), but the pedigrees are not influenced, in the case of Coker 201 and Mc. Nair 220 the pedigree and origin are same and result fits for those varieties (Fig.4.3.3.2). Cluster III has five sub clusters, similar to each other (80%). In cluster IIIA one genotypes (Coker 201), that was similar to cluster IIIB, which had three genotypes (Sarmast, Allapo 45 and Acalla 1517), that groups was similar to cluster IIIC, which had one genotype (B 53 62), that group was similar to cluster IIID, which had two genotypes (Qalandari and Prococe 1), that group was similar to cluster IIIE, which had two genotypes (NIAB 313 and B 765), most of the genotypes in cluster III belongs to different origins and pedigrees, except Sarmast, Acalla 1517 and Qalandari had same pedigree influence (Fig.4.3.3.2). Cluster IV has one variety (MNH 93), similar to cluster II and III (50%). Cluster V had two varieties (Rehmani and NIAB 78), both have different origen and pedigree showed (82%) genetic similarity. Cluster VI has genotype *G. stocksii*, a wild species, and in cluster VII two genotypes (S6 x DNH 40 and *G. stocksii x G. hirsutum*) showed (14%) similarity with rest. On the basis of SSR data their genetic looks very narrow. The coefficient of similarity for all of varieties was found to be in the range (-0.14-95%).

Table 4.4. Number of alleles of each SSR locus in different cotton genotypes.

Locus	**Total number of alleles**	**PIC Vale**	**Locus**	**Total number of alleles**	**PIC Value**
BNL 1434	5	0.70	MGHES 29	1	0.00
BNL 1878	3	0.59	MGHES 31	1	0.00
BNL 1679	3	0.64	NAU 920	4	0.51
BNL 1681	1	0.00	NAU 1195	2	0.5
BNL 1694	2	0.5	NAU 2748	2	0.42
MGHES 06	1	0.00	NAU 2777	4	0.64
MGHES 13	4	0.48	NAU 3232	2	0.55
MGHES 21	4	0.55	Gh 137	2	0.5
MGHES 25	2	0.5			

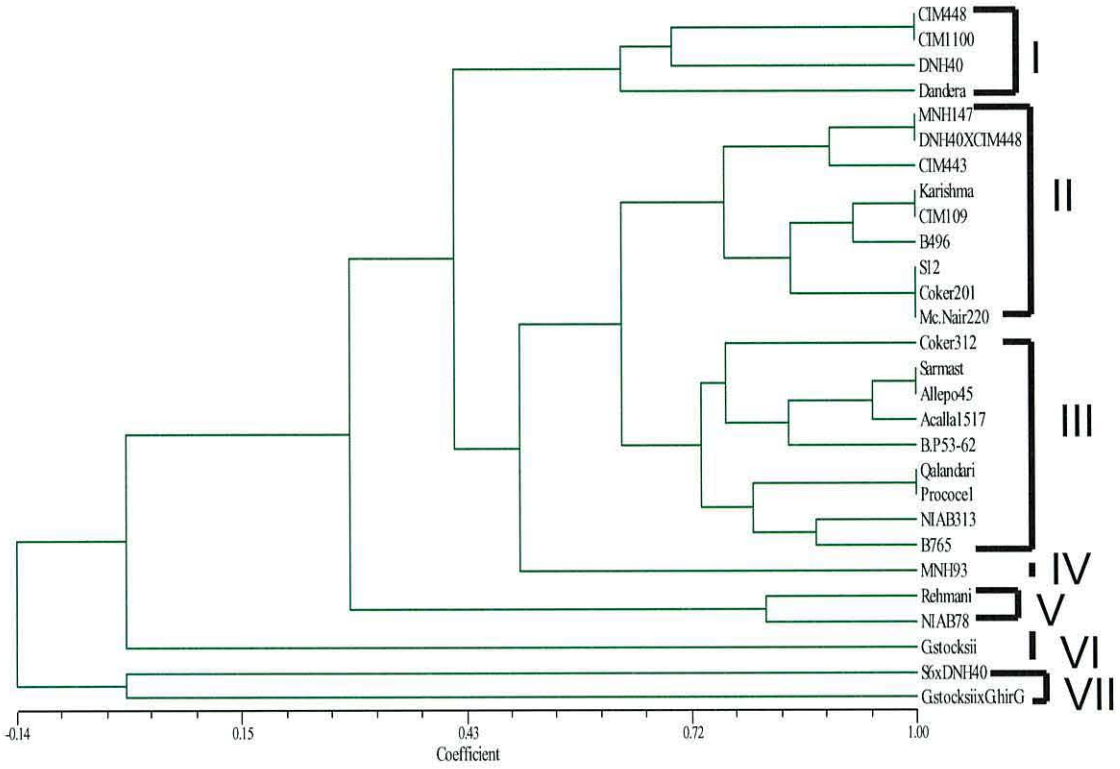

Fig. 4.3.3.1. Dendrogram showing how the twenty eight cotton genotypes clustered on the basis of SSR.

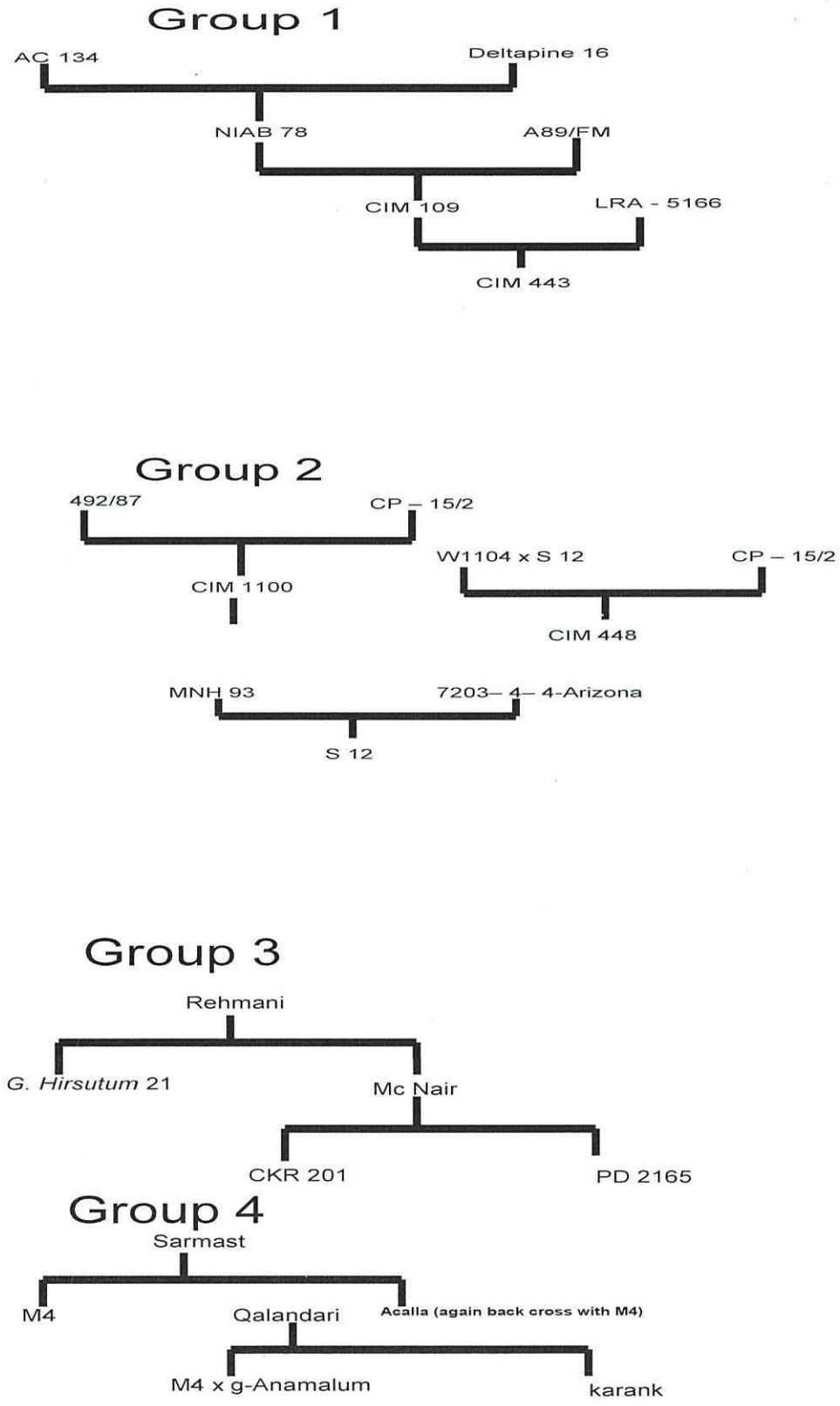

Fig 4.3.3.2. Groups of genotypes on the bases of their pedigree. (Naheed Naz. 2009 and M. Rahman, D. Hussain, and Y. Zafar. 2002)

4.4 Discussion.

4.4.1 Diversity analysis

In the present study, microsatellites or SSR primers was successful in detecting polymorphisms in 43 cotton genotypes. All primer pairs amplified one locus each. Similar results were previously obtained by Gutierrez *et al.*, (2002) and Lui *et al.,* (2000). Out of the 17 primer pairs studied, 13 primer pairs showed polymorphisms when using the automated DNA sequencer. In contrast, only 2 primer pairs showed polymorphism with gel electrophoresis. Vemireddy, *et al.,* (2007) and Tang *et al.*, (2004) reported that, the capillary electrophoresis produces accurate and consistent result superior in comparison to gel electrophoresis of plant material. The number of alleles and polymorphic information content were important parameters for the evaluation of the genetic diversity. The value of gene diversity (PIC) increased with the number of alleles at each locus. Each of the microsatellite primer pairs detected variation at a single locus with alleles varying from 1 – 5 per locus. Five alleles were detected at BNL 1434 locus. Four alleles were detected at MGHES 13, MGHES 21, NAU 920 and NAU 2777. The genetic diversity varied among the genotypes, genetic variation (PIC) ranged between 0.42 – 0.70 and most of the markers were polymorphic (76 – 91%). In this regard, Adawy (2007) investigated the genetic diversity of cotton varieties with 21 SSR primers. He found a total of 78 alleles. The number of polymorphic alleles was 47 and the average level of polymorphism was 60.3%. PIC value ranges from 0.06 to 0.98. In this regards Seetharam *et al.*, (2009) found that the PIC value for the SSR loci ranged from 0.064 to 0.72 in 35 rice SSR primers. However, in comparison with the result from previous studies using microsatellites and other types of markers on the genetic diversity of cotton cultivar, were very narrow (Erkilinc & Karaca 2005, Saha, *et al.*, 2003, Iqbal *et al.,* 1997, Gutierrez *et al.*, 2002, and Chaudhary *et al.,* 2010). In contrast, very little variation was observed with the gel electrophoresis system. Only two markers (11%) were polymorphic and that

diversity was displayed by two genotypes. the cotton genotypes were monomorphic and genetically homogenous for the SSR studied.

4.4.2 Diversity observed in clustering analysis.

The cluster analyses of the genotypes (Fig.3.1.4.3) supported the pedigree information of cotton genotypes (Fig 4.3.3.2). It was observed that the genetic similarity was high (up to 95%) between the genotypes. Lui *et al.,* (2000) found a similar result. Chaudhary *et al.*, (2010) used RAPD markers and found five groups of clusters and the similarity was ranging from 0.48 to 0.86.

4.5 CONCLUSION

In this study a SSR DNA fingerprinting technique was used to investigate the genomic status of the 43 cotton varieties. We observed out of 17 SSR primer pairs, 13 primer pairs resulted in polymorphic bands. Overall this study indicated,

1) That the cotton varieties have genetic variation to some extent, the genetic basis is very narrow, and it is essential for breeders to find some new germplasms for the development of new varieties.

2) Result indicated that possibly some polymorphisms existing with in the varieties but could not be detected due to conventional agarose gel system used.

3) Increase number of SSR primers would increase the number of polymorphic markers.

Chapter 5

Developing a mapping population for cotton

5.1 Introduction.

Cotton belongs to the family Malvaceae and genus Gossypium. Four species of cotton are grown commercially in the world. These species are cultivated as allotetraploid species having (AADD) genomes. One of these *Gossypium hirsutum* L., is known as American or upland cotton. This produces 90% of total world production. A second is *Gossypium barbadense* L., known as Egyptian or Pima cotton. This is characterised by being the best quality of fiber and by possessing an extra long staple (Zhang *et al*, 2002). Progress in the mapping of the cultivated cotton genome is slow. This is due to its large genome, polyploidy level and to an insufficient number of DNA markers (Mei *et al*., 2004). According to Lin *et al* (2010) the worldwide cotton community has given priority to the D genome species *Gossypium raimondii* for complete sequencing as the representative of the spectrum of diversity among the eight *Gossypium* genome types and three polyploidy clades.

Identification of quantitative trait loci in a genome depends on heritability of the trait and a sufficient population sample size (Berke and Rocheford, 1999). For mapping, different segregating populations, such as those generated by back crosses, recombinant inbred lines (RILs), F2 & F3 populations, near isogenic lines (NIls) and diploid hybrids are commonly used (Sarfraz, 2006). Molecular mapping populations have been developed mostly from different species of cotton (Zhang, et al., 2002). The first tetraploid cotton linkage maps constructed with Restriction Fragment Length Polymorphism (RFLP) were reported by Reinish *et al*., 1994. Several genetic linkage maps of cotton have been developed using Random Amplification of Polymorphic DNA (RAPD) and Simple Sequence Repeat (SSR)

techniques (Zhang *et al.*, 2002) and RFLP, RAPD and SSR (Zuo *et al.*, 2000). Lin, *et al.*, (2010) reported that at least a dozen published genetic maps exist for *Gossypium barbadense* crossed with high yielding *Gossypium hirsutum.* They also reported that those maps collectively include > 5,000 public DNA markers (~ 3,300 RFLP, 700 AFLP, > 2000 SSR and 100 SNP).

In cotton, the most reported genetic traits are fiber quality and yield (Lacape *et al.*, 2004, Kohel *et al.*, 2001, Zhang *et al.*, 2003, Shen *et al.*, 2007). In addition, Song and Zhang (2009) identified 26 single QTL for 7 plant architecture traits. In the work of Song and Zhang (2009) the phenotypic variation explained by an individual QTL ranged from 9.6 % to 44.6 %. The 11 QTL found for fruit branch angle, plant height, main stem leaf size and fruiting branch internodes length explained 2.3 to 15.3 % of phenotypic variation in these traits.

Stella *et al.* (2009) observed that out of 25 SSRLP (Simple sequence repeat length polymorphism) primer pairs only 4 primer pairs revealed polymorphic sequences. They suggested that it is essential to find new germplam for the development of new varieties. An increase in the number of SSR primers would increase the number of polymorphism markers. As will be seen, we were not successful in this during this work in this chapter, however. The difficulties are illustrated by the work of Wang *et al.* (2006), who reported that during construction of a molecular linkage map, 2130 SSRs primers were screened, but only 67 polymorphic pairs between two parents were found. They also observed limited polymorphism between two others (ZMS 12 and 8891). Amir *et al.* (2002) obtained the same type of result with RAPD primers. They used 157 primers and found only 2 polymorphic bands.

Cotton cultivation depends on the climatic conditions. It is a warm-season crop. The temperature required from germination to harvest ranges from 20-30 ^{0}C (Reddy *et al.*, 1991 and Bhatti, 1975), but in Pakistan, the summer temperature often reaches 48-50 °C (Ashraf *et al.*, 1994) which is supra-optimal. Due to climatic condition in the UK, it was not possible to grow the cotton plant out of doors to make a segregation population. I wished to explore how this might

be done, however, using the limited artificial growth facilities available in Bangor. The chances of success were remote, but it would provide an exercise in how to proceed. There was always a possibility that a positive result would be found. This chapter focuses on the phenotypic characterization of traits for cotton breeding and genetics using a rudimentary segregating population developed by backcross of an inter specific (*G. hirsutum* x *G. barbadense*).

5.2 Material and methods.

We used three varieties of cotton which, in previous studies on salt tolerance had been rated as salt tolerant and salt susceptible. Two salt susceptible varieties, MNH 147 (*G. hirsutum*) (Ashraf and Ahmed, 2000) and Dandara (*G. barbadense*) (Rathert, 1982) and one salt tolerant variety CIM 448 (*G. hirsutum*) (Noor, *et al.*, 2001) along with their F2 (CIM 448 x Dandara and MNH 147 x CIM 448) were used. One backcross generation (BC1) was made for each.

Plants were grown in Pen-y-Ffridd Research Station, Bangor University, Bangor, UK. (Fig 5.2.1). The minimum temperature was maintained at 25°C during the16 hour photoperiod and 20°C during the dark period. Supplementary 400 Watt high pressure sodium vapour lamps were used to maintain a minimum photon flux density of photo synthetically active radiation (400 – 700 nm) of 350 μmol m^{-2} s^{-1} during the photoperiod. The experimental material was sown on 29 11 2007, in plastic trays ((P-84) (Plantpak, Malvern, England), using John Innes compost No. 1 soil. Eighteen seeds of each cultivar were sown and watered every day with tap water. After fifteen days of sowing, six healthy and uniform seedling of each cultivar were selected, and transplanted to two litre plastic pots.

Fig. 5.2.1. Cotton genotypes grown for backcross generation in Pen-y-Ffridd Research Station, Bangor University, Bangor, UK.

Hand emasculation and pollination techniques were used for crossing (standard method used at cotton section Tando Jam). Flowers of the female parents were emasculated in the early morning of opening and the pistil of the emasculated flower was covered with a white paper bag. This helped to keep the stigma moist and avoid pollination from any foreign pollen. The next morning, pollen from the paternal parent was dusted in the pistil. The flower was then covered with the white paper bag. Pollination was done before 10 am to maximize seed setting and boll retention. Twenty one crosses (11 crosses of [CIM 448 x Dandara] x CIM 448 and 10 crosses of [MNH 147 x CIM 448] x CIM 448) were made. Eight crosses were successful, five from [CIM 448 x Dandara] x CIM 448 and three from [MNH 147 x CIM 448] x CIM 448. The cotton seed harvested from these crosses and from their parents were ginned to get seeds that were used in this study.

The back cross population (BC1) along with their parents were grown on a flood bench system see chapter 2 (section 2.3.3) in a green house (Memorial building) on 4-8-2009. The minimum temperature was maintained at 30°C during the16 hour photoperiod and 20°C during the dark period. Supplementary 400 Watt high pressure sodium vapour lamps were used to maintain a minimum photon flux density of photo synthetically active radiation (400 – 700 nm) of 350 µmol m^{-2} s^{-1} during the photoperiod). The plants were treated with salt and control solutions as described in chapter 2 (section 2.3 and 2.3.3). The plants were harvested after fifteen days of salt treatment. The phenotypic parameters were recorded as in chapter 2 (section 2.4) at the time of harvesting. For methods used in further analysis see chapter 2 (sections 2.5, 2.6, 2.7, 2.10, 2.11 and 2.12).

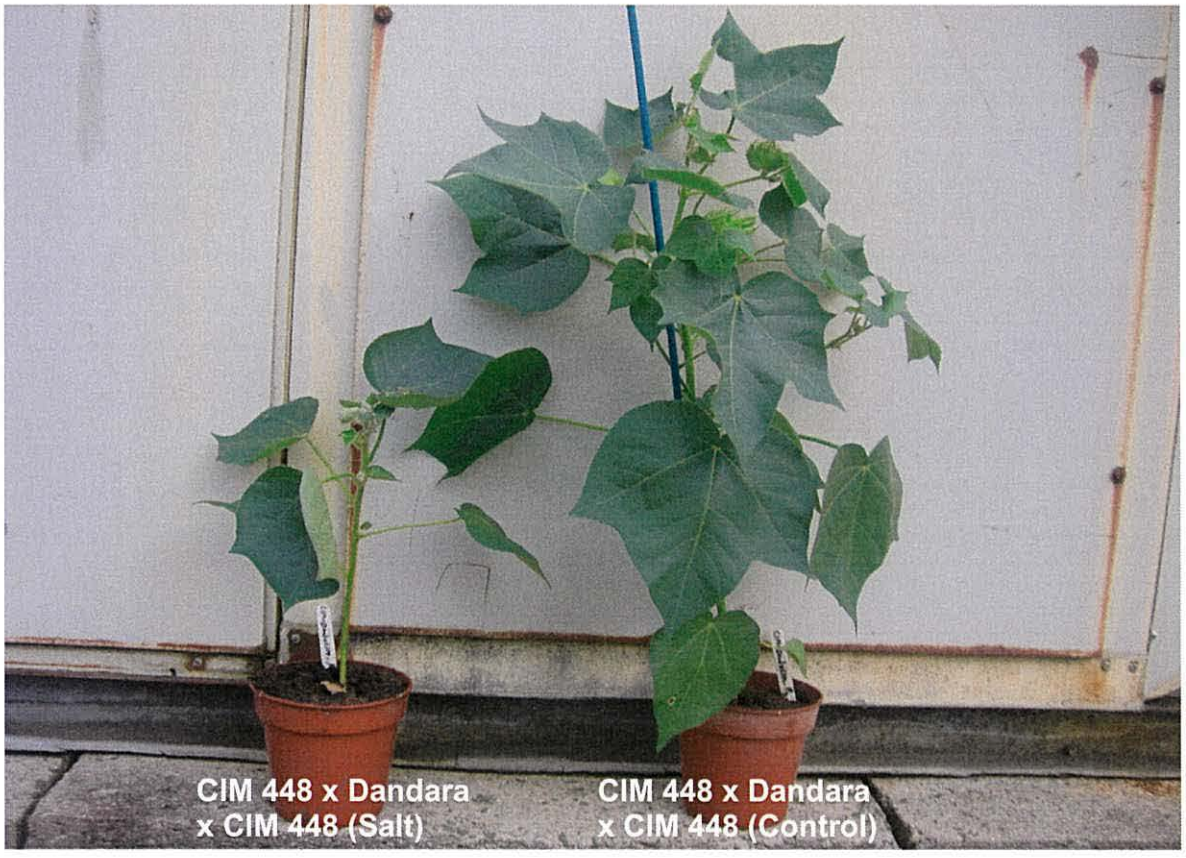

Fig. 5.2.2. One example of growth reduction in backcross generation after salt treatment in green house (Memorial building), Bangor University, Bangor, UK

5.3. Results

5.3.1. The effect of salt on plant height, nodes per plant, fresh weight, dry weight and chlorophyll content.

The effect of salt reduced plant height, fresh, dry weight and chlorophyll content significantly ($P< 0.000$) in all genotypes (Table 5.3.6). The genotypes were not significantly different from each other except of plant height and chlorophyll content ($P < 0.000$). The interaction between treatments and genotypes was not significant in above traits.

The variety Dandara had tallest plant height (54.7 ± 5.5 (4) and 86 ± 3.5 (4) cm) in salt and control treatment respectively, followed by CIM 448 (42.3 ± 15.6 (3) cm) in salt treatment and MNH 147 and CIM 448 x Dandara x CIM 448 (66.5 ± 5.4 (4) and 66.5 ± 8.8 (4) cm) respectively in control treatment. Whereas, the lowest plant height was observed in CIM 448 x Dandara x CIM 448 (38.1 ± 6.2 (4) cm), in salt treatment and in control treatment CIM 448 had the lowest plant height (64 ± 1.4 (4) cm) (Table 5.3.1). It was observed from the data presented from Table 5.3.7 showed that the plant height showed highly significant correlation with Na^+ concentration in old stem and significant correlation with Na^+ old petiole and K^+ concentration in old leaf and young leaf, petiole and stem.

The variety CIM 448 had maximum nodes per plant (9 ± 2 (3) and 12.75 ± 0.5 (4)) in salt and control treatment respectively, followed by MNH 147 x CIM 448 x CIM 448 (8.5 ± 1.3 (3)) in salt treatment and MNH 147 (12.2 ± 1.7 (4)) in control treatment. Whereas, the minimum nodes per plant was observed in CIM 448 x Dandara x CIM 448 and Dandara (7.7 ± 1.2 (4) and 7.7 ± 0.5 (4)) respectively in salt treatment and Dandara (11 ± 1.4 (4)) in control treatment (Table 5.3.1). It was observed from the data presented from Table 5.3.7 showed that the nodes per plant showed no significant correlation with any trait.

The variety MNH 147 had highest fresh weight (40.9 ± 6.4 (4) g) in salt treatment followed by MNH 147 x CIM 448 x CIM 448 (39.2 ± 9.3 (4) g) in salt treatment and in control treatment

CIM 448 x Dandara x CIM 448 had highest fresh weight (136.7 ± 22 (4) g) followed by MNH 147 (125.7 ± 51.1 (4) g). Whereas, the lowest fresh weight was observed in Dandara (34.2 ± 1.3 (4) and 108.9 ± 20.2 (4) g), in salt and control treatment respectively (Table 5.3.1). It was observed from the data presented from Table 3.3.9 showed that the fresh weight showed significant correlation with K^+ concentration in old stem.

The variety MNH 147 had highest dry weight (8.9 ± 1.6 (4) g) in salt treatment followed by CIM 448 x Dandara x CIM 448 (8.1 ± 3.1 (4) g) in salt treatment and in control treatment CIM 448 x Dandara x CIM 448 had highest dry weight (26.5 ± 4.4 (4) g) followed by MNH 147 (21.8 ± 7.5 (4) g). Whereas, the lowest dry weight was observed in CIM 448 (7.8 ± 1.9 (3) and 19.9 ± 0.6 (4) g).), in salt and control treatment respectively. (Table 5.3.1). It was observed from the data presented from Table 3.3.9 showed that the dry weight showed no significant correlation with any trait.

The variety CIM 448 x Dandara x CIM 448 had highest chlorophyll content (48.1 ± 3.2 (4) and 56.4 ± 2.5 (4)) in salt and control treatment respectively followed by CIM 448 (45.1 ± 3.1 (3) and 50.6 ± 3.3 (4))) in salt and control treatment respectively. Whereas, the lowest Chlorophyll content was observed in Dandara (41.2 ± 3.3 (4) and 41.8 ± 4.1 (4)) in salt and control treatment respectively (Table 5.3.1). It was observed from the data presented from Table 3.3.9 showed that the chlorophyll content showed highly significant correlation with Na^+ concentration in young stem and significant correlation in young petiole.

5.3.2. The effect of salt on Na^+concentration in young and old leaves, petiole and stem.

The effect of salt increased Na^+ concentration in all young and old tissues significantly ($P < 0.000$) (Table 5.3.6). The genotypes were not significantly different from each other, except of young and old leaf and young stem. The interaction between genotypes and treatments was not significant, except of young and old petiole and old stem ($P<0.000$).

The variety MNH 147 had highest Na^+ concentration (122.4 ± 23.3 (4) and 2.4 ± 1.8 (4) mM) in salt and control treatment respectively followed by CIM 448 x Dandara x CIM 448 (121.3 ± 40.3 (4) and 1.7 ± 0.6 (4) mM) in salt and control treatment respectively in young leaf. Whereas, the lowest Na^+ concentration was observed in CIM 448 (79.4 ± 42.1 (3) mM) in salt treatment, and control treatment MNH 147 x CIM 448 x CIM 448 had a lowest Na^+ concentration (1.1 ± 0.6 (4) mM) in young leaf (Table 5.3.2). It was observed from the data presented from Table 5.3.7 showed that the Na^+ concentration in young leaf showed positive correlation with Na^+ concentration in young petiole and stem but not significant.

The variety CIM 448 x Dandara x CIM 448 had highest Na^+ concentration (134.3 ± 21.3 (4) mM) in salt treatment followed by MNH 147 (108.6 ± 21.1 (4) mM), and in control treatment MNH 147 had highest Na^+ concentration (4.5 ± 3.8 (4) mM) followed by CIM 448 x Dandara x CIM 448 (2.4 ± 0.6 (4) mM), in young petiole. Whereas, the lowest Na^+ concentration was observed in Dandara (53 ± 23.6 (4) mM) in salt treatment, and control treatment MNH 147 x CIM 448 x CIM 448 had a lowest Na^+ concentration (1.4 ± 1.0 (4) mM) in young petiole (Table 5.3.2). It was observed from the data presented from Table 5.3.7 showed that the Na^+ concentration in young petiole showed positive correlation with Na^+ concentration in young stem but not significant.

The variety MNH 147 had highest Na^+ concentration (146.1 ± 28.6 (4) and 3.9 ± 2.7 (4) mM) in salt and control treatment respectively followed by CIM 448 x Dandara x CIM 448 (133 ± 7.7 (4) and 3 ± 1.2 (4) mM) in salt and control treatment respectively in young stem. Whereas, the lowest Na^+ concentration was observed in Dandara (94.3 ± 57.6 (4) and 1.1 ± 0.6 (4) mM) in salt and control treatment respectively in young stem (Table 5.3.2). It was observed from the data presented from Table 5.3.7 showed that the Na^+ concentration in young stem showed positive correlation with K^+ concentration in old stem.

The variety MNH 147 x CIM 448 x CIM 448 had highest Na^+ concentration (104.8 ± 33 (4) mM) in salt treatment followed by MNH 147 (84.3 ± 20.8 (4) mM) and in control treatment MNH 147 highest Na^+ concentration (8.6 ± 3.4 (4) mM) followed by CIM 448 (6.1 ± 2.7 (4) mM) in old leaf. Whereas, the lowest Na^+ concentration was observed in Dandara (63.0 ± 33.7 (4) and 1.7 ± 1.5 (4) mM) in salt and control treatment respectively in old leaf (Table 5.3.2). It was observed from the data presented from Table 5.3.7 showed that the Na^+ concentration in old leaf showed positive correlation with K^+ concentration in old petiole and stem.

The variety Dandara had highest Na^+ concentration (149.8 ± 18.9 (4) and 9.5 ± 7.7(4) mM) in salt and control treatment respectively, followed by MNH 147 x CIM 448 x CIM 448 (88.6 ± 25.1 (4) mM) in salt treatment and MNH 147 (8.3 ± 2.9 (4) mM) in control treatment. Whereas, the lowest Na^+ concentration was observed in MNH 147 (78.9 ± 8.8 (4) mM) in salt treatment and in control treatment MNH 147 x CIM 448 x CIM 448 had a lowest Na^+ concentration (4.5 ± 1.2 (4) mM) in old petiole (Table 5.3.2). It was observed from the data presented from Table 5.3.7 showed that the Na^+ concentration in old petiole showed highly significant correlation with Na^+ concentration in old stem and K^+ concentration in young stem, however, significant correlation was observed in K^+ concentration in young petiole and old leaf and stem.

The variety Dandara had highest Na^+ concentration (154.5 ± 21.4 (4) and 8 ± 5.7(4) mM) in salt and control treatment respectively, followed by CIM 448 (86.8 ± 17.5 (3) mM) in salt treatment and MNH 147 (6.1 ± 2.1 (4) mM) in control treatment. Whereas, the lowest Na^+ concentration was observed in MNH 147 x CIM 448 x CIM 448 (76.4 ± 31.1 (4) and 3.6 ± 0.6 (3) mM) in salt and control treatment respectively in old stem (Table 5.3.2). It was observed from the data presented from Table 5.3.7 showed that the Na^+ concentration in old stem showed significant correlation with K^+ concentration in old leaf and stem and young leaf, petiole and stem.

5.3.3. The effect of salt on K^+ concentration in young and old leaves, petiole and stem.

The effect of salt on K^+ concentration in most of young and old tissues was significant ($P < 0.000$) except, of old petiole and stem (Table 5.3.6). The genotypes were significant ($P < 0.002$) different from each other, except of old petiole. The interaction between genotypes and treatment was significant of young stem and old petiole and stem.

The variety Dandara had highest K^+ concentration (204.5 ± 17.8 (4) and 159.3 ± 20.7 (4) mM) in salt and control treatment respectively followed by MNH 147 (163.2 ± 28.3 (4) and 156 ± 18.2 (4) mM) in salt and control treatment respectively in young leaf. Whereas, the lowest K^+ concentration was observed in MNH 147 x CIM 448 x CIM 448 (131.1 ± 23.1 (4) mM) in salt treatment, and control treatment CIM 448 had a lowest K^+ concentration (129.7 ± 19.6 (4) mM) in young leaf (Table 5.3.3). It was observed from the data presented from Table 5.3.7 showed that the K^+ concentration in young leaf showed significant correlation with K^+ concentration in young petiole and stem.

The variety Dandara had highest K^+ concentration (330.5 ± 33.1 (4) and 230.8 ± 25.1 (4) mM) in salt and control treatment respectively followed by MNH 147 x CIM 448 x CIM 448 (273.4 ± 22.3 (4) mM) in salt treatment and CIM 448 x Dandara x CIM 448 (220.3 ± 26.3 (4) mM) in control treatment of young petiole. Whereas, the lowest K^+ concentration was observed in CIM 448 (251.6 ± 16.8 (3) mM) in salt treatment, and control treatment MNH 147 x CIM 448 x CIM 448 had a lowest K^+ concentration (196.6 ± 12.3 (4) mM) in young petiole (Table 5.3.3). It was observed from the data presented from Table 5.3.7 showed that the K^+ concentration in young petiole showed highly significant correlation with K^+ concentration in young stem.

The variety Dandara had highest K^+ concentration (307.6 ± 11.5 (4) and 214.4 ± 16.6 (4) mM) in salt and control treatment respectively followed by CIM 448 x Dandara x CIM 448 (226.2 ± 25.1 (4) mM) in salt treatment and MNH 147 (208.5 ± 27.6 (4) mM) in control treatment. Whereas, the lowest K^+ concentration was observed in CIM 448 (204.3 ± 21.1 (4) mM) in salt treatment,

and control treatment MNH 147 x CIM 448 x CIM 448 had a lowest K^+ concentration (195.3 ± 15.5 (4) mM) in young stem (Table 5.3.3). It was observed from the data presented from Table 5.3.7 showed that the K^+ concentration in young stem showed no correlation with any trait.

The variety Dandara had highest K^+ concentration (217.6 ± 30.1 (4) and 180 ± 6.2 (4) mM) in salt and control treatment respectively followed by MNH 147 (131.1 ± 51.3 (4) and 115.3 ± 26.6 (4) mM) in salt and control treatment respectively in old leaf. Whereas, the lowest K^+ concentration was observed in CIM 448 (90.6 ± 29.8 (3) and 54.9 ± 22.4 (4) mM) in salt and control treatment respectively in old leaf (Table 5.3.3). It was observed from the data presented from Table 5.3.7 showed that the K^+ concentration in old leaf showed highly significant correlation with K^+ concentration in young petiole and significant correlation with young leaf and stem.

The variety MNH 147 x CIM 448 x CIM 448 had highest K^+ concentration (270.1 ± 31.9 (4) mM) in salt treatment followed by CIM 448 x Dandara x CIM 448 (267.5 ± 24.4 (4) mM) and in control treatment Dandara had a highest K^+ concentration (293.1 ± 27.9 (4) mM) followed by CIM 448 x Dandara x CIM 448 (262.3 ± 24.7 (4) mM) in old petiole. Whereas, the lowest K^+ concentration was observed in CIM 448 (254.2 ± 5.4 (3) mM) in salt treatment and in control treatment MNH 147 x CIM 448 x CIM 448 had a lowest K^+ concentration (228.1 ± 17.5 (4) mM) in old petiole (Table 5.3.3). It was observed from the data presented from Table 5.3.7 showed that the K^+ concentration in old petiole showed no significant correlation with any trait

The variety MNH 147 had highest K^+ concentration (234.7 ± 11.4 (4) mM) in salt treatment followed by MNH 147 x CIM 448 x CIM 448 (220.9 ± 19.9 (4) mM) and in control treatment CIM 448 x Dandara x CIM 448 had a highest K^+ concentration (243.9 ± 82.1 (4) mM) followed by MNH 147 (190.7 ± 10.6 (4) mM) in old stem. Whereas, the lowest K^+ concentration was observed in Dandara (102.1 ± 16.5 (4) and 163.2 ± 6.4 (4) mM) in salt and control treatment

respectively in old stem (Table 5.3.3). It was observed from the data presented from Table 5.3.7 showed that the K^+ concentration in old stem showed no significant correlation with any trait.

5.3.4. The effect of salt on Na^+/K^+ ratio in young and old leaves, petiole and stem.

The effect of salt decreased K^+/ Na^+ ratio in most of young and old plant tissues significantly ($P < 0.000$) except, of old leaf (Table 5.3.6). The genotypes were not significantly different from each other in all tissues. The interaction between genotypes and treatments was not significantly different.

The variety Dandara had highest K^+/Na^+ ratio (4.2±4 (4)) in salt treatment followed by MNH 147 x CIM 448 x CIM 448 (2.4 ± 1.8 (4)) in young leaf, and in control treatment CIM 448 had highest K^+/Na^+ ratio (230 ± 34 (4)) followed by MNH 147 x CIM 448 x CIM (193 ± 79 (4)). Whereas, the lowest K^+/Na^+ ratio was observed in CIM 448 (2.1 ± 0.9 (4)) in salt treatment and in control treatment the lowest K^+/Na^+ ratio was observed in CIM 448 x Dandara x CIM 448 (78 ± 12.4 (4)) CIM 448 x Dandara x CIM 448followed by MNH 147 (87 ± 38.5 (4)) (Table 5.3.4). It was observed from the data presented from Table 5.3.8 showed that the K^+/Na^+ ratio in young leaf showed no significant correlation with any trait.

The variety Dandara had highest K^+/Na^+ ratio (7.2 ±3.1 (4) and 138 ± 29 (4)) in salt and control treatment respectively followed by MNH 147 x CIM 448 x CIM 448 (3.7 ± 1.3 (4) and 118 ± 28 (4)) in young petiole. Whereas, the lowest K^+/Na^+ ratio was observed in CIM 448 x Dandara x CIM 448 (2.0 ± 0.4 (4)) followed by MNH 147 (2.6 ± 0.5 (4)) in salt treatment and in control treatment MNH 147 had the lowest K^+/Na^+ ratio (72 ± 51 (4)) followed by CIM 448 x Dandara x CIM 448 (98± 32 (4)) in young petiole (Table 5.3.4). It was observed from the data presented from Table 5.3.8 showed that the K^+/Na^+ ratio in young petiole showed highly significant correlation with Ca^{++} concentration in young stem.

The variety Dandara had highest K^+/Na^+ ratio (5.3 ± 5 (4) and 147 ± 14 (4)) in salt and control treatment respectively followed by MNH 147 x CIM 448 x CIM 448 (2.4 ± 1.3 (4)) in salt treatment and CIM 448 (116 ± 42 (4)) in control treatment. Whereas, the lowest K^+/Na^+ ratio was observed in MNH 147 (1.6 ± 0.4 (4)) followed by CIM 448 x Dandara x CIM 448 (1.7 ± 0.2 (4)) in salt treatment and in control treatment MNH 147 had the lowest K^+/Na^+ ratio (70 ± 37 (4)) followed by CIM 448 x Dandara x CIM 448 (81± 45 (4)) in young stem (Table 5.3.4). It was observed from the data presented from Table 5.3.8 showed that the K^+/Na^+ ratio in young stem showed no correlation with any trait.

The variety Dandara had highest K^+/Na^+ ratio (5.6 ± 5.3 (4) and 101 ± 38 (4)) in salt and control treatment respectively followed by MNH 147 (1.7 ± 0.8 (4)) in salt treatment and MNH 147 x CIM 448 x CIM 448 (21 ± 10 (4)) in control treatment. Whereas, the lowest K^+/Na^+ ratio was observed in MNH 147 x CIM 448 x CIM 448 (1.1 ± 0.6(4)) followed by CIM 448 x Dandara x CIM 448 (1.36 ± 0.9 (4)) in salt treatment and in control treatment CIM 448 had the lowest K^+/Na^+ ratio (11± 8 (4)) followed by MNH 147 (16± 8 (4)) in old leaf (Table 5.3.4). It was observed from the data presented from Table 5.3.8 showed that the K^+/Na^+ ratio in old leaf showed highly significant negative correlation with K^+/Na^+ ratio in old petiole and young stem and significant positive correlation with K^+/Na^+ ratio in young petiole Ca^{++} concentration in young stem.

The varieties CIM 448 and CIM 448 x Dandara x CIM 448 had highest K^+/Na^+ ratio (3.4 ± 1.1 (4) and 3.4 ± 0.8 (4)) in salt treatment respectively followed by MNH 147 (3.3 ± 0.2 (4)) and in control treatment Dandara and MNH 147 x CIM 448 x CIM 448 had highest K^+/Na^+ ratio (53 ± 45 (4) and 53 ± 15 (4)) followed by CIM 448 x Dandara x CIM 448 (46 ± 12 (4)) in old petiole. Whereas, the lowest K^+/Na^+ ratio was observed in Dandara (1.7 ± 0.2 (4)) followed by MNH 147 x CIM 448 x CIM 448 (3.2± 0.6 (4)) in salt treatment and in control treatment MNH 147 had the lowest K^+/Na^+ ratio (35 ± 13 (4)) followed by CIM 448 (45 ± 11 (4)) in old petiole (Table 5.3.4). It was observed from the data presented from Table 5.3.8 showed that the K^+/Na^+ ratio in old

petiole showed highly significant negative correlation with K^+/Na^+ ratio in young petiole and Ca^{++} concentration in young stem and significant positive correlation with K^+/Na^+ ratio in young stem.

The varieties MNH 147 x CIM 448 x CIM 448 had highest K^+/Na^+ ratio (3.2 ± 1.0 (4)) in salt treatment followed by MNH 147 (2.9 ± 0.8 (4)) and in control treatment CIM 448 x Dandara x CIM 448 had highest K^+/Na^+ ratio (102 ± 74 (4)) followed by MNH 147 x CIM 448 x CIM 448 (52 ± 9 (4)) in old stem. Whereas, the lowest K^+/Na^+ ratio was observed in Dandara (0.7 ± 0.2 (4)) followed by CIM 448 and CIM 448 x Dandara x CIM 448 (2.3 ± 0.2 (4) and 2.3 ± 0.4 (4)) respectively in salt treatment and in control treatment MNH 147 had the lowest K^+/Na^+ ratio (34 ± 13 (4)) followed by CIM 448 (41 ± 19 (4)) in old stem (Table 5.3.4). It was observed from the data presented from Table 5.3.8 showed that the K^+/Na^+ ratio in old stem showed significant positive correlation with K^+/Na^+ ratio in young stem and Ca^{++} concentration in old stem

5.3.5. The effect of salt on Ca^{++} concentration in young and old leaves, petiole and stem.

The effect of salt on Ca^{++} concentration in young petiole and old leaf was significant ($P < 0.000$) (Table 5.3.6). The genotypes were significantly ($P<0.000$) different from each other in old leaf and stem. The interaction was significant ($P < 0.000$) in old stem.

The variety Dandara had highest Ca^{++} concentration (134.6 ± 13.8 (4) mM) in salt treatment followed by CIM 448 x Dandara x CIM 448 (118.2 ± 18.7 (4) mM) in young leaf, and in control treatment MNH 147 had highest Ca^{++} concentration (119.5 ± 16.2 (4) mM) followed by Dandara (118.2 ± 28.7 (4) mM). Whereas, the lowest Ca^{++} concentration was observed in MNH 147 x CIM 448 x CIM 448 (91.9 ± 13.6 (4) and 106.3 ± 30.8 (4) mM) in salt and control treatment respectively in young leaf (Table 5.3.5). It was observed from the data presented from Table 5.3.8 showed that the Ca^{++} concentration in young leaf showed no significant correlation with any trait.

The variety Dandara had highest Ca^{++} concentration (164.1 ± 3.3 (4) and 136.5 ± 23.3 (4) mM) in salt and control treatment respectively followed by MNH 147 x CIM 448 x CIM 448 (162.1 ± 11.3 (4) mM) in salt treatment, and CIM 448 x Dandara x CIM 448 (131.9 ± 11.0 (4) mM) in control treatment. Whereas, the lowest Ca^{++} concentration was observed in CIM 448 (152.5 ± 12.1 (3) mM) in salt treatment and MNH 147 x CIM 448 x CIM 448 had lowest Ca^{++} concentration (122.8 ± 14.3 (4) mM) in control treatment of young petiole (Table 5.3.5). It was observed from the data presented from Table 5.3.8 showed that the Ca^{++} concentration in young petiole showed no significant correlation with any trait.

The variety Dandara had highest Ca^{++} concentration (137.2 ± 9.7 (4) and 122.1 ± 17.1 (4) mM) in salt and control treatment respectively followed by MNH 147 x CIM 448 x CIM 448 (112.3 ± 21.7 (4) mM) in salt treatment and MNH 147 (117.5 ± 26.8 (4) mM) in control treatment. Whereas, the lowest Ca^{++} concentration was observed in MNH 147 (105.1 ± 13.1 (4) mM) in salt treatment and in control treatment MNH 147 x CIM 448 x CIM 448 had the lowest Ca^{++} concentration (101.8 ± 15.7 (4) (4) mM) in young stem (Table 5.3.5). It was observed from the data presented from Table 5.3.8 showed that the Ca^{++} concentration in young stem showed no correlation with any trait.

The variety Dandara had highest Ca^{++} concentration (214.0 ± 22.9 (4) and 187.7 ± 27.6 (4) mM) in salt and control treatment respectively followed by CIM 448 x Dandara x CIM 448 (200.8 ± 22.8 (4) and 179.2 ± 22.8 (4) mM) in salt and control treatment respectively in old leaf. Whereas, the lowest Ca^{++} concentration was observed in MNH 147 x CIM 448 x CIM 448 (180.5 ± 4.2 (4) and 146.4 ± 6.4 (4) mM) in salt and control treatment respectively in old leaf (Table 5.3.5). It was observed from the data presented from Table 5.3.8 showed that the Ca^{++} concentration in old leaf showed no significant correlation with any trait.

The variety CIM 448 had highest Ca^{++} concentration (163.9 ± 12.4 (3) mM) in salt treatment followed by CIM 448 x Dandara x CIM 448 (162.8 ± 7.2 (4) mM), and in control treatment variety Dandara had a highest Ca^{++} concentration (174.6 ± 38.3 (4) mM), followed by CIM 448 x Dandara x CIM 448 (163.4 ± 21.6 (4) mM) in old petiole. Whereas, the lowest Ca^{++} concentration was observed in MNH 147 x CIM 448 x CIM 448 (160.8 ± 15.8 (4) and 143.8 ± 10.7 (4) mM) in salt and control treatment respectively in old petiole (Table 5.3.5). It was observed from the data presented from Table 5.3.8 showed that the Ca^{++} concentration in old petiole showed highly significant negative correlation with Ca^{++} concentration in young petiole. The variety MNH 147 had highest Ca^{++} concentration (101.1 ± 3.3 (4) and 88 ± 6.5 (4) mM) in salt and control treatment respectively followed by CIM 448 (93.1 ± 9.9 (3) mM), in salt treatment MNH 147 x CIM 448 x CIM 448 (81.4 ± 10.5 (4) mM) in control treatment. Whereas, the lowest Ca^{++} concentration was observed in Dandara (61.1 ± 7.8 (4) and 75.5 ± 10.1 (4) mM) in salt and control treatment respectively in old stem (Table 5.3.5). It was observed from the data presented from Table 5.3.8 showed that the Ca^{++} concentration in old stem showed negative correlation with Ca^{++} concentration in young leaf and stem.

Table 5.3.1. Means and standard deviation of plant height, fresh weight, dry weight and chlorophyll content of cotton genotypes.

	Plant height (mm)		Nodes per plant		FW (g)		DW (g)		Chlorophyll content	
Genotypes	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**
CIM 448	64±1.4	42.3±16	12.8±0.5	9±2	123±8.4	39±10.2	20±0.7	7.8±1.9	50.7±3.3	45.2±3.1
CIM 448 x Dandara x CIM 448	67±8.9	38.1±6.2	12.8±1.3	7.8±13	137±22	35.5±15	27±4.4	8.1±3.1	56.5±2.5	48.1±3.3
Dandara	86±3.6	54.8±5.5	11±1.4	7.8±0.5	109±20.2	34.2±1.3	22±4.3	7.9±0.9	41.9±4.1	41.2±3.3
MNH 147	67±5.4	40±2.4	12.3±1.7	8±1.4	126±51	40.9±6.4	22±7.5	9±01.6	50.6±8.2	49±4.0
MNH 147 x CIM 448 x CIM 448	66±7.4	38.6±0.8	12±0.8	8.5±1.3	123±42	39.2±9.3	20±8.4	8.1±1.9	45.3±6.2	43±4.1

Table 5.3.2. Means and standard deviation of Na^+ concentration in young and old plant tissues of cotton genotypes. (young leaf = Y.L, young petiole = Y.P, young stem = Y.S, old leaf = O.L, old petiole = O.P and old stem = O.S).

	Na^+ (Y.L)		Na^+ (Y.P)		Na^+ (Y.S)		Na^+ (O.L)		Na^+ (O.P)		Na^+ (O.S)	
Genotypes	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**
CIM 448	1.8±1.2	79.4±42	2.1±1.3	93±41	2.1±1.3	116±28.7	6.1±2.8	80.2±43	5.5±1.2	82.7±31	5.2±2.3	87±18
CIM 448 x Dandara x CIM 448	1.8±0.6	121±40	2.4±0.6	134±21	3±1.2	133±7.8	5.5±1.9	80.5±19	6.1±2.1	82.4±18	3±1.9	78±8.6
Dandara	1.8±0.6	83.3±48	1.8±0.6	53±24	1.1±0.6	94.3±57.7	1.8±1.6	63±34	9.6±7.7	149.9±19	8±5.7	155±21
MNH 147	2.4±1.9	122±23	4.6±3.9	109±21	4±2.7	146±28.6	8.6±3.4	84.3±21	8.3±3	79±8.9	6.1±2.1	84±22
MNH 147 x CIM 448 x CIM 448	1.1±0.6	84.6±54	1.5±1	83±35	3±1.9	111±43	3.3±1.6	105±33	4.6±1.3	88.6±25	3.6±0.6	77±31

Table 5.3.3. Means and standard deviation of K^+ concentration in young and old plant tissues of cotton genotypes. (young leaf = Y.L, young petiole = Y.P, young stem = Y.S, old leaf = O.L, old petiole = O.P and old stem = O.S).

	K^+ (Y.L)		K^+ (Y.P)		K^+ (Y.S)		K^+ (O.L)		K^+ (O.P)		K^+ (O.S)	
Genotypes	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**
CIM 448	130±2.	143±19	203±12	252±17	203±11	204±21	55±22	91±30	239±3	254±6	180±8	197±21
CIM 448 x Dandara x CIM 448	134±31	151±29	220±26	266±28	205±27	226±25	102±24	101±46	262±25	268±25	244±82	178±15
Dandara	159±21	205±18	231±25	331±33	214±17	308±12	180±6	218±30	293±28	256±15	163±6	102±17
MNH 147	156±18	163±28	207±16	273±14	209±28	220±33	115±27	131±51	260±5	256±14	191±11	235±11
MNH 147 x CIM 448 x CIM 448	135±29	131±23	197±12	274±22	195±16	226±48	59±24	105±23	228±18	270±32	186±8	221±20

Table 5.3.4. Means and standard deviation of K^+/Na^+ concentration in young and old plant tissues of cotton genotypes. (young leaf = Y.L, young petiole = Y.P, young stem = Y.S, old leaf = O.L, old petiole = O.P and old stem = O.S).

	K^+/Na^+ (Y.L)		K^+/Na^+ (Y.P)		K^+/Na^+ (Y.S)		K^+/Na^+ (O.L)		K^+/Na^+ (O.P)		K^+/Na^+ (O.S)	
Genotypes	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**
CIM 448	230±34	2.1±0.9	116±42	3.1±1.4	116±40	1.9±0.6	11±8	1.4±0.9	45±11	3.4±1.1	41±19	2.3±0.2
CIM 448 x Dandara x CIM 448	78±12.4	1.4±0.7	98±32	2.0±0.4	81±45	1.7±0.2	20±9	1.36±0.9	46±12	3.4±0.8	102±74	2.3±0.4
Dandara	97±27	4.2±4	138±29	7.2±3.1	147±14	5.3±5	103±38	5.6±5.3	53±45	1.7±0.2	42±48	0.7±0.2
MNH 147	87±38.5	1.4±0.5	72±51	2.6±0.5	70±37	1.6±0.4	16±8	1.7±0.8	35±13	3.3±0.2	34±13	2.9±0.8
MNH 147 x CIM 448 x CIM 448	193±79	2.4±1.8	118±28	3.7±1.3	88±49	2.4±1.3	21±10	1.1±0.6	53±15	3.2±0.6	52±9	3.2±1.0

Table 5.3.5. Means and standard deviation of Ca^{++} concentration in young and old plant tissues of cotton genotypes. (young leaf = Y.L, young petiole = Y.P, young stem = Y.S, old leaf = O.L, old petiole = O.P and old stem = O.S).

	Ca^{++} (Y.L)		Ca^{++} (Y.P)		Ca^{++} (Y.S)		Ca^{++} (O.L)		Ca^{++} (O.P)		Ca^{++} (O.S	
Genotypes	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**
CIM 448	123±16	95±33	127±14	153±12	109±7	111±6	157±12	182±29	146±6	164±12	80±2	93±10
CIM 448 x Dandara x CIM 448	125±13	118±19	132±11	159±12	106±19	105±10	179±23	201±23	164±21	163±7	80±8	79±7
Dandara	118±29	135±14	137±23	164±3	122±17	137±10	188±28	214±23	175±38	161±10	76±10	61±8
MNH 147	120±16	115±9	131±18	161±3	118±27	105±13	166±10	194±23	157±11	162±5	88±7	101±3
MNH 147 x CIM 448 x CIM 448	106±31	92±14	123±14	162±11	102±16	112±22	146±6	181±4	144±11	161±16	82±11	93±7

Table 5.3.6. Mean square, degree of freedom and P values of different traits of cotton genotypes.

Variables	Genotypes	DF	Treatment	DF	Genotypes x Treatments	DF
Plant height	493.186**	4	7098.454**	1	22.344^{NS}	4
Nodes/plant	2.143^{NS}	4	150.992**	1	$.960^{NS}$	4
FW	240.457^{NS}	4	71272.956**	1	184.391^{NS}	4
DW	14.449^{NS}	4	1858.313**	1	13.386^{NS}	4
Chlorophyll content	148.481**	4	113.081**	1	24.702^{NS}	4
Na^+ (Y.L)	934.436^{NS}	4	89924.097**	1	887.171^{NS}	4
K^+ (Y.L)	3213.398**	4	2454.248*	1	670.504^{NS}	4
K^+/Na^+ (Y.L)	9048.284^{NS}	4	175229.485**	1	9023.589^{NS}	4
Ca^{++} (Y.L)	929.688^{NS}	4	554.380^{NS}	1	493.501^{NS}	4
Na^+ (Y.P)	1888.934**	4	81835.501**	1	1752.905**	4
K^+ (Y.P)	3347.442**	4	43815.558**	1	958.118^{NS}	4
K^+/Na^+ (Y.P)	22132.481^{NS}	4	212102.150**	1	21826.916^{NS}	4
Ca^{++} (Y.P)	120.774^{NS}	4	8691.542**	1	59.214^{NS}	4
Na^+ (Y.S)	884.729^{NS}	4	133419.422**	1	732.230^{NS}	4
K^+(Y.S)	4083.979**	4	9655.840**	1	2541.999**	4
K^+/Na^+ (Y.S)	29593.579^{NS}	4	188063.972**	1	28113.160^{NS}	4
Ca^{++} (Y.S)	761.754^{NS}	4	85.651^{NS}	1	227.731^{NS}	4
Na^+ (O.L)	488.877^{NS}	4	58125.000**	1	431.455^{NS}	4
K^+ (O.L)	19844.561**	4	6960.439**	1	710.815^{NS}	4
K^+/Na^+ (O.L)	32696.056^{NS}	4	49305.600^{NS}	1	30828.640^{NS}	4
Ca^{++} (O.L)	1793.878**	4	7189.385**	1	42.596^{NS}	4
Na^+ (O.L)	1962.534**	4	77843.687**	1	1635.874**	4
K^+ (O.P)	1018.835^{NS}	4	188.955^{NS}	1	1630.226**	4
K^+/Na^+ (O.P)	101.795^{NS}	4	18162.173**	1	123.304^{NS}	4
Ca^{++} (O.P)	297.777^{NS}	4	236.523^{NS}	1	331.723^{NS}	4
Na^+ (O.S)	2398.084**	4	79955.347**	1	1952.185**	4
K^+ (O.S)	8821.632**	4	389.450^{NS}	1	5646.624**	4
K^+/Na^+ (O.S)	2905.909^{NS}	4	31226.041**	1	2893.332^{NS}	4
Ca^{++} (O.S)	779.519**	4	200.347^{NS}	1	295.224**	4

Table 5.3.7. Correlation of plant height, nodes per plant, fresh and dry weight, chlorophyll content, Na^+ and K^+ concentration in young and old leaf, petiole and stem. (young leaf = Y.L, young petiole = Y.P, young stem = Y.S, old leaf = O.L, old petiole = O.P and old stem = O.S).

	Plant height	Nodes /plant	FW	DW	Chl. content	Na^+ (O.L)	Na^+ (O.P)	Na^+ (O.S)	Na^+ (Y.L)	Na^+ (Y.P)	Na^+ (Y.S)	K^+ (O.L)	K^+ (O.P)	K^+ (O.S)	K^+ (Y.L)	K^+ (Y.P)	K^+ (Y.S)
Plant height	1	-0.278	-0.614	-0.36	-0.689	-0.779	.957(*)	.992(**)	-0.48	-0.809	-0.723	.914(*)	-0.533	-0.872	.891(*)	.879(*)	.897(*)
Nodes/plant		1	0.536	-0.35	-0.144	0.42	-0.409	-0.395	-0.602	-0.106	-0.146	-0.58	-0.166	0.424	-0.63	-0.596	-0.588
FW			1	0.594	0.445	0.652	-0.72	-0.664	0.142	0.248	0.561	-0.567	-0.099	.918(*)	-0.608	-0.635	-0.744
DW				1	0.664	0.217	-0.389	-0.311	0.738	0.373	0.779	-0.044	-0.083	0.579	-0.008	-0.132	-0.257
Chlorophyll Content					1	0.15	-0.765	-0.66	0.868	.904(*)	.980(**)	-0.548	-0.002	0.611	-0.34	-0.642	-0.664
Na^+ (O.L)						1	-0.662	-0.783	0.021	0.278	0.271	-0.686	0.659	0.803	-0.862	-0.581	-0.64
Na^+ (O.P)							1	.975(**)	-0.452	-0.804	-0.781	.935(*)	-0.268	-.902(*)	0.858	.956(*)	.977(**)
Na^+ (O.S)								1	-0.395	-0.77	-0.69	.951(*)	-0.464	-.892(*)	.926(*)	.925(*)	.942(*)
Na^+ (Y.L)									1	0.787	0.871	-0.193	0.174	0.348	-0.03	-0.243	-0.273
Na^+ (Y.P)										1	0.852	-0.71	0.329	0.545	-0.527	-0.729	-0.702
Na^+ (Y.S)											1	-0.533	0.045	0.708	-0.371	-0.618	-0.668
K^+ (O.L)												1	-0.37	-0.775	.950(*)	.978(**)	.958(*)
K^+ (O.P)													1	0.233	-0.515	-0.181	-0.163
K^+ (O.S)														1	-0.8	-0.794	-0.876
K^+ (Y.L)															1	.884(*)	.884(*)
K^+ (Y.P)																1	.988(**)
K^+ (Y.S)																	1

Table 5.3.8. Correlation of K^+ and Ca^{++} concentration and K^+/Na^+ ratio in young and old leaf, petiole and stem.

	K^+/Na^+ (O.L)	$K+/Na^+$ (O.P)	K^+/Na^+ (O.S)	K^+/Na^+ (Y.L)	K^+/Na^+ (Y.P)	K^+/Na^+ (Y.S)	Ca++ (O.L)	Ca++ (O.P)	Ca++ (O.S)	Ca++ (Y.L)	Ca++ (Y.P)	Ca++ (Y.S)
Plant height	.974(**)	-.956(*)	-.922(*)	-0.187	.943(*)	-.962(**)	0.703	-0.326	-0.779	0.667	0.378	.961(**)
Nodes/plant	-0.485	0.446	0.393	.991(**)	-0.23	0.44	-0.841	0.514	0.537	-0.862	-0.718	-0.236
FW	-0.683	0.666	0.822	0.428	-0.561	0.565	-0.775	0.089	.970(**)	-0.686	-0.31	-0.647
DW	-0.252	0.294	0.472	-0.452	-0.39	0.135	-0.015	-0.319	0.552	0.11	0.275	-0.472
Chlorophyll content	-0.617	0.731	0.526	-0.202	-0.856	0.48	-0.162	0.417	0.575	-0.072	-0.358	-0.848
Na^+ (O.L)	-0.782	0.655	.908(*)	0.313	-0.535	0.856	-0.819	-0.128	0.712	-0.81	-0.037	-0.608
Na^+ (O.P)	.974(**)	-.995(**)	-.888(*)	-0.326	.971(**)	-.899(*)	0.743	-0.499	-0.866	0.69	0.572	.983(**)
Na^+ (O.S)	.995(**)	-.978(**)	-.929(*)	-0.307	.941(*)	-.971(**)	0.776	-0.396	-0.822	0.742	0.47	.958(*)
Na^+ (Y.L)	-0.312	0.404	0.313	-0.657	-0.602	0.24	0.226	0.016	0.247	0.308	0.117	-0.603
Na^+ (Y.P)	-0.729	0.807	0.554	-0.15	-.923(*)	0.672	-0.228	0.507	0.453	-0.186	-0.406	-.885(*)
Na^+ (Y.S)	-0.643	0.732	0.626	-0.226	-0.843	0.511	-0.222	0.266	0.665	-0.121	-0.245	-0.861
K^+ (O.L)	.972(**)	-.965(**)	-0.812	-0.524	.895(*)	-.946(*)	0.823	-0.615	-0.739	0.815	0.686	.883(*)
K^+ (O.P)	-0.418	0.295	0.453	-0.215	-0.302	0.611	-0.225	-0.269	0.035	-0.276	0.309	-0.313
K^+ (O.S)	-.890(*)	0.864	.971(**)	0.307	-0.798	0.818	-0.805	0.156	.977(**)	-0.73	-0.316	-0.866
K^+ (Y.L)	.949(*)	-.884(*)	-0.877	-0.558	0.769	-.973(**)	.912(*)	-0.39	-0.747	.916(*)	0.511	0.785
K^+ (Y.P)	.950(*)	-.976(**)	-0.781	-0.542	.916(*)	-0.874	0.797	-0.694	-0.792	0.77	0.769	.903(*)
K^+ (Y.S)	.963(**)	-.982(**)	-0.85	-0.518	.917(*)	-0.877	0.829	-0.599	-0.877	0.787	0.702	.924(*)
K^+/Na^+ (O.L)	1	-.982(**)	-.921(*)	-0.403	.924(*)	-.971(**)	0.827	-0.446	-0.836	0.797	0.536	.939(*)
$K+/Na^+$ (O.P)		1	0.864	0.372	-.970(**)	.917(*)	-0.755	0.556	0.827	-0.714	-0.62	-.972(**)
K^+/Na^+ (O.S)			1	0.277	-0.793	.905(*)	-0.822	0.093	.907(*)	-0.77	-0.238	-0.857
K^+/Na^+ (Y.L)				1	-0.161	0.359	-0.768	0.562	0.43	-0.8	-0.745	-0.153
$K+/Na^+$ (Y.P)					1	-0.852	0.575	-0.539	-0.74	0.525	0.545	.991(**)
K^+/Na^+ (Y.S)						1	-0.809	0.337	0.728	-0.803	-0.416	-0.867
Ca++ (O.L)							1	-0.332	-0.827	.991(**)	0.542	0.617
Ca++ (O.P)								1	0.235	-0.345	-.960(**)	-0.447
Ca++ (O.S)									1	-0.746	-0.421	-0.806
Ca++ (Y.L)										1	0.546	0.558
Ca++ (Y.P)											1	0.48
Ca++ (Y.S)												1

5.3.32. Principal component analysis of phenotypes.

In this project I have been trying to correlate the differences of the values of such components (derived from physiological data) with known genetic (and hence breeding history) differences. The genetic differences have been described quantitatively according to the SSR pattern at 17 loci of each variety (see in chapter 4). The aim is to be able to predict the physiological behaviour of a variety from its SSR pattern. This is a "model" exercise for the processes involved in predicting a breeding trait from a genetic marker – marker assisted breeding.

This experiment was designed to perform this analysis on three independent varieties (called "parents" for convenience; CIM 448, Dandara and MNH 147) and sixteen BC1 derived from crosses between three (CIM 448, Dandara and MNH 147) of these "parents". This "models" the real-world situation where selection for traits would be made at each generation.

Application of PCA to the mean values of seven physiological parameters (Na^+ young and old petiole, Na^+ old stem, K^+ young stem, K^+ old petiole and K^+ old stem and Ca^{++} old stem) generated Figure 5.3.31. This reveals several grouping according to low and high Na^+, K^+ and Ca^{++}. The clearest are generated by PC1 and PC2, which account for 49% and 37% of the total variance respectively. Here we see varieties CIM 448 and MNH 147 (group 1) grouping apart from Dandara (group 11). Two crosses were used in this experiment. Both have parents in the same groups (MNH 147 and CIM 448) with regard to PC1, and in PC 2 both crosses have both parents in same group (Dandara and CIM 448).

Principal component 1 (PC1) is a function of Na^+ in young and old petiole, Na^+ in old stem and K^+ in young stem. Principal component 2 (PC2) is a function of K^+ in old petiole and stem and Ca^{++} in old stem. The Ward Linkage correlation coefficient distance for these individual parameters is shown in Fig 5.3.32. There is, perhaps, an expected division between the two PCs – for example Na^+ in young petiole and old stem being dependent of each other (except K^+

young stem), and K^{+} in old petiole and old stem, Ca^{++} in old stem being independent in this respect.

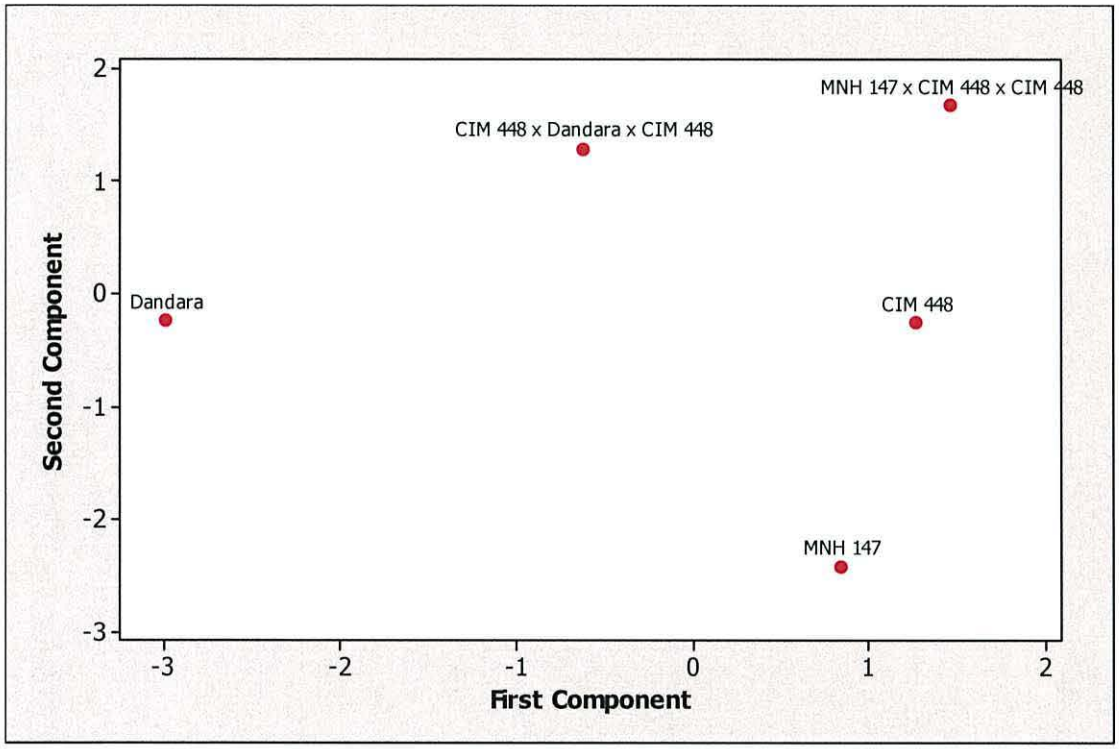

Fig 5.3.31. Scatter plot of three cotton varieties with their BC1 based on principal component analysis generated by significant physiological traits bases.

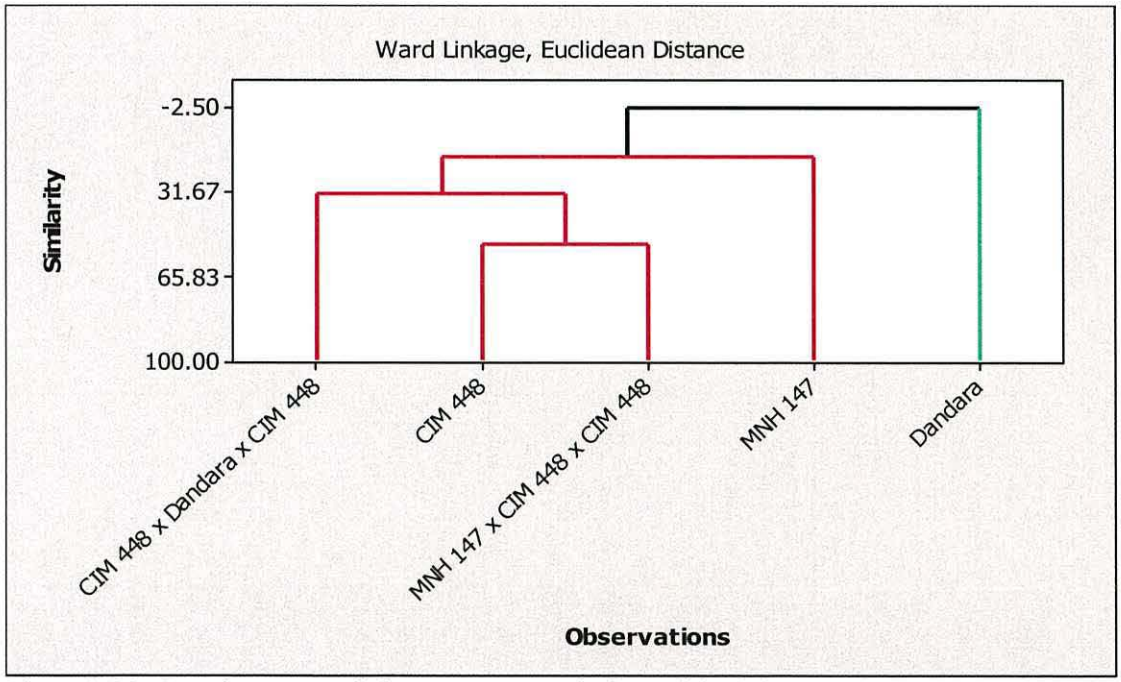

Fig 5.3.32. Dendrogram of three cotton varieties with their BC1 based on principal component analysis generated by significant physiological traits bases.

5.3.33 Fragment analysis using agarose gel electrophoresis

The results of PCR amplification of a number of SSR loci in the three varieties and their crosses (BC1) using 17 microsatellite (SSR) primer pairs are summarized in Table 5.2. The experiment detected a total of 17 alleles at 17 loci, with average of 1.0 alleles per locus (Fig. 5.3.33, 5.3.34 & 5.3.35). No polymorphism was found in this study. Fifteen primer pairs gave the expected size of fragment. Two primers NAU 2777 and 1195 gave the larger size (1000 bp) than expected. Eleven alleles are present on ten different chromosomes (Fig. 5.3.36).

Table 5.2. Number of alleles of each SSR locus in different cotton genotypes.

S.NO	Locus	Total number of alleles	S.NO	Locus	Total number of alleles
1	BNL 1434	1	10	MGHES 29	1
2	BNL 1878	1	11	MGHES 31	1
3	BNL 1679	1	12	NAU 920	1
4	BNL 1681	1	13	NAU 1195	1
5	BNL 1694	1	14	NAU 2748	1
6	MGHES 06	1	15	NAU 2777	1
7	MGHES 13	1	16	NAU 3232	1
8	MGHES 21	1	17	Gh 137	1
9	MGHES 25	1			

Table 5.3. Cotton genotypes and the track number identifies used in the illustrators of gels predicted in this Table.

Track NO.	Genotypes	Track NO.	Genotypes
1	Dandara	11	MNH 147
2	CIM 448	12	CIM 448
3	CIM 448 x Dandara x CIM 448 P1	13	MNH 147 x CIM 448 x CIM 448 P1
4	CIM 448 x Dandara x CIM 448 P2	14	MNH 147 x CIM 448 x CIM 448 P2
5	CIM 448 x Dandara x CIM 448 P3	15	MNH 147 x CIM 448 x CIM 448 P3
6	CIM 448 x Dandara x CIM 448 P4	16	MNH 147 x CIM 448 x CIM 448 P4
7	CIM 448 x Dandara x CIM 448 P5	17	MNH 147 x CIM 448 x CIM 448 P5
8	CIM 448 x Dandara x CIM 448 P6	18	MNH 147 x CIM 448 x CIM 448 P6
9	CIM 448 x Dandara x CIM 448 P7	19	MNH 147 x CIM 448 x CIM 448 P7
10	CIM 448 x Dandara x CIM 448 P8	20	MNH 147 x CIM 448 x CIM 448 P8

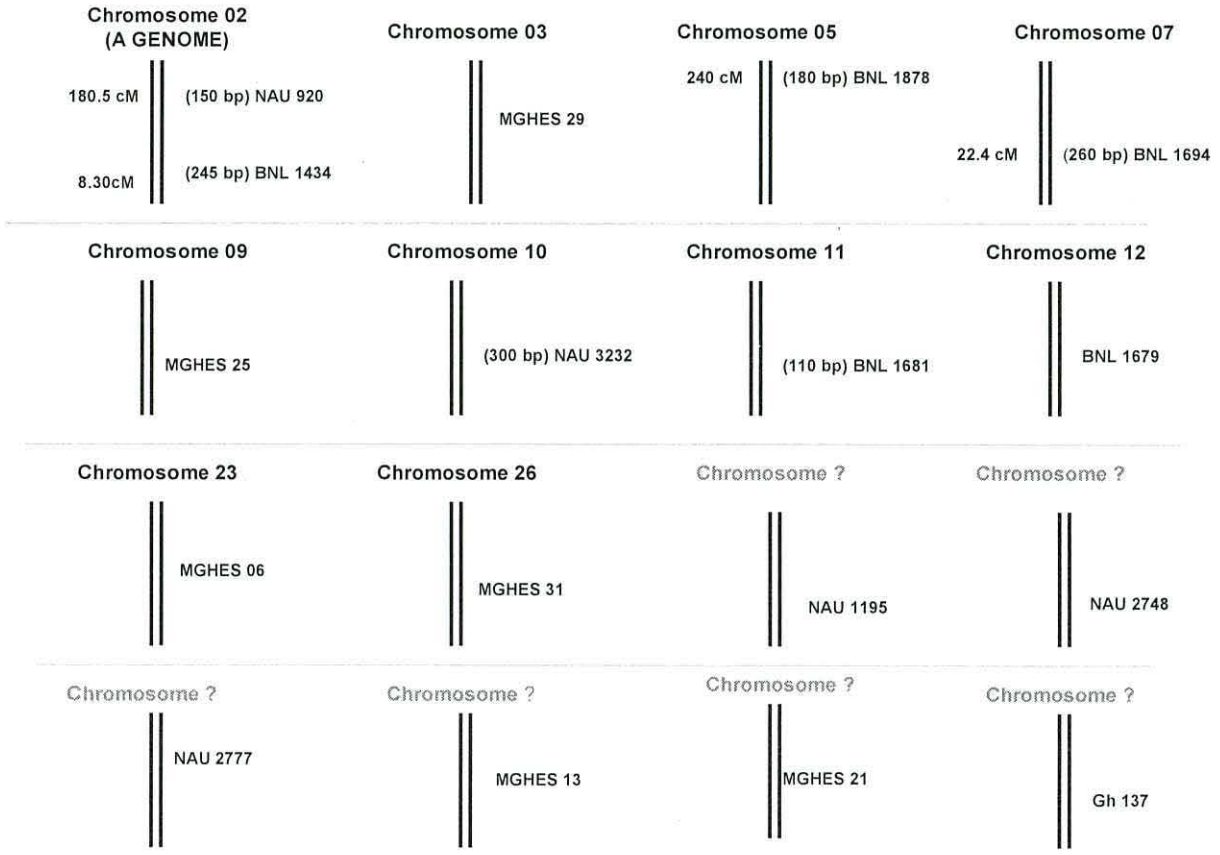

Fig.5.3.33. Genetic map constructed using 43 cotton varieties with 17 SSR primers. Eleven of these on 10 chromosomes were confirmed by different literature. (Nguyen *et al.*, 2004, Song *et al.*, 2005 and Wang *et al.*, 2006). The locations of six primers are unknown.

5.4 Discussion

5.4.1 Effect of salinity on physiological characters

In this chapter the physiological traits of three varieties and examples of their back crosses were measured. The application of NaCl (150 mM) affected the growth of all genotypes. Plant shoot heights, nodes per plant, fresh and dry weight were significantly reduced by salt stress. Similar results were previously observed by Karl and Lauchli, 2002, Khan, *et al.*, 2004, Meloni, *et al.*, 2003). In this study a highly significant difference was found only within cultivars with respect to plant height. No significant differences were found either between the genotypes or in the interaction between the genotypes to salt treatment in all other traits.

Plant growth may be affected through an initial osmotic effect in this stress when salt concentrations increase in soil solution outside the plant. The water potential gradient decreases, the uptake of water can be slow, making it more difficult for water and nutrients to move through root membranes and into the plant. A second effect is the toxic ion effect during salt stress, in this effect the toxic salt solution enters the plant tissues and reduces the growth (Munns, 2005). These effects may inhibit germination or slow down plant growth. The above two effects are also dependent upon the level of salt in the growth medium (Kent and Lauchli, 1985). The chlorophyll content was significantly decreased by salt stress but no significant differences were found in interaction of the genotypes and salt treatments. Similar results were found by Munis, *et al.*, (2010).

The concentrations of Na^+ in various young and old parts of plants were highly significantly increased by 150 mM salt treatment. Highly significant differences were found in young and old petiole and old stem in genotypes and interaction between genotypes by salt. This result was similar to Ashraf and Saghir (2000) who found that leaves of cotton grown at three different levels (70, 140, 210 mM) of NaCl had high concentration of Na^+. The salt tolerant variety CIM

448 had a lower Na^+ concentration in the shoot than the two other varieties and their crosses. Rathert (1982) claimed that the salt tolerance is associated with low accumulation of Na^+ in shoot. In contrast Ashraf and Saghir (2000) and Leidi and Saiz (1997) claimed salt tolerance association with high Na^+ accumulation in the shoot of cotton plants. Our data indicated that the salt tolerant cotton genotype (CIM 448) excluded the Na^+ ion better (Table 5.4.1) than the salt sensitive genotypes (Dandara, MNH 147 and the crosses).

The highly significant effect in young organs and significant effect in old leaves were found for Potassium (K^+) following salt treatment. The K^+ concentration was increased in various parts of plants by salt stress in most cases. However, the cross (MNH 147 x CIM 448 x CIM 448) shows a decrease in K^+ concentration in young leaves and in MNH 147 and Dandara, K^+ decreased in old petiole. While in Dandara and its cross (CIM 448 x Dandara x CIM 448) K^+ decreased in the old stem. In contrast to the results for cotton, a decrease of K^+ in response to an increase in salinity was observed in many salt tolerant plants (Ashraf and Saghir 2000). However, in our data, the sensitive varieties and their crosses (Dandara, MNH 147 and the crosses) showed decreased K^+ ion in young leaves and old petiole and stem. This could be the result of replacement of Na^+ for K^+ ions. Hu and Schmidhalter, (2005) reported that the K^+ is a competitor of Na^+ under saline conditions.

The Ca^{++} concentration was not significantly affected by salt stress, except in the young petiole and old leaf. Similar result was reported by Abd – Ella and Shalaby (1995). Ca^{++} concentration was increased in various parts of plants by salt stress in most cases. However, the cross (CIM 448 x Dandara x CIM 448) shows a decrease in Ca^{++} concentration in young leaves and stem and old petiole and stem, and in MNH 147 shows a decrease in Ca^{++} concentration in young leaves and stem and Dandara Ca^{++} concentration decreased in old petiole and stem. Kaya *et al*,. (2002) claimed that the Ca^{++} concentration increased in roots and decreased in strawberry leaves.

The K^+/ Na^+ ratio was significantly decreased by salt treatment, except in old leaves. Gorham, *et al.*, (1985) reported that the salt tolerant varieties maintained the high K^+/ Na^+ ratio. However, the variety Dandara had a higher K^+/ Na^+ ratio than other cultivars, despite being previously classified as being sensitive.

5.4.2 Molecular diversity

No polymorphism was found between the varieties and their BC1 population in any of the seventeen SSR primers pairs used in this chapter. Our data shows that 15 primer pairs amplified sequences of the same size as expected and two primers, which belong to expressed sequence tag SSR (EST – SSR) amplified larger size than expected. Chee, *et al.*, (2004) also observed similar results. They found 23% amplified product of PCR primers that was more than 150 nucleotides longer than expected from EST sequences. This could be the gap between PCR product and its EST sequence from data base.

The failure to find polymorphism could be due to the use of an electrophoresis method (Erkilinc & Mehmet Karaca, 2005). Three parents used in this chapter were also screened with 17 primers by the electrophoresis method (see in chapter 5). They were similar to each other. However, it's more likely to be due to the narrow genetic base of cotton (*Gossypium hirsutum L.*) (Stella, *et al.* 2009) or not enough varieties being used.

5.5 Conclusion

In this study, a rudimentary segregating population was developed by back cross. Contrasting parents according to salt stress were selected for the developing mapping population. Ideally, the parents should have been genetically different and large number of individuals should have been analysed for detection of QTL. This was not practicable under the growth facilities available. It remains essential to find some new germplasm for development of new varieties. We observed no polymorphism in any cultivar. Overall this study indicated that might be some

polymorphisms existing with in the cultivars could not be detected due to conventional agarose gel system, so capillary electrophoresis should be used for detecting the QTLs.

Table 5.4.1 Ranking of growth parameters, chlorophyll content, photosynthesis, Na^+, K^+, K^+/Na^+ and Ca^{++} in 150 mM stress. Each entry represents {parameter value – mean}/s.d. (see 2.13 for explanation.

Genotypes	Growth Parameters And chlorophyll content	Na^+	K^+	K^+/Na^+	Ca^{++}	Average	Ranks
Dandara	-0.4338	-0.24201	1.576856	1.718779	1.497745	0.823505	1
MNH 147	0.62885	-0.86821	0.255317	-0.52276	0.366307	-0.0281	2
MNH 147 x CIM 448 x CIM 448	-0.0826	0.928008	-0.22422	-0.02242	-0.84476	-0.0492	3
CIM 448	0.20899	1.165778	-1.03344	-0.38266	-0.95094	-0.19846	4
CIM 448 x Dandara x CIM 448	-0.3214	-0.98357	-0.57451	-0.79094	-0.06835	-0.54775	5

Chapter 6

General discussion, conclusions and recommendations.

6.1. General discussion.

The main purpose of this project was to screen salt tolerant and susceptible varieties of cotton as to their physiological responses to salinity, and to identify the allelic variability and genetic relationship between cotton genotypes through the use of SSR markers. In chapter three, the effect of salt on physiological traits of different cotton genotypes were studied. In chapter four, molecular diversity in cotton genotypes were studied and in chapter five rudimentary mapping populations were developed for breeding and genetics. It must be said that by using small number of varieties, the results are not simple to interpret. This emphasises the value of large volume, high throughput techniques increasingly being used in this area.

In chapter 3 an attempt was made to co-ordinate the "salt tolerant" behaviours of the cotton varieties that were available for the project. This attempt highlighted a number of issues. Of these some were purely "technical" such as the age of the seeds being detrimental to both viability and vigour (Basra *et al.*, 2004). The latter being a particular problem, as changes in seedling vigour due to post-harvest changes in the seeds may well be a confounding issue regarding response to salt.

More intellectually interesting in the possibility those different varieties demonstrate differences in salt tolerance on the basis of different mechanisms (Gorham *et al.*, 1985). This raises a fundamental difference between classical breeding approaches and current "molecular"approaches. The former could be seen as a "holistic" procedure in which an agronomic ally desirable traits such as crop yield responses to a stress could be selected for in

the field. This has proven to be very successful strategy and is the basis of most of the considerable advances in crop breeding over the 20th century. Such a holistic approach is generally not part of molecular-based breeding strategies, especially those based on a single biochemical characterisation- such as the specificity of a K^+/ Na^+transporter.

However, as molecular biology extends its understanding into the realms of systems biology and the controlling networks of the basic biochemical steps, the distinction is becoming blurred. Some gene loci - and presumably the gene sequences themselves- control a numbers of individual biochemical traits that confine to give a holistic (agronomic ally desirable) characteristic. Such master controlling sections of the genome would be ideal targets for the plant breeder, and the ideal subjects of MAS.

This is long way from the results given in this report. However, this is a suggestion here that different cotton varieties may possess different (biochemical) characteristics that may provide salt tolerance. This appears from the data presented, to be especially so when the varieties are members of different species of cotton- or are the offspring of crosses between different species. In a number of cases, for example, *G. stocksii* behaved in a way that was qualitatively different from *G. hirsutum* varieties. Within the *G. hirsutum* paradigm such differences tended to be quantitative.

In this work I was unable to use the most appropriate index of salt tolerance – cotton yield. In its place we have relied on assessments from the literature and the use of surrogate characters. Some of the latters are easier to justify than others. Plant size and photosynthesis ability could be argued as factors likely to influence crop yield in a positive and predictable Faison. Although *G. stocksii* may show some of its tolerance by a slow growth rate. This may be an example of the different mechanisms found a cross the cotton varieties, referred to above. Sodium content, however, is far more contentious High tissue Na^+ could, on one hand, be an

indication of efficient coping strategies within the plant. An example would be efficient segmentation of the ion within vacuoles. Cotton is a moderately halophytic plant and this is a common characteristic of more classical halophytes. On the other hand high Na^+ in tissues could be a sign of weak exclusion mechanisms or poor Na^+/K^+ discrimination. Determination of K^+/Na^+ ratios does not really help in this discussion.

It is not clear, therefore, whether high Na^+ or a high K^+/Na^+ ratio is a desirable or an undesirable characters. In my attempt to being all the characteristics together in chapter 3, I have made the assumption shared by many papers in the literature that it is undesirable/ consider that this will not always be the case.

In the following discussion, therefore, I will deal with groups of such traits individually. From this it may be possible to see some pattern, arising within the different varieties. This will help in the interpretation of the analyses in which I attempt to link the physiological behaviour to give marker prevalence.

6.1.1. Shoot growth, chlorophyll content and photosynthesis under NaCl.

Salinity usually affected the plant growth, but the varieties responded differently under different levels of salinity. The plant height, nodes per plants, fresh and dry weight of cotton plants were altered significantly by NaCl. According to this study, the genotypes Acalla SJ 2, Coker 201, AC 134, *G. hirsutum* x *G. sturtainum*, *G. stocksii*, DNH 40, Dandara, CIM 448, DNH 40 x S6, S6 x DNH 40, MNH 93, S12, Qalandari, Karishma and NIAB 78 showed salt tolerance as experimented by less reduction in plant growth. The reason of the reduction in plant height may be due to the toxic effect of NaCl and imbalance of nutrient amount in cotton plants. My study is the agreement with Sattar *et al.*, (2010), who reported that this reduction is due to high amount of NaCl which reduced water uptake ability of plants and resulting in imbalanced plant

nutrients and osmotic stress, or the combination of both. The plants showed stressful effects on node formation as compared to control plants. Ahmed *et al*, (1991) reported that reduction in plant growth decreased the number and growth of fruiting branches. The reduction in growth under salt stress has been reported in many plants species in maize (Cicek & Husnii, 2002), wheat (Hameed *et al*., 2008), sorghum (Netondo *et al.,* 2004) and barley (Gorham, *et al*., 1990).

In this study, chlorophyll content was reduced by salt stress. The reduction in chlorophyll contents under salt stress was also observed in previous studies (Akhtar *et al*., 2010, Khan *et al*., 2009). The reduction in chlorophyll content with salt stress may be due to reduction in photosynthetic capacity at a given internal CO_2 concentration (Tavakkoli, *et al*., 2010). Indeed I found that transpiration rate, stomatal conductance and CO_2 uptake reduced significantly from control plants. A similar finding was observed by (Zhang, *et al.,* 2009). Therefore, reduction in photosynthesis by increasing salinity may be due to lower stomatal conductance, depression of specific metabolic processes in the carbon uptake, inhibition in photochemical capacity or a combination of these reported by (Jamil *et al*., 2007).

It was investigated in most of genotypes the growth of plants was stopped with increasing salt level up to 250 mM. A similar finding was observed by (Anjum, *et al.,* 2005). In contrast Tuteja (2007) reported that the halophytes can grow at over 250 mM salinity level, and that`cotton is a salt tolerant crop.

6.1.2. Na^+ uptake by all young and old parts of plants under NaCl.

It has also been suggested that salt tolerance is associated with low Na^+ uptake, salt exclusion and its compartmentation within the cell or within the plant (Gorham, 1990 and Akhtar *et al*., 2010). Na^+ uptake in all young and old plants parts was increased significantly by salt treatments as compared to control treatments. The Na^+ uptake response of genotypes was different significantly. It was observed in most of genotypes Na^+ increased less in young parts than in old parts. Khan *et al.,* (2004) also observed lower Na^+ increases in younger leaves than

in older leaves in cotton. This has also been reported for green beans (Yasar *et al.*, 2006) and sorghum (Lacerda *et al.*, 2003). In contrast, it was found in some genotypes that a lower Na^+ increase occured in old parts than in young plant parts. According to this study, the genotypes MNH 93, Stonvelle 312, DNH 40 x CIM 448, *G. hirsutum x G. sturtainum*, MNH 147, MNH 147 x S6, S6 x DNH 40, DNH 40, *G. stocksii* x *G. hirsutum*, MNH 147 x CIM 448, S6 x Dandara, Rehmani and NIAB 78 apparently showed salt tolerance as the result of less uptake of Na^+ in their old and young parts of plants. The genotypes Sarmast, CIM 448, NIAB 313 x DNH 40, DNH 40 x S6, *G. stocksii,* Dandara, CIM 1100 and Qalandari showed salt susceptibility due to higher uptake of Na^+ in their old and young parts of plants. More Na^+ in leaf sap of sensitive cotton genotypes was also observed by Ahmed *et al.*, (2002). The higher concentrations disturb the different metabolic activities (Khan *et al.*, 2010). The higher amount of Na^+ in plant could be due to increased concentration in rooting medium, diffusion through damaged membranes and/or lower efficiency of exclusion mechanism (Akhtar *et al.*, 2010).

6.1.3. K^+ uptake by all young and old parts of plants under NaCl.

K^+ uptake in some young and old plant parts were increased significantly by salt treatments as compared to control treatments. Similar results were reported by Leidi and Saiz, (1997), Jafri and Ahmed, (1994), Karl and Lauchli, (2002) in cotton, by Leonova, *et al.*, (2004) in barley and by Yasar, *et al.*, (2006) in green beans. However, in contrast I found K^+ uptake in some young and old plant parts to be decreased significantly by salt treatments as compared to control treatments. Similar results have been reported in cotton (Hebbar, *et al.*, 2000, Akhtar, *et al.*, 2010 and Ahmed, *et al.*, 2002) and in barley (Tavakkoli, *et al.*, 2010) where decreased K^+ accumulation following different salt treatment was seen. The genotypes *G. stocksii* x *G. hirsutum, G. hirsutum* x *G. sturtianum*, Dandara, DNH 40 x S6, MNH 147 x S6, MNH 147, Rehmani, CIM 1100, Mc. Nair 220 and MNH 93 maintain high K^+ in the old and young parts

of plants in salinity and Dandara x S6, NIAB 313, *G. stocksii*, DNH 40 x CIM 448, NIAB 313 x DNH 40, NIAB 78 and S12 maintain low K^+ in the old and young parts of plants in high salinity. Higher uptake of K^+ might be maintaining the osmotic adjustment in salt tolerant plants. Leonova, *et al.*, (2004) observed in barley and Munis, *et al.*, (2010) in cotton, that following salt treatment, salt tolerant genotypes increased accumulation of K^+ content while in salt sensitive genotypes a decreased in K^+ as compared to control treatment was seen. In contrast, Khan *et al.*, (2004) reported that the susceptible genotypes showed greater K^+ content than salt tolerant genotypes. My study is in agreement with Gorham *et al.*, (1990), who reported that low concentration of Na^+ and high concentration of K^+ is a discrimination character in salt tolerant plant.

6.1.4. K^+/Na^+ ratio of all young and old parts of plants under NaCl.

Salt tolerant plants have the ability to maintain the higher K^+/Na^+ ratio. That selection of salt tolerance using K^+/Na^+ ratio as a successful criterion was reported by Ahmed *et al.*, (2002). My results are agreement with this. In my study, the genotypes Stonvelle 213, MNH 93, Mc. Nair 220. 149 F, DNH 40 x CIM 448, S6 x Dandara, MNH 147 x S6, *G. stocksii* x *G. hirsutum*, NIAB 313 x DNH 40 MNH 147 x CIM 448 and CIM 448 maintained a higher K^+/Na^+ ratio in salt stress than did other genotypes. The K^+/Na^+ ratio in all plant parts was decreased significantly by salt treatment. Similar findings were reported in previous studies (Jafri and Ahmed, 1994, Munis *et al.*, 2010, Abd – Ella and Shalaby., 1993). In contrast, Hebbar *et al.*, (2000) observed that the K^+/Na^+ ratio was not affected at higher salinity level.

6.1.5. Ca^{++} uptake by all young and old parts of plants (Constant $CaCl_2$) under (150 mM) NaCl.

In this study, the effect of 150 mM NaCl showed a decrease in Ca^{++} concentration in most young and old plant parts. The decrease in Ca^{++} was also observed in genotypes Rehmani and CIM 1100 showed higher uptake of Ca^{++} content in most of its young and old plant parts. S

12, B 756, Sarmast and NIAB 78 showed lower uptake of Ca^{++} content in most of their young and old plant parts. Kent and Lauchli, (1985) and Ahmed, *et al,*. (2002) also reported decrease in Ca^{++} in cotton by NaCl stress. No interaction was found between genotypes and treatments. In contrast, Abdul Ella and Shalaby (1993) also reported that no changes in Ca^{++} concentration of cotton leaves were observed by high NaCl stress. In this study no evidence of Ca^{++} being related to salt tolerance was found.

6.1.6. Molecular diversity.

An objective of this study was to estimate genetic diversity and relationship between different cotton cultivars by analysis the pattern of DNA polymorphism. Similar studies were reported previously for different cotton species (Lacape, *et al.*, 2007, Bertini, *et al.*, 2006, Wang *et al.*, 2004), for wheat (Salem, *et al.*, 2007), for barley (Chaabane, *et al.*, 2009), for millet (Hu, *et al.*, 2009) and for rice (Chakravarthi & Rambabu, 2006). When using capallary electrophoresis (CEQ 8000), molecular markers used in this study showed that most of SSR markers were highly polymorphic and useful for estimating the genetic variation in cotton genotypes). However, when using gel electrophoresis, out of 17 SSR primers, only two primers were seen to be polymorphic (BNL 1434 & BNL 1878) although most of the primer pairs produced clear and strong amplification products. Tang *et al.*, (2004) also suggested that the capillary electrophoresis produces more accurate and consistent result in comparison to gel electrophoresis.

In chapter 4 (Table 4.4) a wide range of genetic diversity was described with a range of PIC 0.00 to 0.70. Different PIC values were reported in cotton by Khan, *et al.*, (2009) and Adawy, (2007). A total of 43 alleles were observed following the use of 17 primer pairs. The number of allele per pair (locus) ranged from 1 to 5, with a mean of 2.5 per locus. This is toward the lower end of the range reported in the literature. For example 3.6/ locus were found by Khan, *et al*, (2009) and Guang and Xiong, (2007), 2- 11 allele/locus by Hussein, *et al.*, (2007) and 3

-17 alleles/locus by Lacape, *et al.*, (2007). The maximum number of alleles per locus was found for BNL 1434. Adawy (2007) who also studied this locus also found 5 alleles to be present. In this study the coefficient of similarity for all of varieties was found to be in the range of -0.14 to 95% see in chapter 3 (Fig (3.3.13). The reason for this similarity is, presumably that most varieties have the same ancestors. Sheidai *et al.*, (2010) reported 53% average genetic similarity in tetrapliod cotton cultivars. My result showed that the position of many genotypes in the same dendrogram groupings was related to their known pedigrees (chapter 4). For example the members of group I, CIM 448 and CIM 1100 have the same parents and the member of the group Coker 201 and Mc. Nair 220 (group II) have the same parents and origin.

In this study the result showed the clear separation of cotton species. However, *G. stocksii* is a wild species which was in a separate group, but S6 x DNH 40 and *G. stocksii* x *G. hirsutum* showed in same group. This is noteworthy as three different species are represented. This would suggest that the future use of, different genotypes from dendrogram of different clusters should used for hybridisation to create genetic diversity between cotton varieties.

6.1.7. Mapping population.

For a perfect experimental mapping population, a breeder needs two varieties which are different to each other. For example one could be phenotypic ally highly tolerant and one susceptible as well as genotypic ally different to each other. In this study a rudimentary segregating population was developed by back cross of an inter specific (*G. hirsutum* x *G. barbadense*) as CIM 448 x MNH 147 and intra specific (*G. hirsutum* x *G. hirsutum*) as MNH 147 x CIM 448.

The result showed that in most of the traits the genotypes responded significantly differently to each other. Examples are Plant height, chlorophyll content, Na^+ uptake in young petiole and old petiole and stem, K^+ uptake in all young and old leaf and stem and Ca^{++} uptake in young stem and old leaf and stem. Dandara lost more fresh weight, dry weight and chlorophyll content and showed an increase K^+ and Ca^{++} uptake and a high K^+/Na^+ ratio in most of plant parts in

comparison with CIM 448, MNH 147 and back cross (BC1) populations. Dandara belongs to *G. barbadense* and is reported to be salt sensitive (Rathert, 1983). However, no polymorphism was found in between two different species at any of the loci studied. However, as I discussed above it was the result of a rudimentary segregation population and may not be represent time of the cross as a whole.

6.2. Conclusion

In the light of this project, it can be concluded that:

Plant height, nodes per plant, fresh weight, dry weight, chlorophyll content, transpiration rate, stomatal conductance and CO_2 uptake were reduced by high salinity. Previously identified salt tolerant genotypes reduced less in growth and photosynthesis parameters under NaCl treatment in comparison to control treatment.

In all parts of plants Na^+ uptake increased significantly and K^+ increased in most of plant parts by salt treatment. Ca^{++} uptake decreased in most of plant parts. Salt tolerant genotypes accumulated less Na^+ and had higher uptake of K^+ in their plant parts than salt susceptible genotypes. However, it was concluded that the different results from previous studies were due to different genotypes being used, different age of plants and different parts of plants. It was also observed in this study that the flood bench system was better for salt treatment than regular addition of solution. According to this study, the genotypes Acalla SJ2, DNH 40 x CIM 448, Rehmani, 149 F, MNH 93, Stonville 213, DNH 40, CIM 448, Coker 213, DNH 40 x S6, S6 x DNH 40, Mc Nair 220, MNH 147 x S6, S6 x Dandara, MNH 147 and MNH 147 x CIM 448 showed more salt tolerance than other varieties. It also showed that the crosses of *G. hirsutum* with *G. sturtainum* and *G. stocksii* and the wild species *G. stocksii*, showed salt tolerance as manifested by less reduction in plant growth, chlorophyll content, photosynthesis, less uptake of Na^+ in their old and young parts of plants, high K^+/Na^+ ratio and high uptake of K^+ and Ca^+. The variety S6 was not available for this study. However, the consistent apparent salt

tolerance of crosses containing this parent and another not of reported tolerance would represent that it may be a promising source of salt tolerant genes.

Out of seventeen SSR primers used, 13 showed polymorphism. Fragment analysis using the sequencer was more successful than gel electrophoresis for detecting such polymorphism. In this genotypic study most of the results was in agreement with their pedigrees and origin. This suggested that SSR primers are reliable and informative for cotton the genome.

6.3. Recommendations.

1. There are many methods of salt tolerance; these include (1) Na^+ exclusion and (2) Na^+ sequestration. In this study salt tolerance was apparently found by both methods. The genotypes which belong to *G. hirsutum* such as CIM 448, DNH 40, Rehmani, MNH 93 showed salt tolerance by Na^+ exclusion, however, *G. stocksii*, showed salt tolerance by Na^+ sequestration. In future, it will be interesting to investigate the distribution of Na^+ exclusion and sequestration in salt tolerant species through field experiment.

Literature cited

Abd – Ella, M. K. and Shalaby E. E. (1993). Cotton response to salinity and different potassium – sodium ratio in irrigation water. *Journal of Agronomy and Crop Sciences.* 170: 25 – 31.

Adams, K. L. and Wendel, J. F. (2004). Exploring the genomic mysteries of polyploidy in cotton. *Biological Journal of the Linnean Society.* 82: 573–581.

Adawy, S. (2007). An evaluation of the utility of simple sequence repeat loci (SSR), expressed sequence tags (ESTs) and expressed sequence tag microsatellites (EST – SSR) as molecular markers in cotton. *Journal of Applied Sciences Research.* 3: 1581 – 1588.

Adawy, S., Ebtissam H. A., Hussein A. and Hanaiya A. E. (2006). Molecular characterization and genetic relationships among cotton genotypes. 2- AFLP Analysis. *Arab Journal of Biotechnology.* 9: 477 – 492.

Ahmad, S., Khan N., Iqbal M. Z., Altaf H. and Hassan M. (2002). Salt tolerance of cotton (*Gossypium hirsutum* L.). *Asian Journal of Plant Sciences.* 6: 715- 719.

Akhtar, J., Anwarul-Haq, M., Khalil, A., Saqib, M. and Saeed, M. A. (2005). Performance of cotton cultivars under saline conditions. *Bioline International.* 17: 29-36.

Akhtar, J. and Azhar, F. M. (2001). Response of *G. hirsutum* L. hybrids to NaCl salinity at the seedling stage. *International Journal of Agriculture and Biology.* 3: 233- 235.

Akhtar, J., Saqib A. Z., Sarfraz, M., Saleem, I. and Haq, M. A. (2010). Evaluating salt tolerant cotton genotypes at different levels of NaCl stress in solution and soil culture. *Pakistan Journal of Botany.* 42 (4) 2857 – 2866.

Akhtar, L. H., Gorham, J. and Nasrullah, M. (1999). Effects of NaCl and $CaCl_2$ on callus tissue of *G. hirsutum* L. cv. Acala SJ2. *Pakistan Journal of Scientific and Industrial Research.* 42: 260-272.

Ali, Y., Aslam, Z., Asif, M. (2004). A high yielding and salt tolerant new cotton variety-NIAB-999. *International Journal of Biology and Biotechnology.* 1: 181-185.

Ali, Y., Aslam Z. and Hussain F. (2005). Genotype and environment interaction effect on yield of cotton under naturally salt stress condition. *International Journal of Environment Science and Technonology.* 2: 169 – 173.

Amir, A. H., Tanwir, A. M., Hasnain, R. and Rehman, M. (2002). Genetics of leaf nectaries in upland cotton. *Asian Journal of Plant Sciences.* 1: 423 -424.

Anjum, R., Amir, A., Rahmat, U., Jahangir, M and Yousaf, M. (2005). Effect of soil salinity/sodicity on the growth and yield of different varieties of cotton *International Journal of Agriculture and Biology.* 4: 606- 608.

Ashraf, M. (1994). Breeding for salinity tolerance in plants. *Critical Review Plant Science*. 13: 17-42.

Ashraf, M. (2002). Salt tolerance of cotton: Some new advances. *Critical Review Plant Science*. 21: 1-30.

Ashraf, M. (2004). Some important physiological selection criteria for salt tolerance in plants. *Flora*. 199: 361–376.

Ashraf, M. and Mc Neilly, T. (1988). Variability in tolerance of nine wheat cultivars *Journal of Agronomy and crop Sciences*. 160: 14-20.

Ashraf, M., Saeed, M. M. and Qureshi, M. J. (1994). Tolerance to high temperature in cotton *(Gossypium hirsutum L.)* at initial growth stages. *Environmental and Experimental Botany*. 34: 275-283.

Ashraf, M. and Saghir, A. (2000). Influence of sodium chloride on ion accumulation, yield components and fibre characteristics in salt-tolerant and salt- sensitive lines of cotton *Gossypium hirsutum*. *Field Crops Research*. 2: 115-127.

Asif, M., Javed, I. M. and Zafar, Y. (2008). Genetic analysis for fiber quality traits of some cotton genotypes. *Pakistan Journal of Botany*. 40: 1209-1215.

Asif, M., Rehman, M.., Javed, I. M. and Zafar, Y. (2009). Parentage confirmation of cotton hybridsusting molecular markers. *Pakistan Journal of Botany*. 41: 695-701

Bagci, S. A., Hasan, E. and Ahmet, Y. (2003). Determination of the salt tolerance of some barley genotypes and characteristics affecting tolerance. *Turk Journal of Agriculture*. 27: 253 – 260.

Bajracharya, J., Steele, K. A., Jarvis, D. I., Sthapit, B. R. and Witcombe, J. R.. (2006). Rice landrace diversity in Nepal: Variability of agro- morphological traits and SSR markers in landraces from a high – altitude site. *Field Crop Research*. 95: 327–335.

Bhatti, A. (1975). Treatment of cotton seed for germination. *Plant and Soil*. 41: 681-683.

Bhatti, M. A. and Azhar, F. M. (2002). Salt tolerant of nine *G. hirsutum* L. varieties to NaCl salinity at early stage of plant development. *International Journal of Agriculture and Biology*. 4: 544-546.

Basal, H. (2010). Response of cotton (*Gossypium hirsutum* L.) genotypes to salt stress. *Pakistan Journal of Botany*. 42: 505–511.

Basra, S. M. A., Ashraf, M, Iqbal, N., Khaliq, A. and Ahmed, R. (2004). Physiological and biochemical aspects of pre-sowing heat stress on cottonseed. *Seed Science and Technology*. 32: 837-846.

Belkhodja, R., Morales, F., Abadia, A., Gomez, A. J., and Abadia, J. (1994). Chlorophyll florescence as a possible tool for salinity tolerance screening in barley (*Hordeum vulgare* L.). *Plant Physiology*. 104: 667 -673.

Berke, G. and Rocheford, T. R. (1999). Quantitative trait loci for tassel traits in maize. *Crop Science*. 39:1439-1443.

Bernatzky, R. and Tanksley, S.D. (1986). Towards a saturated linkage map in tomato based on isozome and random cDNA sequences. *Genetics*. 112: 887–898.

Bertrand, C. Y. C and David, J.M. (2008). Marker – assisted selection: an approach for precision plant breeding in the twenty first century. *Biological Sciences*. 363: 557-572.

Bertini, C. H. C., Ivan, S., Tocio, S., Everaldo, G. B. and Maurillio, A. M. (2006). Characterization and genetic diversity of cotton cultivars using microsatellites. *Genetics and Molecular Biology*. 29: 321–329.

Bolek, Y. S. Kamal, M. Z., Alan, E. P., Alois, A. B, Clint, W. M, Peggy, M. T. and Umesh, O. K. R. (2005). Mapping of verticillium wilt resistance genes in cotton. *Plant Science*. 168: 1581-1590.

Boscaiu, M. G. B., Naranjo, M. A., Vicente, O., Boira, H. (2007). Responses of halophytes to salt stress. *Animal Science and Biotechnologies*. 64: 63-64.

Brown, M. S. (1980). The identification of chromosomes of *G. hirsutum* L. by means of translocations. *Heredity*. 71: 266-274.

Brugnoli, E. and Lauteri, M. (1991). Effects of salinity on stomatal conductance, photosynthetic capacity, and carbon isotope discrimination of salt-tolerant *(Gossypium hirsulum L.)* and salt-sensitive *(Phaseolus vulgaris L.)* C3 non-halophytes. *Plant Physiology*. 95: 628-635.

Chaabane, R., Mouldi, F., Hammadi, B. S., Barek, M. B. N., Chedly, R., Ahmad, N. and Mahmoud, S. (2009). Molecular characterization of Tunisian Barley (*Hordeum vulgare* L.) genotypes using microsatellites (SSRs) markers. *European Journal of Scientific Research*. 36: 6–15.

Chakavarthi, B. K. and Rambabu, N. (2006). SSR marker based DNA fingerprinting and diversity study in rice (*Oryza sativa* L.). *African Journal of Biotechnology*. 5: 684–688.

Chaudhuri, K. and Choudhuri, M. A. (1997). Effects of short-term NaCl stress on water relations and gas exchange of two jute species. *Biologia. Plantarum*. 40: 373–380.

Chaudhary, L., Sindhu, A., Kumar, M., Kumar, R. and Saini, M. (2010). Estimation of genetic divergence among some cotton varieties by RAPD analysis. *Journal of Plant Breeding and Crop Science*. 2: 039–043.

Chee, P. W., Rong, J., Coplin, W. D., Schulze, S. R. and Paterson, A. H. 2004. EST derived PCR based markers for functional gene homologues in cotton. Genome. 47: 449-462.

Chistiakov. D. A., Bart, H. and Filip, A. M. V. (2006). Microsatellites and their genomic distribution, evolution, function and applications: A review with special reference to fish genetics. *Agriculture*. 255: 1-29.

Cho, Y.G., Ishii, T., Temnykh, S., Chen, X., Lipovich, L., McCouch, S. R., Park, W. D., Ayres, N. and Cartinhour, S. (2000). Diversity of microsatellites derived from genomic liberaries and Gene Bank sequences in rice (*Oryza sativa* L.). *Tag Theoretical and Applied Genetics*. 100: 713-722.

Cicek, N. and Husnii, C. (2002). The effect of salinity on some physiological parameters in two maize cultivars. *Plant Physiology*. 28: 66–74.

Cixin, H., Juqiang, Y., Guoxin, S. Lianhai, F., Holaday, A. S., Auld, D., Blumwald, E. and Zhang, H. (2004). Expression of an *Arabidopsis* vacuolar Sodium/Proton antiporter gene in cotton Improves photosynthetic performance under salt conditions and increases fiber yield in the field. *Plant Cell and Physiology*. 5: 1848-1854.

Colautti, R. I., Manca, M., Viljanen, M., Ketelaars, H. A., Burgi, H., Macisaac, H. J. and Heath, D. D. (2005). Invasion genetics of the Eurasian spiny waterflea: evidence for bottlenecks and gene flow using microsatellites. *Molecular Ecology*. 14: 1869 – 1879.

Cramer, G. (2004). Sodium – Calcium interaction under salinity stress. In Lauchli A., and Luttege U., (eds). *Salinity: Environment – Plant – Molecules*. Kulwer Academic Publication. 205-221.

Endrizzi, J. E., Turcotte, E. L., Kohel, R. (1985). Genetics, cytology and evolution of *Gossypium. Advances in Genetics*. 23: 271-375.

Erkilinc, A. and Karaca, M. (2005). Assessment of genetic variation in some cotton varieties (*Gossypium hirsutum* L.) grown in Turkey using microsatellite. *Akdeniz Universitesi Ziraat Fakultesi Dergisi*. 18: 201-206.

Eynard, A., Lal, R. and Wiebe, K. (2005). Crop response in salt affected soils. Journal of Sustainable Agriculture. 27: 5-50.

Farooq, S. and Azam, F. (2002). Molecular markers in plant breeding – II. Some pre requisites for use. *Pakistan Journal of Biological Sciences*. 5: 1141–1147.

FAS (Foreign Agriculture Services). USDA. (2005). *Cotton world market and trade*. 12 September. 2005.

Flowers, T. J. and Yeo, A. R. (1995). Breeding for salinity resistance in crop plants – where next. *Australian Journal of Plant Physiology*. 22: 875 – 884.

Flowers, T. J., Hajibagheri, M. A. and Clipson, N. J. W. (1986). Halophytes. *Quarterly Review of Biology*. 25: 313–337.

Francia, E., Tacconi, G., Crosatti, C., Barabaschi, D., Bulgarelli, D., Dall, E. A. and Vale, G. (2005). Marker assisted selection in crop plants. *Plant Cell, Tissue and Organs*. 82: 317-337.

Frelichowski, J. E., Michael, J., Palmer, B., Jeffrey, D. M., Tomkins, P., Cantrell, R., David, G., Stella, M., Yu, J., Kohel, R. J. (2006). Cotton genome mapping with new microsatellites from Acala Maxxa BAC- end. *Molecular Gene Genomics*. 275: 479-491.

Fryxell, P. A. (1971). Phenotic analysis and the phylogeny of the diploid species of *Gossypium* L. *Evolution*. 25: 554-562.

Fryxell, P. A., Craven, L. A. and Stewart, M. J. (1992). A revision of Gossypium Sect. Grandicalyx (Malvaceae), including the description of six new species. *Systematic .Botany*. 17: 91-114.

Fukino, N, Taneishi, M., Saito, T., Nishijima, T. and Hirai, M. (2002). Construction of a linkage map and genetic analysis for resistance to cotton aphid and powdery mildew in melon. *Acta. Horticulturae*. 588: 283- 286.

Gale, J., Kohl, H. C. And Robert, M. H. (1967). Changes in water balance and photosynthesis of onion, bean and cotton plants under saline condition. *Physiologia Plantarum*. 20: 408-420.

Ganesan, M., and Jayabalan, N. (2005). Salt tolerance adaptation development in cotton cultivars (*Gossypium hisutum* L.) through regeneration. *Plant Cell Biotechnology and Molecular Biology*. 6: 127-132.

Ghoulam, C., Ahmed, F. and Khalid, F. (2002). Effects of salt stress on growth, inorganic ions and proline accumulation in relation to osmotic adjustment in five sugar beet cultivars. *Environmental and Experimental Botany*. 47: 39–50.

Glasel, J.A. (1995). Validity of nucleic acid purities monitored by A260/A280 absorbance ratios. *Biotechniques*. 18**:** 62-63.

Glenn, E. P., Brown, J. J. and Blumwald, E. (1999). Salt tolerance and crop potential of helophytes. *Critical Reveiw Plant Science*. 18: 227–255.

Gorham, J. (1992). Salt tolerance in plants. *Scientific Progress, Oxford*. 76: 273-285.

Gorham, J. and Bridges, J. (1995). Effect of calcium on growth and leaf ion concentration of *Gossypium hirsutum* grown in saline hydroponic culture. *Plant and soil*. 176: 219-227.

Gorham, J., Wyn Jones, R. G. and Mc Donnell, E. (1985). Some mechanisms of salt tolerance in crop plants. *Plant and Soil*. 89: 15-40.

Gorham, J., Mc. Donnell, E. and Wyn Jones, R. G. (1984). Salt tolerance in the Triticeae: *Leymus sabulosus. Journal of Experimental Botany*. 35: 1200 -1209.

Gorham, J., Bristol, A., Young, E. M., Wyn Jones, R. G. and Kashour, G. (1990). Salt tolerance in the *Triticeae*: K/Na discrimination in barley. *Journal of Environmental Botany*. 41: 1095–1101.

Gossett, D. R., Banks, S. W., Millhollon, E. P. and Lucas, M. C. (1996). Antioxidant response to NaCl stress in a control and an NaCl tolerant cotton cell line grown in the presence of paraquat, buthionine sulfoximine, and exogenous glutathione. *Plant Physiology*. 112: 803-809.

Greenway, H. and Munns, R. (1980). Mechanism of salt tolerance in non-halophytes. *Annual. Review of Plant Physiology*. 31: 149-190.

Guang, C. and Xiong, M. D. (2006). Genetic diversity of source germplasm of upland cotton in China as determined by SSR marker analysis. *Acta Genetica Sinica*. 33: 733-745.

Guillermo, V. B., Edward, L. L., Paterson, A. H. and Peng, W. C. (2005). Pedigree- vs. DNA marker-based genetic similarity estimates in cotton. *Crop Science*. 45: 2281–2287.

Gurmani, A. R., Bano, A., Din, J., Khan, S. U. and Hussain, I. (2009). Effect of phytohormone on growth and ion accumulation of wheat under salinity stress. *African Journal of Biotechnology*.8: 1887–1894.

Gutierrez, O. A., Basu, S. Saha, S., Jenkins, J. N., Shoemaker, D. B., Cheatham, C. L. and McCarty, J. C. (2002). Genetic Distance among selected cotton genotypes and its relationship with F_2 performance. *Crop Science*. 42: 1841–1847.

Hakim, M. A., Juraimi, A. S., Begum, M., Hanafi, M. M., Ismail, M. R. and Selamat, A. (2010). Effect of salt stress on germination and early seedling growth of rice. African *Journal of Biotechnology*. 9: 1911–1918.

Hameed, A., Shazia, N., Tahira, I., Hina, S. and Ahsanul Haq, M. (2008). Effects of NaCl salinity on seedlings growth, senescence, catalase and protease activities in two wheat genotypes differing in salt tolerance. *Pakistan Journal of Botany*. 40: 1043–1051.

Harland, S. C. (1936). Haploids in poly embryonic seeds of Sea Island cotton. *Heredity*. 17: 24-27.

Hawkins, J. S., HyeRan, K., John D. N, Wing, A R. and Wendel, J. F. (2006). Differential lineage-specific amplification of transposable elements is responsible for genome size variation in *Gossypium*. *Journal of Sustainable Agriculture*. 27: 5-50.

Hebbar, K. B., Venugopalan, M. V. and Rao, M. R. K. (2000). Effect of salinity on cotton growth and development: Sodium can not substitute for potassium in cotton. *Journal of Plant Biology*. 27: 271–276.

Hoagland, D. R. and Arnon, D. I. (1950). The water-culture method for growing plants without soil. *California Agricultural Experiment Station Circular*. 347: 1-32.

Hoffman, G. J., Rawlins, S. L., Garber, M. J. and Cullen, E. M. (1971). Water relations and growth of cotton as influenced by salinity. *Agronomy Journal*. 63: 822-826.

Hu, X., Jianfei, W., Ping, L. and Hongsheng, Z. (2009). Assessment of genetic diversity in broomcorn millet (*Panicum miliaceum* L.) using SSR markers. *Genetics and Genomics*. 36: 491-500.

Hu, Y. and Schmidhalter, U. (2005). Drought and Salinity. A comparison of their effects on mineral nutrition of plants. *Plant and Nutrition Soil Sciences*. 168: 541-549.

Hussein, E. H. A., Marwa, H. A. O., Mona, H. H. and Sami, S. A. (2007). Molecular characterization of cotton genotypes using PCR based markers. *Journal of Applied Sciences Research*. 3: 1156–1169.

Iqbal, M. J., Aziz, N., Saeed, N. A., Zafar, Y. and Mailk, K. A. (1997). Genetic diversity of some elite cotton varieties by RAPD analysis. *Tag Theoretical and Applied Genetics*. 94: 139–144.

Jacome, A. G., Oliveira, R. H., Fernández, P. D., Gheyi, H., Souza, A. P., Goncalves, A. C. A. (2003). Growth of cotton genotypes as affected by the irrigation salinity level. *Acta Scientiarum - Agronomy*. 25: 305–313.

Jafri, A. Z. and Ahmed, R. (1994). Plant growth and ionic distribution in cotton *G. hirsutum* L. under saline environment. *Pakistan Journal of Botany*. 26: 105-114.

Jamil, M., Shafiq, R., Kui, J. L., Jeong, M. K., Hyun, S. K. and Eui, S. R. (2007). Salinity reduced growth PS2 photochemistry and chlorophyll content in radish. *Scientia Agricola*. 64:111- 118.

Kao, W. Y., Tsai, H. C., and Tsai, T. T. (2001). Effect of NaCl and nitrogen availability on growth and photosynthesis of seedlings of a mangrove species, (*Kandelia candel* L.) Druce. *Journal of Plant Physiology*. 158: 841–846.

Karl, H. M. and Lauchli, A. (2002). Effect of salt stress on growth and cation compartmentation in leaves of two plant species differing in salt tolerance. *Plant Physiology*. 159: 137–146.

Kawasaki, S., Borchert, C., Deyholos, M., Wang, H., Brazille, S., Kawai, K., Galbraith, D. and Bohnert, H. J. (2001). Gene expression profiles during the initial phase of salt stress in rice. *Plant Cell*. 13: 889–905.

Kaya, C., Kirnak, H., Higgs, D. and Saltali, K. (2002). Suplimentary calcium enhances plant growth and fruit yield in strawberry cultivars grown at high (NaCl) salinity. *Scientia Horticulturae*. 93: 65–74.

Kent, L. and Lauchli, A. (1985). Germination and seedling growth of cotton: Salinity calcium interaction. *Plant Cell Environment*.8: 155–159.

Khan, A. I., Yong, B. F. and Ifttikhar, A. K. (2009). Genetic diversity of Pakistani cotton cultivars as related by simple sequence repeat markers. *International Journal of the Faculty of Agriculture and Biology*. 4: 21-30.

Khan, A. N., Qureshi, R. H. and Ahmed, N. (2005). Na distribution and rate of transport in cotton cultivars under saline conitions. *International Journal of Biology and Biotechnology*. 2: 169-171.

Khan, A. N., Qureshi, R. H. and Ahmed, N. (2004). Effect of external sodium chloride salinity on ionic composition of leaves of cotton cultivars 1. Cell sap, sodium and potassium. *International Journal of Agriculture and Biology*. 5: 781-783.

Khan, A. N., Qureshi, R. H. and Ahmed, N. (2004). Salt tolerance of cotton cultivars in relation to relative growth rate in saline environments. *International Journal of Agriculture and Biology*. 6: 786-787.

Khan, M. A., Stewart, J. M. D. and Murphy, J. B. (1999). Evaluation of the *Gossypium* gene pool for foliar terpenoid aldehydes. *Crop Sciences*. 39: 253-258.

Khan, M.A., Shirazi, M. U., Ali, M. K., Mujtaba, S. M., Islam, E., Mumtaz, S., Shereen, A., Ansari, R.U. and Yasin, M. A. (2009). Role of proline, K/Na ratio and chlorophyll content in salt tolerance of wheat (*Triticum aestivum* L.). *Pakistan Journal of Botany*. 41: 633-638.

Khan, T. M., Saeed, M., Mukhtar, M. S. and Khan, A. M. (2001). Assessment of variation for salinity tolerance in some hybrids of cotton. *G. hirsutum* L. *International Journal of Agriculture and Biology*. 3: 167-170.

Kohel, R. J., John, Y., Yong, H. P. and Gerard, R. L. (2001). Molecular mapping and charachterization of traits controlling fiber quality in cotton. *Euphytica*. 121:163-172.

Kuznetsov, V. V., Khydyrov, B. T., Roshchupkin, B. V., and Borisova, N. N. (1990). Common systems of cotton resistance to salinity and high temperatures: facts and hypotheses. *Fiziologiya Rasteni*. 37: 987-996.

Kuznetsov, V. V., Khydyrov, B. T., Shevyakova, N. I., and Rakitin, V. Y. (1992). Heat shock induction of salt tolerance in cotton. Involvement of polyamines, ethylene and praline. *Soviet Plant Physiology*. 38: 877-883.

Kwon, T. R., Siddiqui, Z. S. and Harris, P. J.C. (2009). Effects of supplemental calcium on ion accumulation, transport and plant growth of salt sensitive *Brassica rapa* landrace. *Journal of Plant Nutrition*. 32: 644–667.

Lacape, J. M., Nguyen, T. B., Thibvilliers, S., Bojinov, B. and Courtois, B. (2003). A combined RFLP–SSR–AFLP map of tetraploid cotton based on a *Gossypium hirsutum* x *Gossypium barbadense* backcross population. *Genome*. 46: 612–626.

Lacerda de, C. F., Jose, C., Marco, A. O., Hugo, A. R., and Jose, T. P. (2003). Solute accumulation and distribution during shoot and leaf development in two sorghum genotypes under salt stress. *Experimental and Environmental Botany*. 49: 107-120.

Lacape, M., Dessauw, D., Rajab, M., Noyer, J. L. and Hau, B. (2007). Microsatellites diversity in tetraploid *Gossypium* germplasm: assembling a highly informative genotyping set of cotton SSRs. *Molecular Breeding*. 19: 45–58.

Lacape, J. M., Nguyen, T. B., Courtoisa B., Belotb, J. L., Gibanda, M., Gawryziaka, G., Sandrina, R. and Haua, B. (2004). QTL analysis of cotton fiber quality using multiple *Gossypium hirsutum* x *Gossypium barbadense* back cross generations. *Crop Sciences*. 45: 123-140.

Lacape, J. M., Nguyen, T. B., Courtois, B., Belot, J. L., Marc, J., Gourlot, J. P., Gawryziak, G. Sandrine, R. and Bernard, H. (2005). QTL analysis of cotton fiber quality using multiple *Gossypium hirsutum* x *Gossypium barbadense* back cross generations. *Crop Science*. 45: 123-140.

Leidi, E. O. and Saiz, J. F. (1997). Is salinity tolerance related to Na^+ accumulation upland cotton (*Gossypium hirsutum L.)* seedling? *Plant Soil*. 190: 67-75.

Leonova, T. G., Goncharova, E. A., Khodorenko, A. V. and Babakov, A. V. (2005). Charachteristics of salt tolerant and salt susceptible cultivars of barley. *Russian Journal of Plant Physiology*. 52: 774–778.

Lin, Z., He, D., Zhang, X., Nie, Y., Guo, X., Feng, C. and Stewart, J. M. D. (2005). Linkage map construction and mapping QTL for cotton fiber quality using SRAP, SSR and RAPD. *Plant Breeding*. 124: 180-187.

Lin, L., Gary, J. P., John, E. B., James, C. E., Rosana, O. C., Lisa, K. R., Changsoo, K., Cornelia, L., Junkang, R., Haibao, T., Xiyin, W., Michele, B., Amy, H. C., Kriten, C., Kristi, C., Ethan, E., Wolfgang, G., Corrinne, G., Jennifer, I., Santhosh, K., Dave, K., Jaime, O., Nabila, T., Eareana, U., Marina, W., Yeisoo, Y., Andrea, Z., Mehboob, R., Daniel, G. P., Rod, A. W., Wendel, J. F and Andrew, H. P.. (2010). A draft physical map of a D- genome cotton species (*Gossypium raimondii*). *Genomics*.11: 1-17.

Lira, S. R. H. and Hernandez, R. S. P. (1988). Characterization of salt tolerance in genotype of cotton. *Informes de Investigaciones, Pronapa*. 299-317.

Liu, B. and Wendel, J. F. (2003). Epigenetic phenomena and the evolution of plant allopolyploids. *Molecular Phylogenetic Evolution*. 29: 365-379.

Liu, S. S., Saha, D. S., Burr, B. and Cantrell, R. G. (2000). Chromosomal assignment of microsatellite loci in cotton. *Journal of Heredity*. 91: 326-332.

Luce, C., Noyer, J. L, Tharreau, D., Ahmadi, N., Feyt, H (2001). The use of microsatellite markers to examine the genetic resources of rice. (*Oryza sativa* L.) adapted to European condition. Proc Int Symp On Molecular markers. *Acta Hort*. 546: 221–234.

Mei, M. N., Syed, H., Gao, W., Thaxton, P. M., Smith, C. W., Stelly, D. M. and Chen, Z. J. (2004). Genetic mapping and QTL analysis of fiber related traits in cotton (Gossypium). *Tag Theoretical and Applied Genetics*. 108: 280-291.

Meloni, D. A., Oliva, M. A., Ruiz, H. A., and Martinez, C. A. (2001). Contribution of proline and inorganic solutes to osmotic adjustment in cotton under salt stress. *Journal of Plant Nutrition*. 24: 599–612.

Meloni, D. A., Oliva, M. A., Martinez, C. A. and Cambraia, J. (2003). Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. *Environmental Journal of Experimental Botany*. 49: 69-76.

Mondini, L., Arshiya, N. and Mario, A. P. (2009). Assessing plant genetic diversity by molecular tools. *Diversity*. 1: 19–35.

Moreau, L S., Lemarie, A., Charcosset, A. and Gallais, A. (2000). Economic efficiency of one cycle of marker-assisted selection. *Crop Science*. 40: 329-337.

Munns, R. (2005). Gene and salt tolerance: breeding them together. *New Physiologist*. 167: 645–663.

Munns, R and Mark, T. (2008). Mechanisms of salinity tolerance. *Plant Biology*. 59: 651–681.

Munis, F. H., Lili, T., Khurram, Z., Jlafu, T., Fenglin, D. and Xianlong, Z.. (2010). Critical osmotic, ionic and physiological indicators of salinity tolerance in cotton (*Gossypium hirsutum* L.) for cultivar selection. *Pakistan Journal of Botany*. 42: 1685 – 1694.

Muravenko, O .V., Fedotov, A. R., Fedorova, L. I. and Zelenin, A. V. (1998). Comparison of chromosome BrdU – Hoechst – Giemsa banding pattern of the A1 and $(AD)_2$ genomes of cotton. *Genome*, 41: 616-625.

Murtaza, G., Ghafoor, A. and Qadir, M. (2006). Irrigation and soil management strategies for using saline sodic water in cotton-wheat rotation. *Agriculture and Water Management*. 81: 98-114.

Naheed, G. and Rasul, G. (2009). Recent water requirement of cotton crop in Pakistan. *Pakistan Journal of Meteorology*. 6: 75-84.

Nawaz, K., Aqsa, T., Iqra, K. H., and Majeed, A. (2010). Induction of salt tolerance in two cultivars of sorghum (*Sorghum bicolor* L) by exogenous application of proline at seedling stage. *World Applied Science Journal*. 10: 93–99.

Neale, D. B., Devey, M. E., Jermsted, K. D., Ahuja, M. R., Alosi, M. C. and Marshal, K. A. (1992). Use of DNA markers in forest tree improvement research. *New Forests*. 6: 391-407.

Nekrutenko, A and Baker, R. J. (2003). Sub-genome specific markers in tetraploid cotton *G.hirsutum*: Amplification of evolutionary analysis of polyploidy. *Gene*. 306: 99-106.

Netondo, G. W., John, C. O. and Erwin, B. (2004). Sorghum and salinity; Response of growth, water relations and ion accumulation to NaCl salinity. *Crop Science*. 44: 797–805.

Nguyen, T. B., Giband, M., Brottier, P., Risterucci, A. M. and Lacape, J. M. (2004). Wide coverage of the tetraploid cotton genome using newly developed microsatellite markers. *Tag Theoretical and Applied Genetics*. 109: 167–175.

Ni, J, Colowit, P. M., Mackill, D. J. (2002). Evaluation of genetic diversity in rice subspecies using microsatellite markers. *Crop Science*. 42: 601–607.

Noor, E., Azhar, F. M., and Khan, A. A. (2001). Differences in responses of *G. hirsutum* L. varieties to NaCl salinity at seedling stage. *International Journal of Agriculture and Biology*. 3: 345-347.

Pasapula, V., Shen, G., Kuppu, S., Paez, V. J., Mendoza, M., Hou, P., Chen, J., Qiu, X., Zhu, L., Zhang, X., Auld, D., Blumwald, E., Zhang, H., Gaxiola, R., and Payton, P. (2010). Expression of an Arabidopsis vacuolar H+ pyrophosphatase gene (AVP1) in cotton improves drought and salt tolerance and increase fiber yield in the field conditions. *Plant Biotechnology*. 9: 88–99.

Preetha, S. and Raveendern, T. S. (2008). Molecular marker technology in cotton. *Biotechnology and Molecular Biology Review*. 3: 32–45. April 2008.

Qadir, M. and Shams, M. (1997). Some agronomic and physiological aspects of salt tolerance in cotton *(Gossypium hirsutum* L.). *Journal of Agronomy and Crop Science*. 179: 101-106.

Qurashi, S. N. D., Sukumar, S., Ramesh, V. K., and Jenkins, J. N. (2004). EST-SSR: A new class of genetic markers in cotton. *Molecular Biology and Physiology*. 8: 112-123.

Rafiq, A. C. (2006). New frontiers in cotton production. *International Cotton Advisory Committee*. 1-3.

Rahman, M., Hussain, D. and Zafar, Y. (2002). Estimation of genetic divergence among Elite cotton cultivars genotypes by DNA fingerprinting technology. *Crop Science*. 42: 2137-2144.

Rajguru, S. N., Banks, S. W., Gossett, D. R., Lucas, M. C., Fowler, T. E., Jand Millhollon, E. P. (1999). Antioxidant response to salt stress during fiber development in cotton ovules. *The Journal of Cotton Sciences*. 3: 11–18.

Randall, L. S. and Wendel, J. F. (2002). Differential evolutionary dynamics of duplicated paralogous Adh Loci in allotetraploid cotton (*Gossypium*). *Molecular Biology and Evolution*. 19: 597-607.

Rashid, A., Rahmat, K. and Sarfaraz, K. M. (2009). Response of wheat to soil amendments with poor quality irrigation water in salt affected soil. *World Journal of Agriculture Sciences*. 5: 422-424.

Rathert, G. (1982). Influence of extreme K: Na ratios and high substrate salinity on plant metabolism of crops differing in salt tolerance. VII. Relation between carbohydrates and degradative enzymes in salt tolerant and salt sensitive cotton genotypes during initial salinity stress. *Journal of Plant Nutrition*. 5: 1401-1413.

Reddy, V. R., Reddy, K. R. and Baker, D. N. (1991). Temperature effect on growth and development of cotton during the fruiting period. *Agronomy Journal*. 83: 211-217.

Reinisch, A. J., Dong, J. M., Rubaker, C. L. B., Stelly, D. M., Wendel, J. F. and Peterson, A. H. (1994). A detailed RFLP map of cotton *Gossypium hirsutum, Gossypium barbadense* L. chromosome organization and evolution in a disomic polyploidy genome. *Genetics*. 138: 829-847.

Rong, J., Feltus, F. A., Liu, L., Lin, L. and Paterson, A. H. (2010). Gene copy number evolution during tetraploid cotton radiation. *Heredity*. 105: 463–472.

Saha, S., Mehmet, K., Johnie, N. J., Allan, E. Z., Umesh, O. K. R. and Ramesh, V. K. (2003). Simple sequence repeats as useful resources to study transcribed genes of cotton. *Euphytica*. 130: 355–364.

Salem, K. F. M., El Zanaty, A. M. and Esmail, R. M. (2008). Assessing wheat (*Triticum aestivum* L.) genetic diversity using morphological characters and microsatellite markers. *World Journal of Agricultural Sciences*. 4: 538–544.

Sarah, P. O. and Whitton, J. (2000). Polyploid incidence and evaluation. *Annual Review of Genetics*. 34: 401-437.

Sattar, S., Hussain, T. and Javaid, A. (2010). Effect of NaCl salinity on cotton (*Gossypium arboretum* L.) grown on medium and in hydroponic cultures. *The Journal of Animal & Plant Sciences*. 20: 87 – 89.

Seemann, N. J. R., and Critchley, C. (1985). Effect of salt stress on the growth, ion content, stomatal behaviour and photosynthetic capacity of a salt sensitive species, *Phaseolus vulgaris* L. *Planta.* 164: 151-162.

Seetharam, K., Thirumeni, S. and Paramasivam, K. (2009). Estimation of genetic diversity in rice (*Oryza sativa* L.) genotypes using SSR markers and morphological characters. *African Journal of Biotechnology*. 8 : 2050–2059.

Sekhar, S. C. and Bhaskara, R. V. K. (2011). Cottonseed oil as health oil. *Pertanika Journal of Tropical Agricultural Science*. 34: 17-24.

Sheidai, M., Leyla, B., Ahmad, M., Zahra, N. M., and Omran, A. (2010). Genetic diversity in F2 back cross progenies of cotton. *Gene Conserve*. 9: 167-187.

Shen, X., Wangzhen, G., Qiongxian, L., Xiefei, Z., Youlu, Y. and Tainzhen, Z. (2007). Genetic mapping of quantitative traits for fiber quality and yield trait by RIL approach in up land cotton. *Euphytica*.155: 371-380.

Sima, N. A. K. K., Askari, H., Mirzaei, H. H., Hossein, H. and Pessarakli, M. (2009). Genotype dependent differential responses of three forage species to calcium supplement in saline conditions. *Journal of Plant Nutrition*. 32: 579–597.

Singh, K. N., and Chatrath, R. (1992). Genetic variability in grain yield and its component characters and their associations under salt stress conditions in tissue culture lines of bread wheat (*Triticum aestivum* L. em Thell.) *Wheat Information Service*. 75:46-53.

Song, X. and Zhang, T. (2009). Quantitative trait loci controlling plant architectural traits in cotton. *Plant Science*. 177: 317–323.

Song, X., Wang, K., Guo, W., Zhang, J., and Zhang, T. (2005). A comparison of genetic maps constructed from haploid and BC1 mapping populations from the same crossing between *Gossypium hirsutum* L. and *Gossypium barbadense* L. *Genome*. 48: 378–390.

Stephen, P. M. and Rita, H. M. (2008). Molecular plant breeding as the foundation for 21 century crop improvement. *Plant Physiology*, 147: 969–977.

Stella, K. K., Ulloa, M., Sack, E. and Steward, McD. J. (2009). Assessing genetic diversity in *Gossypium arboreum* L. cultivars using genomic and EST – derived microsatellites. *Genetica*. 136: 141-147.

Sukumar, S., Johnie, N. J., Jixiang, W., Jack, C. M., Gutiérrez, O. A., Richard, G. P., Roy, G. C. and David, M. S. (2006). Effects of chromosome-specific introgression in upland cotton on fiber and agronomic traits. *Genetics*. 172: 1927–1938.

Sun, C. Q, Wang, X. K, Li, J. C. and Yushimura, A (2001). Comparison of the genetic diversity of common wild rice (*Oryza rufipogon* Griff.) and cultivated rice (*Oryza sativa* L.) using RFLP markers. *Tag Theoretical and Applied Genetics*. 102: 157–162.

Tang, T., Jianzi, H., Yang, Z. and Suhua, S. (2004). High-throughput S-SAP by fluorescent multiplex PCR and capillary electrophoresis in plants. *Journal of Biotechnology*. 114: 59–68.

Tavakkoli, E., Pichu, R. and Glenn, K. M. (2010). The response of barley to salinity stress differs between hydroponic and soil systems. *Functional Plant Biology*. 37: 621–633.

Torberi, H. A. and Reeves, D. W. (1994). Fertilizer nitrogen requirement for cotton production as affected by tillage and traffic. *Soil Science Society of American Journal*. 58: 1416 –1423.

Turner, J. E. (2003). Characterization of the acetate non – utilizing mutant 1 of *Arabidopsis*. University of Wales. Thesis. Page 58.

Tuteja, N. (2007). Mechanisms of high salinity tolerance in plants. *Methods in Enzymology*. 428: 419–438.

Ulloa, M. and Meredith, W. R. J. (2000). Genetic linkage map and QTL analysis of agronomic and fiber quality traits in an interspecific population. *Journal of Cotton Science.* 4: 161–170.

Ulloa, M., Meredith, W. R. J., Shappley, Z. W., Kahler, A. L. (2002). RFLP genetic linkage maps from four F2, 3, populations and a join map of *Gossypium hirsutum* L. *Tag Theoretical and Applied Genetics*. 104: 200-208.

Vemireddy, L. R., Sunil, A. and Javaregowda, N. (2007). Capillary Electrophoresis is Essential for Microsatellite Marker Based Detection and Quantification of Adulteration of Basmati Rice (*Oryza sativa*). *Journal of Agricultural Food and Chemistry*. 55: 8112–8117.

Venkatesh, N. K., Basavaraj, M. K., Manjula, S. M., Lalitadas, A. D. and Narayanan, S. S. (2009). The Worldwide gene pools of *G. arboreum* L. and *G. herbaceum* L., and their improvement. *Genetics and Genomics of Cotton*. 3: 1-29.

Wang, P.Z., Han, Z. G. and Tianzhen, Z. (2004). EST –SSR Based genetic diversity assessment of Xinjiang cultivars in upland cotton. *Journal of Genetics and Molecular Biology*. 15: 171–181.

Wang, Z. G., Guo, J. M., Zhu, Y. C., Chen, X. and Zhang, T. Z. (2006). Molecular tagging and mapping of quantitative trait loci for lint percentage and morphological marker genes in upland cotton. *Journal of Integrative Plant Biology*. 48: 320-326.

Wang, B., Wangzhen, G., Xiefei, Z., Yaoting, W., Naitai, H. and Tianzhen, Z. (2006). QTL mapping of fiber quality in an elite hybrid derived RIL population of upland cotton. *Euphytica*. 152: 367–378.

Wendel, F. J. and Cronn C. R.. 2003. Polyploidy and Evolutionary History of Cotton. Advances in Agronomy. 78: 139-186.

Witcombe, J. R. and Virk, D.S. (2001). Number of crosses and population size of participatory and classical plant breeding. *Euphytica*. 122: 451–462.

Wu, C. A., Guo, D. Y., Qing, W. M. and Cheng, C. Z. (2004). The cotton GhNHX1 gene encoding a novel putative Na^+/H^+ antiporter plays an important role in salt stress. *Plant and Cell Physiology*. 45: 600–607.

WuWei, Y., Liu, J. D., Fan, B. X. and Hu, Q. M.. (1997). The effect of salt on the fibre characteristics of upland cotton. *China Cottons*. 24: 17-18.

Yasar, F., Uzal, O., Tufenkci, S. and Yildiz, K. (2006). Ion accumulation in different organs of green bean genotypes grown under salt stress. *Plant Soil Environment*. 10: 476–480.

Yeo, A. (1998). Molecular biology of salt tolerance in the context of whole-plant physiology. *Journal of Experimental Botany*. 49: 915–929.

Yeo, A. R., Lee, K. S., Izard, P., Boursier, P. J. and Flowers, T. J. (1991). Short and long term effects of salinity on leaf growth in rice (*Oryza sativa* L.). *Journal of Experimental Botany*. 42: 881-888.

Yu, J., Yu, S., Lu, C., Wang, W., Fan, S., Song, M., Lin, Z. X., Zhang, X. L. and Zhang, J. (2007). High density linkage map of cultivated allotetraploid cotton based on SSR, TRAP, SRAP and AFLP markers. *Journal of Integrative Plant Biology*. 49: 716-724.

Zachary, W., Shappley, J., Jenkins N., Jun Z. and Jack, C. M, J. (1998). Quantitative Trait Loci associated with agronomic and fiber traits of upland cotton. *Journal of Cotton Sciences*. 2:112-123.

Zhang, H. X. and Blumwald, E. (2001). Transgenic salt-tolerant tomato plants accumulate salt in foliage but not in fruit. *Nature Biotechnology*. 19: 765–768.

Zhang, B., Liu, F., Wang, Q., and Zhang, W. (2001). Selection of salt tolerance in cotton tissue culture and plant regeneration from NaCl tolerant embryogenic callus. *Israel Journal of Plant Sciences*. 49: 187-191.

Zhang, J., Guo, W. and Zhang, T. (2002). Molecular linkage map of allotetraploid cotton *(Gossypium hirsutum L.* x *Gossypium barbadense* L. with a haploid population. *Tag Theoretical and Applied Genetics*. 105: 1166-1174.

Zhang, Z., Zhou, B., Chen, S., Shen, X. and Zhang, X. (2003). Construction of gene pool with superior fibre properties in upland cotton through interspecific hybridisation between *G.hirsutum* and *Gossypium* wild species. *Acta Agronomica Sinica*. 29: 514-519.

Zhang, T., Youlu, Y., John, Y., Wangzhen, G. and Russell, J. K. (2003). Molecular tagging of a major QTL for fiber strength in up land cotton and its marker assisted selection. *Tag Theoretical and Applied Genetics*. 106: 262–268.

Zhang, H., Hezhong, D., Weijiang, L., Yi, S., Shouyi, C. and Xiangqiang, K. (2009). Increased glycine betaine synthesis and salinity tolerance in AhCMO transgenic cotton lines. *Molecular Breeding*. 23: 289-298.

Zhang, Z., Xiao, Y., Luo, M., Li, X., Luo, X.. H., Lei, L. D. M., and Pei, Y. (2005). Construction of a genetic linkage map and QTL analysis of fiber – related traits in upland cotton (*Gossypium hirsutum* L.). *Euphytica*. 144: 91-99.

Zhong, H. and Lauchli, A. (1993). Spatial and temporal aspects of growth in the primary root of cotton seedlings: Effects of NaCl and $CaCl_2$. *Journal of Experimental Botany*. 44: 763-771.

Zuo, K., Sun, J., Zhang, X., Nie, Y., Liu, J. and Feng, C. (2000). Constructing a linkage map of upland cotton ((*Gossypium hirsutum* L.) using RFLP, RAPD and SSR Markers. *Journal of Huazhong Agricultural University*. 19: 190-193.

Appendix

Table 1 Correlation of plant height, nodes per plant, fresh and dry weight, Na^+ and K^+ concentration in young and old leaf, petiole and stem. (150mM). (young leaf = Y.L, young petiole = Y.P, young stem = Y.S, old leaf = O.L, old petiole = O.P and old stem = O.S).

	Plant height	Nodes /plant	FW	DW	Na^+ (O.L)	Na^+ (O.P)	Na^+ (O.S)	Na^+ (Y.L)	Na^+ (Y.P)	Na (Y.S)	K^+ (O.L)	K^+ (O.P)	K^+ (O.S)	K^+ (Y.L)	K^+ (Y.P)	K^+ (Y.S)
Plant height	1	0.46	.696(**)	.783(**)	.739(**)	0.139	-0.223	.705(**)	.681(**)	0.212	-0.09	.500(*)	0.412	-0.039	0.46	.511(*)
Nodes/plant		1	.726(**)	.646(**)	0.306	-0.188	-0.247	0.267	0.261	0.08	-0.34	0.02	.615(*)	-0.172	0	-0.14
FW			1	.924(**)	.569(*)	0.287	-0.122	.725(**)	.762(**)	.575(*)	0.04	0.21	0.342	0.089	0.3	0.309
DW				1	.655(**)	0.279	-0.027	.784(**)	.757(**)	.561(*)	-0.03	0.307	0.374	0	0.35	0.409
Na^+ (O.L)					1	.556(*)	0.094	.781(**)	.681(**)	0.455	-0.07	0.167	0.154	-0.084	0.25	.637(**)
Na^+ (O.P)						1	0.472	.681(**)	.653(**)	.723(**)	0.432	-0.25	-0.465	0.265	0.25	0.428
Na^+ (O.S)							1	0.201	0.149	0.336	0.322	-0.11	-0.494	0.278	-0.1	0
Na^+ (Y.L)								1	.931(**)	.789(**)	0.315	0.251	0.003	0.18	.500(*)	.608(*)
Na^+ (Y.P)									1	.719(**)	0.403	0.252	0.117	0.09	0.42	.589(*)
Na^+ (Y.S)										1	.518(*)	0	-0.3	0.349	0.45	0.456
K^+ (O.L)											1	0.119	-0.403	.547(*)	.636(**)	0.275
K^+ (O.P)												1	0.264	0.056	0.32	0.148
K^+ (O.S)													1	-.544(*)	-0.2	0.016
K^+ (Y.L)														1	.579(*)	-0.06
K^+ (Y.P)															1	0.451
K^+ (Y.S)																1

Continue

Table 1 Correlation of K^+/Na^+ ratio in young and old leaf, petiole and stem. (young leaf = Y.L, young petiole = Y.P, young stem = Y.S, old leaf = O.L, old petiole = O.P and old stem = O.S).

	K^+/Na^+ (O.L)	K+/Na^+ (O.P)	K^+/Na^+ (O.S)	K^+/Na^+ (Y.L)	K^+/Na^+ (Y.P)	K^+/Na^+ (Y.S)
Plant height (mm)	-.841(**)	-.747(**)	0.414	-.752(**)	-.873(**)	-.547(*)
Nodes/plant	-.652(**)	-0.483	0.143	-0.486	-.636(**)	-.642(**)
FW (g)	-.579(*)	-.743(**)	0.484	-.779(**)	-.777(**)	-.787(**)
DW (g)	-.630(**)	-.800(**)	0.408	-.706(**)	-.765(**)	-.755(**)
Na^+ (O.L)	-.624(**)	-.814(**)	0.443	-.527(*)	-.649(**)	-0.317
Na^+ (O.P)	0.2	-0.461	0.253	-0.247	0.008	-0.01
Na^+ (O.S)	0.5	0.073	-0.289	0.361	0.369	0.265
Na^+ (Y.L)	-0.4	-.803(**)	.539(*)	-.573(*)	-.525(*)	-.509(*)
Na^+ (Y.P)	-0.4	-.767(**)	0.407	-.638(**)	-.591(*)	-.508(*)
Na^+ (Y.S)	0.1	-0.494	0.476	-0.29	-0.108	-0.419
K^+ (O.L)	0.5	-0.094	-0.077	-0.03	0.197	-0.122
K^+ (O.P)	-0.3	-0.121	0.275	-0.219	-0.444	-0.414
K^+ (O.S)	-.731(**)	-0.354	-0.032	-0.274	-.656(**)	-0.45
K^+ (Y.L)	0.4	-0.067	0.233	-0.021	0.114	-0.29
K^+ (Y.P)	-0.1	-0.415	0.205	-0.457	-0.21	-0.415
K^+ (Y.S)	-0.3	-.611(*)	0.362	-0.442	-0.354	-0.037
K^+/Na^+ (O.L)	1	.626(**)	-0.288	.639(**)	.894(**)	0.464
K^+/Na^+ (O.P)		1	-0.386	.680(**)	.744(**)	.617(*)
K^+/Na^+ (O.S)			1	-0.355	-0.4	-0.416
K^+/Na^+ (Y.L)				1	.725(**)	.501(*)
K^+/Na^+ (Y.P)					1	.700(**)
K^+/Na^+ (Y.S)						1

Table 2 Correlation of plant height, nodes per plant, fresh and dry weight, Na^+ and K^+ concentration in young and old leaf, petiole and stem. (250mM). (young leaf = Y.L, young petiole = Y.P, young stem = Y.S, old leaf = O.L, old petiole = O.P and old stem = O.S).

	Plant height	Nodes /plant	FW	DW	Na^+ (O.L)	Na^+ (O.P)	Na^+ (O.S)	Na^+ (Y.L)	Na^+ (Y.P)	Na^+ (Y.S)	K^+ (O.L)	K^+ (O.P)	K^+ (O.S)	K^+ (Y.L)	K^+ (Y.P)	K^+ (Y.S)
Plant height	1	0.106	.791(**)	.626(**)	.897(**)	-0.322	-0.351	.630(**)	-.837(**)	-0.483	-.634(**)	0.313	0.157	-.573(*)	.828(**)	.869(**)
Nodes/plant		1	0.496	.598(*)	0.015	-.582(*)	-.780(**)	-0.164	-0.237	0.049	-.637(**)	-0.249	.579(*)	0.262	-0.087	0.217
FW			1	.930(**)	.657(**)	-0.47	-0.485	0.459	-.743(**)	-0.417	-.819(**)	0.001	0.343	-0.437	.700(**)	.781(**)
DW				1	0.469	-.554(*)	-.579(*)	0.296	-.608(*)	-0.429	-.830(**)	-0.029	.503(*)	-0.261	0.495	.673(**)
Na^+ (O.L)					1	-0.137	-0.226	.804(**)	-.590(*)	-0.184	-.544(*)	0.37	0.03	-.693(**)	.727(**)	.624(**)
Na^+ (O.P)						1	.727(**)	0.202	.579(*)	0.389	0.447	0.155	-.669(**)	-0.221	-0.093	-0.347
Na^+ (O.S)							1	-0.032	0.389	0.074	.511(*)	-0.135	-0.4	-0.165	-0.185	-0.297
Na^+ (Y.L)								1	-0.206	0.058	-0.226	.608(*)	-0.434	-.824(**)	.711(**)	0.307
Na^+ (Y.P)									1	.727(**)	.597(*)	-0.059	-0.38	0.287	-.692(**)	-.910(**)
Na^+ (Y.S)										1	0.238	-0.096	-0.305	-0.009	-0.46	-.737(**)
K^+ (O.L)											1	0.247	-.632(**)	0.316	-0.295	-.695(**)
K^+ (O.P)												1	-0.484	-0.372	0.485	0.088
K^+ (O.S)													1	0.314	-0.278	0.333
K^+ (Y.L)														1	-.636(**)	-0.356
K^+ (Y.P)															1	.699(**)
K^+ (Y.S)																1

Continue

Table 2 Correlation of K^+/Na^+ ratio in young and old leaf, petiole and stem. (young leaf = Y.L, young petiole = Y.P, young stem = Y.S, old leaf = O.L, old petiole = O.P and old stem = O.S).

	K^+/Na^+ (O.L)	K+/Na^+ (O.P)	K^+/Na^+ (O.S)	K^+/Na^+ (Y.L)	K^+/Na^+ (Y.P)	K^+/Na^+ (Y.S)
Plant height	-.855(**)	0.061	0.084	-.724(**)	.643(**)	.558(*)
Nodes/plant	-0.47	-0.126	.695(**)	0.057	0.013	0.156
FW	-.785(**)	-0.113	0.249	-.499(*)	.616(*)	.586(*)
DW	-.721(**)	-0.13	.499(*)	-0.372	0.461	.597(*)
Na^+ (O.L)	-.755(**)	0.189	-0.073	-.731(**)	.503(*)	0.38
Na^+ (O.P)	.663(**)	0.226	-.639(**)	0.126	-0.165	-0.209
Na^+ (O.S)	.623(**)	0.131	-.760(**)	0.226	-0.11	-0.125
Na^+ (Y.L)	-0.372	0.292	-0.335	-.723(**)	0.382	0.179
Na^+ (Y.P)	.810(**)	0.24	-0.189	0.449	-.753(**)	-.684(**)
Na^+ (Y.S)	0.4	0.182	-0.151	0.059	-.595(*)	-.761(**)
K^+ (O.L)	.770(**)	0.012	-.543(*)	0.402	-0.325	-0.489
K^+ (O.P)	-0.092	0.088	-0.155	-0.366	0.281	0.195
K^+ (O.S)	-.547(*)	-0.187	.724(**)	0.07	-0.019	0.357
K^+ (Y.L)	0.291	-0.083	0.391	.693(**)	-.523(*)	-0.289
K^+ (Y.P)	-.532(*)	-0.123	-0.263	-.623(**)	.813(**)	.535(*)
K^+ (Y.S)	-.764(**)	-0.063	0.22	-0.493	.690(**)	.759(**)
K^+/Na^+ (O.L)	1	0.024	-0.449	.639(**)	-0.462	-.508(*)
K^+/Na^+ (O.P)		1	-0.061	-0.082	-0.396	-0.29
K^+/Na^+ (O.S)			1	-0.026	-0.203	0.159
K^+/Na^+ (Y.L)				1	-0.362	-0.231
K^+/Na^+ (Y.P)					1	.807(**)
K^+/Na^+ (Y.S)						1

Table 3. Mean square, degree of freedom and P values of cotton genotypes treated with 150mM NaCl.

Variables	Genotypes	DF	Treatment	DF	Genotypes x Treatments	DF
Plant height	126403.826**	15	886158.356**	1	14027.099^{NS}	15
Nodes/plant	9.893**	15	19.031**	1	2.790^{NS}	15
FW	9756.309**	15	70636.456**	1	2740.376^{NS}	15
DW	186.490**	15	808.147**	1	48.288^{NS}	15
Na^+ (y.l)	10294.130**	15	465462.981**	1	9705.122**	15
K^+ (y.l)	6333.992^{NS}	15	14373.201^{NS}	1	8099.073**	15
K^+/Na^+ (y.l)	158.025**	15	18547.632**	1	77.969^{NS}	15
Na^+ (y.p)	193.697^{NS}	15	13443.789**	1	189.450^{NS}	15
K^+ (y.p)	8810.591**	15	239764.138**	1	2945.995^{NS}	15
K^+/Na^+ (y.p)	300.202^{NS}	15	27464.434**	1	390.808^{NS}	15
Na^+ (y.s)	298.314^{NS}	15	18322.774**	1	247.636^{NS}	15
K^+(y.s)	10861.434**	15	276324.279**	1	3656.250^{NS}	15
K^+/Na^+ (y.s)	258.173^{NS}	15	30805.169**	1	348.999^{NS}	15
Na^+ (o.l)	532.571**	15	2006.405**	1	53.651^{NS}	15
K^+ (o.l)	8153.240**	15	1520.119^{NS}	1	1983.683^{NS}	15
K^+/Na^+ (o.l)	532.571**	15	2006.405**	1	53.651^{NS}	15
Na^+ (o.p)	519.891^{NS}	15	26678.194**	1	270.269^{NS}	15
K^+ (o.p)	4621.995**	15	93771.939**	1	997.149^{NS}	15
K^+/Na^+ (o.p)	246.048**	15	12770.725**	1	126.110^{NS}	15
Na^+ (o.s)	642.117^{NS}	15	92151.722**	1	622.560^{NS}	15
K^+ (o.s)	4407.473**	15	19514.588**	1	1451.760^{NS}	15
K^+/Na^+ (o.s)	119.598^{NS}	15	8788.822**	1	138.619^{NS}	15

Table 4. Mean square, degree of freedom and P values of cotton genotypes treated with 250mM NaCl.

Variables	Genotypes	DF	Treatment	DF	Genotypes x Treatments	DF
Plant height	253703.748**	15	3666365.823**	1	30200.761**	15
Nodes/plant	31.231^{NS}	15	170.998**	1	7.338**	15
FW	16883.657**	15	325262.898**	1	5109.619**	15
DW	1460.044**	15	21361.582**	1	486.780^{NS}	15
Na^+ (y.l)	10294.130**	15	465462.981**	1	9705.122**	15
K^+ (y.l)	6333.992^{NS}	15	14373.201^{NS}	1	8099.073**	15
K^+/Na^+ (y.l)	158.025**	15	18547.632**	1	77.969^{NS}	15
Na^+ (y.p)	3623.743^{NS}	15	479271.740**	1	3672.227^{NS}	15
K^+ (y.p)	34712.702**	15	450810.992**	1	8403.807^{NS}	15
K^+/Na^+ (y.p)	172.859^{NS}	15	45102.527**	1	173.105^{NS}	15
Na^+ (y.s)	4660.503**	15	683522.396**	1	4851.413**	15
K^+(y.s)	27652.340**	15	456366.521**	1	22523.556**	15
K^+/Na^+ (y.s)	198.359^{NS}	15	46613.038**	1	268.487^{NS}	15
Na^+ (o.l)	3673.804**	15	583341.715**	1	2976.422**	15
K^+ (o.l)	7707.867**	15	66349.292**	1	3592.495**	15
K^+/Na^+ (o.l)	82.013**	15	5298.286**	1	33.803^{NS}	15
Na^+ (o.p)	1262.721^{NS}	15	399459.256**	1	1450.170^{NS}	15
K^+ (o.p)	12378.760**	15	166786.296**	1	7191.972NS	15
K^+/Na^+ (o.p)	166.945**	15	18055.219**	1	177.930**	15
Na^+ (o.s)	3374.180**	15	221707.977**	1	3673.208**	15
K^+ (o.s)	16558.524**	15	9163.312^{NS}	1	6168.712^{NS}	15
K^+/Na^+ (o.s)	91.425**	15	3730.904**	1	55.287^{NS}	15

Table 5. Means and standard deviation of plant height, nodes per plant, fresh weight and dry weight of cotton genotypes treated with 150 mMNaCl.

	Plant height (mm)		Nodes per plant		FW (g)		DW (g)	
Genotypes	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**
CIM 448	556± 97	476±40	9.6±0.9	9.0±1.6	100.9±43.1	71.3±44.8	10.6±6.2	6.0±1.8
Dhandra	672±127	456±122	6.8±2.9	7.6±2.8	63.6±55.3	69.2±25.6	5.7±3.9	5.6±4.1
Dhandra x S6	866±41	595±62	9.8±1.0	8.0±1.2	181.2±19.9	96.2±42.3	19.8±11.3	9.4±1.2
DNH 40	503±109	454±39	7.5±0.6	8.4±1.1	98.2±48.8	77.5±1.6	5.0±2.5	6.3±1.4
DNH 40 x CIM 448	623±131	446±23	9.1±1.9	8.6±1.5	146.9±40.7	83.2±37.0	23.1±29.2	8.3±2.2
DNH 40 x S6	625±126	490±44	9.6±1.3	8.6±1.0	154.5±56.1	91.7±37.0	18.0±8.2	9.2±1.9
G. stocksii	190±35	163±17	5.4±2.1	6.5±1.0	19.1±36.4	21.5±38.7	0.3±4.1	0.2±3.2
G. stocksii x G. hirsutum	654±70	429±54	10.2±1.3	8.7±1.0	165.5±43.8	82.1±56.9	12.3±3.7	7.0±1.7
G.hirsutum x G. sturtianum	480±93	431±68	7.8±1.6	8.3±0.8	44.6±30.4	63.3±44.8	3.2±1.8	3.2±1.0
MNH 147	654±11	471±38	9.0±1.4	8.4±1.5	105.0±30.3	55.6±36.0	8.6±5.6	6.2±1.9
MNH 147 x CIM 448	645±91	483±38	9.2±1.1	9.0±1.6	116.7±42.3	24.1±36.2	10.7±1.7	6.9±2.2
MNH 147 x S6	735±99	462±96	10.3±1.0	9.2±1.8	110.3±15.9	81.2±47.8	13.1±2.9	7.7±2.6
NIAB 313	608±106	435±58	10.0±1.7	8.4±1.5	110.2±79.4	62.8±39.4	9.3±4.9	4.2±1.2
NIAB 313 x DNH 40	610±42	480±30	10.0±1.4	7.0±2.0	114.0±96.0	26.8±43.0	7.6±4.5	3.1±4.0
S6 x Dhandra	835±71	593±46	11.0±0.8	8.3±1.0	193.8±72.5	96.5±47.6	20.3±7.3	10.5±2.5
S6 x DNH 40	658±147	473±43	10.3±0.8	9.6±0.9	144.5±48.2	90.1±36.0	13.6±3.6	8.8±3.8

Table 6. Means and standard deviation of plant height, nodes per plant, fresh weight and dry weight of cotton genotypes treated with 250 mMNaCl.

	Plant height (mm)		Nodes per plant		FW (g)		DW (g)	
Genotypes	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**
CIM 448	912±121	496±58	16.6±3.6	13.0±2.1	227±37	65±13	45.2±12.5	19.6±9.0
Dhandra	1180±136	688±36	12.2±2.3	8.3±2.1	137±71	55±15	44.2±39.9	12.7±3.8
Dhandra x S6	1070±77	618±41	14.0±3.5	11.8±4.5	212±34	75±6	58.2±18.1	17.9±2.6
DNH 40	937±72	567±109	14.4±2.4	10.5±1.6	179±67	53±12	50.3±34.1	15.4±5.9
DNH 40 x CIM 448	942±85	495±43	14.3±2.2	13.3±1.5	215±73	72±14	53.3±27.3	23.5±13.0
DNH 40 x S6	932±123	608±145	14.0±1.7	9.5±2.1	277±46	82±19	81.1±28.3	22.5±10.2
G. stocksii	258±104	156±57	7.8±3.2	8.2±1.5	7±6	4±3	0.9±8.5	0.4±2.2
G. stocksii x G. hirsutum	1025±159	525±30	15.7±2.2	11.8±1.5	234±36	59±12	42.9±11.4	22.2±8.4
G.hirsutum x G. sturtianum	1006±140	418±157	13.8±1.6	9.5±3.4	126±35	32±11	27.0±16.4	8.5±4.8
MNH 147	996±146	487±88	14.8±1.3	12.7±1.6	184±57	51±12	40.5±24.2	13.2±4.8
MNH 147 x CIM 448	983±62	503±54	15.2±2.2	14.0±1.6	219±44	76±12	61.0±27.9	23.3±15.0
MNH 147 x S6	952±377	572±83	12.7±4.4	12.2±1.5	174±77	73±7	41.5±21.2	20.1±2.6
NIAB 313	1026±94	530±108	16.2±2.9	11.3±3.5	167±26	50±9	49.4±11.7	13.4±4.8
NIAB 313 x DNH 40	987±68	530±66	14.7±3.1	12.0±1.0	182±31	58±11	59.2±48.6	13.4±2.7
S6 x Dhandra	1126±52	653±35	14.0±1.8	10.0±2.0	216±21	72±4	69.2±6.2	17.9±4.5
S6 x DNH 40	1012±93	584±103	15.6±2.7	10.6±0.9	258±78	77±9	73.4±29.9	22±2.6

Table 7. Means and standard deviation of Na^+ concentration in young and old leaf, petiole and stem of cotton genotypes treated with 150 mMNaCl. (young leaf = Y.L, young petiole = Y.P, young stem = Y.S, old leaf = O.L, old petiole = O.P and old stem = O.S).

	Na^+ (Y.L)		Na^+ (Y.P)		Na^+ (Y.S)		Na^+ (O.L)		Na^+ (O.P)		Na^+ (O.S)	
Genotypes	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**
CIM 448	8.2±5.7	27.4±14.4	5.6±1.8	16±9	4.5±25	17±11	11.7±3	28±14	9.3±4	24.0±4.5	12.1±4.4	46.1±9.6
Dhandra	6.4±1.9	48.1±51.2	4.8±2.7	35±41	13.2±21	35±46	13.9±4	55±43	19.9±19	70.5±58.2	13.0±2.3	78.0±53.5
Dhandra x S6	7.6±1.8	116±34.4	5.1±2	40±10	5.4±1.7	53±24	10.2±1	65±20	10.4±3.9	57.5±18.4	13.5±5.2	55.1±42.7
DNH 40	4.6±2	22.2±10.1	5.5±2.3	20±6	3.3±0.7	23±10	10.8±4	31±4.7	8.8±1.8	24.4±2.6	9.3±5.1	41.7±12
DNH 40 x CIM 448	6.7±2.5	40.1±20.1	5.2±1.1	23±12	4.8±2.4	38±19	10.2±2	36±13	7.6±1.7	28.4±6.0	6.9±2.6	54.9±26.1
DNH 40 x S6	4.9±1.4	52.5±49.9	4.4±1.7	29±22	5.9±1.8	28±29	7.5±4	38±24	7.9±2.9	38.1±17.9	10.8±1.0	68.8±49.4
G. stocksii	3.8±0.4	44±1.8	4.3±1.3	8.5±6	3.7±0.8	34±47	4±1	6.5±4.6	4±1.1	42.1±42.7	7.4±5.1	90.8±81.6
G. stocksii x G. hirsutum	10.4±4.2	51.4±12.7	4.8±1.8	29±9	5±1.6	33±13	14.1±2	52±4.8	12.8±3.4	43.5±9.1	7.8±2.3	42.6±13.9
G.hirsutum x G. sturtianum	6.2±3.3	19.7±8.3	4.9±1.1	21±5	3.9±0.4	18±5.8	7.2±2	25±8.2	7.9±2	25.7±4.3	10.1±3.8	52.6±9.6
MNH 147	19.2±4.6	29.7±10.7	4.5±1.1	18±5	3.6±1	23±8.5	15.2±7	49±13	8.5±1.9	35.2±9.3	9.0±3.2	73.6±9
MNH 147 x CIM 448	9.2±1.5	66.4±44.5	4.7±2.3	30±15	5.3±1	33±16	9.5±1	52±38	7.8±2.7	40.0±17.1	11.5±3.3	90.2±51
MNH 147 x S6	7.7±3.7	58. ±42.1	4.4±1.2	22±6	4.5±1.4	19±6.2	8.4±3	31±10	8.7±2.9	28.9±6.1	8.8±2.1	47.3±13.8
NIAB 313	7.3±2.4	33±29.3	5.9±4.2	22±19	3.5±1	22±14	8.1±1	36±18	10.3±5.1	28.5±10.5	14.7±15	53.3±11.5
NIAB 313 x DNH 40	7.9±1.7	19.9±32	4.2±1.2	91±4	5.1±2	30±10	22.1±6	39±16	9.4±4.5	26.4±5.0	8.4±0.4	56.2±23
S6 x Dhandra	7±2.3	101±18.2	5.3±1.6	47±12	3.4±0.4	45±2`	9±4	51±21	11.4±6.3	46.9±10	9.5±4.0	83.3±6.1
S6 x DNH 40	4.8±0.9	68.8±72.6	4.8±1.9	28±34	4.5±2	42±59	7.2±3	53±11	82.2	43.3±23.3	12.6±3.8	68.8±28.9

Table 8. Means and standard deviation of Na^+ concentration in young and old leaf, petiole and stem of cotton genotypes treated with 250 mMNaCl. (young leaf = Y.L, young petiole = Y.P, young stem = Y.S, old leaf = O.L, old petiole = O.P and old stem = O.S).

	Na^+ (Y.L)		Na^+ (Y.P)		Na^+ (Y.S)		Na^+ (O.L)		Na^+ (O.P)		Na^+ (O.S)	
Genotypes	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**
CIM 448	8±2	170±91	11±4	170±67	8±3	238±102	13±7	166±66	13±7	132±51	19±5	72±38
Dhandra	9±2	225±69	8±5	107±48	6±2	107±34	15±3	211±86	11±5	171±51	17±3	164±86
Dhandra x S6	9±2	230±56	10±3	108±27	8±3	176±54	23±10	190±53	17±6	119±42	27±13	68±26
DNH 40	7±3	64±34	7±3	119±27	7±3	147±51	1±4	154±28	13±7	107±19	13±2	74±36
DNH 40 x CIM 448	10±4	103±76	8±4	131±83	9±4	133±71	17±13	101±16	11±4	97±30	30±37	61±34
DNH 40 x S6	12±4	177±54	10±4	113±39	10±5	132±66	22±11	193±68	19±10	128±38	26±9	159±65
G. stocksii	5±2	35±20	6±2	240±54	4±2	20±1007	9±2	23±16	5±1	196±39	8±2	199±78
G. stocksii x G. hirsutum	9±4	107±70	10±4	120±22	8±2	103±47	15±9	135±19	19±6	95±9	18±6	47±42
G.hirsutum x G. sturtianum	5±1	76±62	10±5	137±58	6±1	130±29	11±8	116±74	9±3	87±21	18±6	117±37
MNH 147	10±4	103±58	11±7	114±41	8±3	115±64	14±6	149±41	15±5	107±23	15±6	74±46
MNH 147 x CIM 448	9±3	45±31	6±2	86±29	9±2	99±39	12±6	121±76	12±3	104±37	15±5	80±46
MNH 147 x S6	7±3	64±44	8±3	125±28	7±3	151±64	11±4	132±43	8±2	126±28	12±5	76±54
NIAB 313	6±2	115±61	9±2	116±92	9±2	171±53	11±5	148±59	14±7	114±39	19±9	72±33
NIAB 313 x DNH 40	10±3	61±48	9±1	90±37	9±3	146±91	13±6	139±14	14±3	100±15	11±3	107±64
S6 x Dhandra	10±4	218±30	9±5	100±8	9±1	124±31	15±7	193±69	18±8	125±13	23±8	71±66
S6 x DNH 40	8±3	94±58	8±2	65±18	8±4	77±34	13±6	131±27	20±9	115±32	19±9	116±32

Table 9. Means and standard deviation of K^+ concentration in young and old leaf, petiole and stem of cotton genotypes treated with 150 mMNaCl. (young leaf = Y.L, young petiole = Y.P, young stem = Y.S, old leaf = O.L, old petiole = O.P and old stem = O.S).

	K^+ (Y.L)		K^+ (Y.P)		K^+ (Y.S)		K^+ (O.L)		K^+ (O.P)		K^+ (O.S)	
Genotypes	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**
CIM 448	131±13	129±18.7	231±37	292±22	208±15	228±70	95.1±38	76±27	252±44	297.7±28.2	180.3±9	193±64.9
Dhandra	116±39	137±17.7	185±104	279±17	170±46	310±47	115±54	126±28	220±74	233.9±10.5	126.2±62	172±14.9
Dhandra x S6	172±14	218±75.2	266±58	377±94	348±103	364±17	95.1±23	135±57	249±57	301.8±76	177.2±23	169±62.9
DNH 40	138±8.4	145±53.6	167±14	265±52	178±24	279±32	76.6±39	76±16	191±4.4	256.9±55	149.3±43	198±45.8
DNH 40 x CIM 448	139±23	122±23.8	195±24	298±42	203±20	291±58	101±37	97±50	224±70	299.8±19.3	218.3±68	231±30.2
DNH 40 x S6	159±15	144±25.6	177±78	259±12	205±44	330±28	88.2±41	76±32	223±34	276.4±75.3	170.7±23	213±43.6
G. stocksii	156±9.1	202±21.2	178±53	277±37	179±32	250±71	138±47	167±15	223±74	247.7±10.7	174±59	127±60.2
G. stocksii x G. hirsutum	135±19	109±13.9	195±68	266±98	182±26	324±26	74.8±37	116±65	221±18	279.8±48.1	178.5±4.1	228±22.2
G.hirsutum x G. sturtianum	168±35	191±21.9	208±27	315±25	214±74	284±44	163±19	163±56	256±56	283.6±10.5	182.6±25	217±41.1
MNH 147	186±82	170±76.2	211±17	318±28	166±58	251±120	76.9±16	89±31	217±40	282.6±55.8	189.6±27	197±55.3
MNH 147 x CIM 448	160±48	120±16.8	227	200±14	205	255±104	105±71	67±19	223±24	285.1±92.7	176±13	216±33.8
MNH 147 x S6	135±9.3	132±20.3	193±26	296±37	174±18	269±50	118±41	89±9.9	19±440	269.5±32.1	206±17	242±41.6
NIAB 313	143±27	116±12.5	209±33	264±68	181±34	294±37	80.7±25	84±20	219±26	273.7±55.5	181.9±29	215±17.8
NIAB 313 x DNH 40	152±43	102±24	215±42	250±42	205±20	302±34	77.3±18	70±12	208±21	268±43	188.2±6.2	198±20
S6 x Dhandra	212±13	171±89.9	249±27	381±17	217±17	350±45	121±32	206±94	275±26	327.5±16.3	164.4±16	199±16
S6 x DNH 40	155±26	140±42.2	224±24	325±21	204±33	339±30	117±44	114±1.4	203±66	223.4±90.2	161.2±29	201±2..8

Table 10. Means and standard deviation of K^+ concentration in young and old leaf, petiole and stem of cotton genotypes treated with 250 mMNaCl. (young leaf = Y.L, young petiole = Y.P, young stem = Y.S, old leaf = O.L, old petiole = O.P and old stem = O.S).

	K^+ (Y.L)		K^+ (Y.P)		K^+ (Y.S)		K^+ (O.L)		K^+ (O.P)		K^+ (O.S)	
Genotypes	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**
CIM 448	173±26	152±83	313±47	361±36	274±20	271±75	114±23	78±37	268±38	383±41	192±50	241±43
Dhandra	177±28	111±64	282±38	481±63	257±56	472±19	168±39	112±39	303±66	446±88	172±38	160±58
Dhandra x S6	230±33	110±40	401±76	509±104	318±43	378±83	181±38	98±24	309±42	400±74	147±9	105±35
DNH 40	163±22	185±47	242±35	381±75	295±29	357±81	132±39	102±56	265±149	339±46	183±37	257±43
DNH 40 x CIM 448	161±19	176±67	266±51	386±61	216±84	380±37	115±30	79±25	224±21	340±33	253±66	244±27
DNH 40 x S6	208±34	108±18	372±49	443±56	272±70	414±87	145±53	50±4	321±43	272±86	176±32	238±35
G. stocksii	185±43	198±30	261±36	254±61	219±35	132±82	189±25	236±64	339±14	337±51	216±9	77.8±35
G. stocksii x G. hirsutum	160±30	169±55	269±65	367±33	225±42	401±36	117±27	70±22	300±38	405±46	216±26	268±29
G.hirsutum x G. sturtianum	159±11	213±54	256±87	340±97	211±29	249±85	150±32	206±77	273±23	379±91	200±28	223±81
MNH 147	286±56	187±49	369±80	393±52	342±32	385±28	150±40	59±12	369±217	341±39	298±69	277±17
MNH 147 x CIM 448	182±32	233±34	279±34	425±44	219±36	457±63	138±50	92±33	243±29	316±29	169±26	242±20
MNH 147 x S6	171±35	210±36	239±30	357±43	217±41	388±78	116±24	95±35	218±38	311±50	191±23	246±37
NIAB 313	157±22	162±39	280±58	383±72	319±89	343±95	148±52	90±48	310±114	336±38	196±27	213±59
NIAB 313 x DNH 40	141±20	142±21	249±55	361±14	215±80	404±138	163±33	73±20	277±57	317±47	194±22	274±15
S6 x Dhandra	185±93	123±7	410±66	584±25	306±45	408±112	199±42	139±33	346±67	454±25	164±26	102±53
S6 x DNH 40	189±20	139±28	297±75	509±116	256±54	466±46	153±39	97±58	276±50	365±69	179±26	234±39

Table 11. Means and standard deviation of K^+Na^+ ratio in young and old leaf, petiole and stem of cotton genotypes treated with 150 mMNaCl. (young leaf = Y.L, young petiole = Y.P, young stem = Y.S, old leaf = O.L, old petiole = O.P and old stem = O.S).

	K^+/Na^+ (Y.L)		K^+/Na^+ (Y.P)		K^+/Na^+ (Y.S)		K^+/Na^+ (O.L)		K^+/Na^+ (O.P)		K^+/Na^+ (O.S)	
Genotypes	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**
CIM 448	16±14	5±4	41±12	18±9	46±39	14±13	8±17	3±2	27±12	12±1	15±7	4±2
Dhandra	18±11	3±18	47±22	12±11	45±19	8±9	8±10	2±13	11±14	3±9	10±6	2±1
Dhandra x S6	23±6	2±0	47±16	8±2	39±14	8±2	9±6	2±1	24±13	5±3	13±6	3±21
DNH 40	30±32	6±13	49±15	7±9	13±22	9±30	7±7	3±1	22±4	11±4	16±9	5±1
DNH 40 x CIM 448	21±11	3±3	53±19	9±2	36±14	10±4	10±11	3±3	30±10	11±3	31±18	4±3
DNH 40 x S6	32±10	3±4	39±25	8±34	46±21	11±9	12±9	2±3	28±13	7±3	16±2	3±6
G. stocksii	41±5	17±17	52±5	3±4	63±11	8±3	35±9	26±15	56±23	6±16	24±26	1±2
G. stocksii x G. hirsutum	13±15	2±1	40±30	9±9	35±20	12±9	5±6	2±1	17±8	6±1	23±7	5±2
G.hirsutum x G. sturtianum	27±17	10±14	47±11	18±4	42±17	8±5	23±3	7±3	33±10	11±5	18±15	4±2
MNH 147	10±8	6±4	36±20	12±18	41±15	10±7	5±7	2±1	26±6	8±2	21±7	3±1
MNH 147 x CIM 448	17±5	2±2	30±15	13±2	38±15	14±6	11±0	1±2	29±16	7±3	15±5	2±2
MNH 147 x S6	18±8	2±2	41±28	9±5	54±12	12±11	14±2	3±1	22±8	9±2	24±8	5±3
NIAB 313	20±6	4±4	38±7	13±6	64±35	7±4	10±2	2±1	21±11	10±4	12±15	4±1
NIAB 313 x DNH 40	19±10	5±3	44±11	13±4	51±28	14±26	4±4	2±2	22±9	10±2	22±0	4±4
S6 x Dhandra	30±32	2±1	43±14	15±4	55±17	16±5	14±5	4±3	24±10	7±2	17±7	2±0
S6 x DNH 40	32±11	2±4	41±25	33±58	48±13	7±33	16±7	2±2	25±13	5±4	13±6	3±2

Table 12. Means and standard deviation of K^+Na^+ ratio in young and old leaf, petiole and stem of cotton genotypes treated with 250 mMNaCl. (young leaf = Y.L, young petiole = Y.P, young stem = Y.S, old leaf = O.L, old petiole = O.P and old stem = O.S).

	K^+/Na^+ (Y.L)		K^+/Na^+ (Y.P)		K^+/Na^+ (Y.S)		K^+/Na^+ (O.L)		K^+/Na^+ (O.P)		K^+/Na^+ (O.S)	
Genotypes	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**
CIM 448	21±4	1±2	29±9	2±2	35±16	2±2	9±13	1±0	20±12	3±1	10±4	3±2
Dhandra	19±7	1±3	34±16	5±5	41±16	4±1	12±1	1±1	30±10	4±2	10±3	1±0
Dhandra x S6	26±7	1±0	40±10	5±1	42±13	2±1	8±3	1±0	16±8	4±1	6±3	2±0
DNH 40	22±12	3±2	36±18	3±1	44±53	2±1	10±4	1±0	19±12	4±0	14±2	4±2
DNH 40 x CIM 448	17±7	2±4	34±23	3±4	24±19	3±2	7±7	1±0	20±9	4±2	8±5	4±3
DNH 40 x S6	18±6	1±0	38±20	4±3	28±14	3±2	7±7	0±0	18±5	3±1	7±4	2±1
G. stocksii	38±10	6±14	43±13	1±6	50±37	1±2	22±2	10±13	25±19	3±1	26±5	0±0
G. stocksii x G. hirsutum	19±12	2±2	27±6	3±1	29±5	4±3	8±5	1±0	14±6	3±2	12±5	6±5
G.hirsutum x G. sturtianum	36±6	3±3	27±17	3±2	34±12	2±1	14±25	2±1	21±6	3±1	11±6	2±1
MNH 147	29±22	2±2	33±42	4±1	41±43	3±3	11±8	0±0	21±9	3±1	19±28	4±4
MNH 147 x CIM 448	21±10	5±5	48±28	5±2	26±5	5±2	12±10	1±2	20±5	3±1	12±4	3±2
MNH 147 x S6	25±10	3±4	28±11	3±1	31±17	3±1	10±5	1±0	22±11	3±1	15±9	3±4
NIAB 313	24±9	1±1	32±4	3±20	36±12	2±1	14±10	1±0	28±7	3±2	10±5	3±2
NIAB 313 x DNH 40	15±5	2±9	30±9	4±2	25±16	3±3	12±7	1±0	26±9	3±1	18±7	3±2
S6 x Dhandra	19±16	1±0	48±21	6±0	33±2	3±1	14±12	1±1	17±10	2±1	7±5	1±1
S6 x DNH 40	24±9	2±2	38±17	8±4	31±18	6±5	12±11	1±1	65±18	2±2	9±8	2±1

Table 13 Correlation of plant height, nodes per plant, fresh and dry weight, Na^+ and K^+ concentration in young and old leaf, petiole and stem. (150mMwithout flood benches). (young leaf = Y.L, young petiole = Y.P, young stem = Y.S, old leaf = O.L, old petiole = O.P and old stem = O.S).

	Plant height	Nodes /plant	FW	DW	Na^+ (O.L)	Na^+ (O.P)	Na^+ (O.S)	Na^+ (Y.L)	Na^+ (Y.P)	Na^+ (Y.S)	K^+ (O.L)	K^+ (O.P)	K^+ (O.S)	K^+ (Y.L)	K^+ (Y.P)	K^+ (Y.S)
Plant height	1	0.055	0.566	0.469	-1.000(*)	-.997(*)	-0.891	-0.424	-0.938	0.196	-1.000(*)	0.864	0.682	-0.943	0.41	0.44
Nodes/plant		1	0.854	0.908	-0.078	0.02	0.404	0.881	0.294	0.99	-0.085	0.551	0.768	0.28	0.933	0.921
FW			1	0.993	-0.585	-0.503	-0.13	0.506	-0.246	0.919	-0.591	0.904	0.989	-0.26	0.984	0.989
DW				1	-0.489	-0.401	-0.017	0.601	-0.134	0.958	-0.495	0.85	0.966	-0.148	.998(*)	.999(*)
Na^+ (O.L)					1	0.995	0.88	0.403	0.93	-0.219	1.000(**)	-0.875	-0.699	0.935	-0.432	-0.461
Na^+ (O.P)						1	0.922	0.491	0.961	-0.123	0.995	-0.824	-0.625	0.965	-0.341	-0.372
Na^+ (O.S)							1	0.789	0.993	0.27	0.877	-0.541	-0.275	0.991	0.048	0.015
Na^+ (Y.L)								1	0.712	0.804	0.397	0.09	0.373	0.701	0.651	0.626
Na^+ (Y.P)									1	0.155	0.927	-0.636	-0.386	1.000(**)	-0.069	-0.102
Na^+ (Y.S)										1	-0.226	0.664	0.851	0.141	0.975	0.967
K^+ (O.L)											1	-0.878	-0.703	0.933	-0.437	-0.467
K^+ (O.P)												1	0.958	-0.647	0.814	0.833
K^+ (O.S)													1	-0.4	0.947	0.957
K^+ (Y.L)														1	-0.084	-0.117
K^+ (Y.P)															1	.999(*)
K^+ (Y.S)																1

Table 14 Correlation of K^+/Na^+ ratio in young and old leaf, petiole and stem. (150mM without flood benches). (young leaf = Y.L, young petiole = Y.P, young stem = Y.S, old leaf = O.L, old petiole = O.P and old stem = O.S).

	K^+/Na^+ (O.L)	K+/Na^+ (O.P)	K^+/Na^+ (O.S)	K^+/Na^+ (Y.L)	K^+/Na^+ (Y.P)	K^+/Na^+ (Y.S)
Plant height	0.887	0.888	0.773	-0.892	0.97	-0.469
Nodes/plant	0.509	0.507	-0.59	-0.5	-0.189	-0.908
FW	0.883	0.882	-0.084	-0.877	0.349	-0.993
DW	0.823	0.822	-0.197	-0.817	0.24	-1.000(**)
Na^+ (O.L)	-0.898	-0.899	-0.758	0.903	-0.964	0.489
Na^+ (O.P)	-0.85	-0.852	-0.819	0.856	-0.985	0.401
Na^+ (O.S)	-0.581	-0.583	-0.977	0.59	-0.975	0.017
Na^+ (Y.L)	0.041	0.039	-0.902	-0.03	-0.632	-0.601
Na^+ (Y.P)	-0.673	-0.674	-0.945	0.681	-0.994	0.134
Na^+ (Y.S)	0.627	0.625	-0.469	-0.618	-0.047	-0.958
K^+ (O.L)	-0.901	-0.902	-0.754	0.905	-0.962	0.495
K^+ (O.P)	.999(*)	.999(*)	0.349	-.998(*)	0.716	-0.85
K^+ (O.S)	0.942	0.942	0.064	-0.939	0.484	-0.966
K^+ (Y.L)	-0.683	-0.685	-0.94	0.691	-0.996	0.148
K^+ (Y.P)	0.785	0.783	-0.26	-0.778	0.177	-.998(*)
K^+ (Y.S)	0.805	0.803	-0.228	-0.798	0.209	-.999(*)
K^+/Na^+ (O.L)	1	1.000(**)	0.394	-1.000(**)	0.749	-0.823
K+/Na^+ (O.P)		1	0.396	-1.000(**)	0.75	-0.822
K^+/Na^+ (O.S)			1	-0.404	0.904	0.197
K^+/Na^+ (Y.L)				1	-0.756	0.817
K^+/Na^+ (Y.P)					1	-0.24
K^+/Na^+ (Y.S)						1

Table 15. Means and standard deviation of plant height, nodes per plant, fresh weight and dry weight of cotton genotypes treated with 150mMwithout flood benches)

	Plant height (mm)		Nodes per plant		FW (g)		DW (g)	
Genotypes	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**
CIM 448	669±170	592±85	14.6±2.1	9.7±1.6	42±10.2	18.7±3.2	6.8±2	4.5±0.8
MNH 147	587±180	567±146	10.7±3.8	9.3±2.7	25±13.4	13.9±6.7	4±2.5	3.3±1.7
MNH 147 x CIM 448	646±232	602±60	14±1.8	9.2±1.6	41±13.8	15.6±4.	6.5±2.5	3.6±1.3

Table 16. Means and standard deviation of plant Na^+ concentration in young and old leaf, petiole and stem of cotton genotypes treated with 150mMwithout flood benches). (young leaf = Y.L, young petiole = Y.P, young stem = Y.S, old leaf = O.L, old petiole = O.P and old stem = O.S).

	Na^+ (Y.L)		Na^+ (Y.P)		Na^+ (Y.S)		Na^+ (O.L)		Na^+ (O.P)		Na^+ (O.S)	
Genotypes	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**
CIM 448	7.8±1.6	218±111	8.1±3.8	149±41	9.9±4.4	212±54	11±6.6	91.3±88	11.7±4	110.6±67	18±7.4	120±60
MNH 147	10±4.9	206±126	9.9±4	169±72	37±51	155±73	19±24	158±111	12.9±10	134.1±62	18±6.2	126±71
MNH 147 x CIM 448	10±4.9	184±46	7.8±3.3	121±38	9.8±3.2	152±43	11±6.6	67.6±44	8.6±3.2	97.9±35	14±3	105±86

Table 17. Means and standard deviation K^+ concentration in young and old leaf, petiole and stem of cotton genotypes treated with 150mMwithout flood benches). (young leaf = Y.L, young petiole = Y.P, young stem = Y.S, old leaf = O.L, old petiole = O.P and old stem = O.S).

	K^+ (Y.L)		K^+ (Y.P)		K^+ (Y.S)		K^+ (O.L)		K^+ (O.P)		K^+ (O.S)	
Genotypes	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**
CIM 448	179±32	215±62	336±85	459±63	266±52	383±52	113±21	196±51	349±73	457.4±85	205±32	282±66
MNH 147	195±57	233±63	323±97	421±92	332±94	358±84	110±29	229±36	318±49	356.1±143	241±42	279±128
MNH 147 x CIM 448	180±35	190±49	336±84	428±59	281±49	364±56	122±60	185±82	285±36	431.4±66	197±27	280±105

Table 18. Means and standard deviation of K^+/Na^+ ratio in young and old leaf, petiole and stem of cotton genotypes treated with 150mM without flood benches). (young leaf = Y.L, young petiole = Y.P, young stem = Y.S, old leaf = O.L, old petiole = O.P and old stem = O.S).

	K^+/Na^+ (Y.L)		K^+/Na^+ (Y.P)		K^+/Na^+ (Y.S)		K^+/Na^+ (O.L)		K^+/Na^+ (O.P)		K^+/Na^+ (O.S)	
Genotypes	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**	**Control**	**Salt**
CIM 448	23±4.2	1.1±0.4	46.5±15.0	3.3±1.0	30±9.5	2±0.8	12±4.3	3.7±2.2	32.2±1	5.3±2.7	13±4.2	2.8±1.3
MNH 147	22±10	1.6±1.0	36.2±15.7	2.7±0.9	28±22.0	2.8±1.2	9.7±5.3	1.8±0.8	31.9±1	2.9±1.0	14±3.8	2.7±1.6
MNH 147 x CIM 448	22±10	1.2±0.6	47.6±14.0	3.9±1.3	30±6.6	2.6±1.0	12±4.1	3.3±1.3	35±7.0	4.8±1.4	14±2.9	3.8±2.1

Table 19 A binary data matrix was constructed on the bases of presence or absence of peaks. A "1" represents the presence of the allele and a "0" its absence. A "-"indicates the variety was not tested.

	BNL 1434					MGHES 13				MGHES 25		NAU 1195		BNL1694		Gh 137		MGHES 21				MGHES 21
	M1	M2	M3	M4	M5	M1	M2	M3	M4	M1	M2	M1	M2	M1	M2	M1	M2	M1	M2	M3	M4	M1
	225	266	258	323	354	200	211	254	158	221	159	219	227	259	287	242	258	199	221	260	354	269
CIM 448	0	0	1	0	0	0	1	1	0	1	1	1	1	1	1	-	-	1	1	0	0	1
MNH 147	1	0	1	1	1	0	1	1	0	-	-	-	-	-	-	1	1	0	1	0	0	-
Dandera	0	0	1	0	1	0	1	1	1	-	-	-	-	-	-	-	-	0	1	0	0	-
Karishma	0	1	1	0	0	0	0	1	0	-	-	-	-	-	-	-	-	0	1	0	0	-
S12	0	0	1	0	0	0	0	1	0	-	-	-	-	-	-	-	-	0	1	0	0	-
Rehmani	0	1	1	0	1	0	1	0	0	-	-	-	-	-	-	-	-	0	1	1	1	-
NIAB 78	0	1	1	0	0	1	1	0	0	-	-	-	-	-	-	-	-	0	1	1	1	-
CIM 1100	0	0	1	0	0	0	1	0	0	-	-	-	-	-	-	-	-	0	1	0	0	-
Coker 201	0	1	1	0	1	0	0	1	0	-	-	-	-	-	-	-	-	0	1	1	1	-
DNH 40	0	0	1	0	0	0	1	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-
G. stocksii* x *G. hirsutum	0	0	0	0	1	-	-	-	-	-	-	-	-	-	-	-	-	0	1	1	0	-
Qalandari	0	1	0	0	1	-	-	-	-	-	-	-	-	-	-	-	-	0	1	0	0	-
Sarmast	0	0	1	0	1	0	0	1	0	-	-	-	-	-	-	-	-	0	1	0	0	-
Allepo 45	0	0	1	0	1	0	0	1	0	-	-	-	-	-	-	-	-	-	-	-	-	-
Acalla 1517	0	0	1	0	1	1	0	1	0	-	-	-	-	-	-	-	-	0	1	0	1	-
B 496	0	1	1	0	1	-	-	-	-	-	-	-	-	-	-	-	-	0	1	0	1	-
MNH 93	1	0	1	0	0	0	0	1	0	-	-	-	-	-	-	-	-	0	1	0	1	-
CIM 443	0	0	1	1	1	0	0	1	0	-	-	-	-	-	-	-	-	0	1	0	0	-
G. stocksii	0	0	1	0	0	0	0	1	0	-	-	-	-	-	-	-	-	0	1	1	0	-
NIAB 313	0	0	1	0	0	0	1	1	0	-	-	-	-	-	-	-	-	0	1	0	0	-
Coker 312	0	0	1	0	0	0	0	1	0	-	-	-	-	-	-	-	-	0	1	0	0	-
B.P 53-62	0	0	1	0	0	0	0	1	0	-	-	-	-	-	-	-	-	-	-	-	-	-
B 765	0	1	1	0	0	0	0	1	0	-	-	-	-	-	-	-	-	0	1	0	0	-
Mc. Nair 220	0	0	1	0	0	0	0	1	0	-	-	-	-	-	-	-	-	-	-	-	-	-
CIM 109	-	-	-	-	-	0	0	1	0	-	-	-	-	-	-	-	-	-	-	-	-	-
Prococe 1	-	-	-	-	-	0	0	1	0	-	-	-	-	-	-	-	-	-	-	-	-	-
DNH 40 X CIM448	0	0	1	0	0	0	0	1	0	-	-	-	-	-	-	-	-	-	-	-	-	-
S 6 x DNH 40	0	0	1	0	1	0	0	1	1	-	-	-	-	-	-	-	-	0	1	1	0	-

Continue	NAU 2748		NAU 3232		NAU2777				BNL 1878			BNL 1679			MGHES 31		BNL 1681	MGHES 29	NAU 920			
	M1	M2	M1	M2	M1	M2	M3	M4	M1	M2	M3	M1	M2	M3	M1	M2	M1	M1	M1	M2	M3	M4
	199	205	192	187	226	250	104	185	189	214	308	162	181	103	219	258	278	229	211	354	258	158
CIM 448	1	0	1	1	1	0	0	0	1	1	1	1	1	0	1	0	1	1	1	0	0	1
MNH 147	-	-	1	1	1	0	0	0	1	1	0	0	0	1	1	1	-	-	1	0	0	0
Dandera	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0	0	1
Karishma	1	0	0	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0	0	0
S12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0	0	0
Rehmani	1	0	0	1	0	0	1	1	-	-	-	-	-	-	-	-	-	-	1	0	0	0
NIAB 78	-	-		-	0	0	1	1	-	-	-	-	-	-	-	-	-	-	1	0	0	0
CIM 1100	1	0	0	1	0	1	0	1	-	-	-	-	-	-	-	-	-	-	1	0	0	0
Coker 201	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	0	0
DNH 40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0	0	0
G. stocksii* x *G. hirsutum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0	1	0
Qalandari	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	0	0
Sarmast	1	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	0	0
Allepo 45	1	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	0	0
Acalla 1517	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	0	0
B 496	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0	0	0
MNH 93	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	1	0
CIM 443	1	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0	0	0
G. stocksii	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0	0	0
NIAB 313	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	0	0
Coker 312	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0	0	0
B.P 53-62	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	0	0
B 765	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	0	0
Mc. Nair 220	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0	0	0
CIM 109	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0	0	0
Prococe 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	0	0
DNH 40 X CIM448	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0	0	0
S 6 x DNH 40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0	1	1