

Genetics of salt stress tolerance in upland cotton (*Gossypium hirsutum* L.)

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Soil salinity is a great threat to cotton production worldwide. Soil salinity stress reduces plant growth mainly through three mechanisms osmotically induced water stress, specific ion toxicity due to high concentration of sodium and chloride ions, and nutrient ion imbalance due to high level of Na⁺ and Cl⁻ which reduces the uptake of K⁺, NO⁻, PO₄. Plant adaptation to environmental stresses involves the expression of specific stress-related genes. Consequently, engineering genes that protect and maintain the function and structure of cellular components can enhance tolerance to salinity stress. Engineered cotton plants have been reported to perform much better than their wildtypes either in greenhouse or field conditions under salinity stress. However, engineered cotton with improved salt-tolerance is still far behind the requirements of commercial production due to its limited salinity tolerance or poor agronomic performance. This review highlights recent advances in genetic improvement, particularly molecular breeding for salinity tolerance of cotton. It is suggested that future research should focus on the development of specific cotton cultivars with high salt tolerance through a combination of traditional breeding and molecular technology.

Keywords: *Gossypium hirsutum*; salt; breeding; genetic

I. INTRODUCTION

Cotton, a woody perennial shrub, is taxonomically classified within the family Malvaceae and genus *Gossypium*. The genus *Gossypium* includes approximately 50 species split across two ploidy levels, 45 diploid ($2n=2x=26$) and at least five allotetraploid ($2n=4x=52$) (Fryxell, 1992; Brubaker *et al.*, 1999), whose aggregate geographic ranges encompasses most tropical and subtropical regions of the world (Sauer, 1993). Cotton is major fiber crop grown in Pakistan. It is mainly grown in Sindh and Punjab provinces where climate is hot (Ahmad and Makhadm, 1992). In addition, cotton is the second important oilseed crop in the world after soybeans (Khan, *et al.*, 2002). In the economy of Pakistan agriculture adds nearly 21% to national income (GDP). It is reported that share of cotton to the value added in agriculture is 8.6 % and about 1.8 to GDP (Anonymous, 2012). In Pakistan, due to continuous use of low quality irrigation water for agriculture purpose, about 5.7×10^6 ha of arable land had been rendered saline (Mujtaba *et al.*, 2003). The problem of soil salinity is of frequent occurrence in arid and semi-arid regions (Ashraf and Fatima, 1994; Lin *et al.*, 1997; Khan *et al.*, 2001). Salinity in topsoil and subsoil is one of the major abiotic environmental stresses to crop production (Grewal, 2010). Worldwide, soil salinity is becoming a serious threat to agricultural productivity (Cha-um *et al.*, 2006). Nearly 10% of the total land surface is covered with different types of salt-affected soils. At present, there are nearly 954 million hectares of saline soils on the earth's surface. All these salt affected soils are distributed throughout the world. More than 80 million hectares of such soils are in Africa, 50 million hectares in Europe, 357 million hectares in Australasia, nearly 147 million hectares in Central, North and South America. Similarly, a large bulk of about 320 million hectares and land in South and South East Asia is under the grip of salinity. The total geographical areas of Pakistan is 80.0 million hectares or 197.0 million acres, with a very good canal irrigated system of about 62,400 km long and mainly confined to Indus plain covering an area of 19.43 million hectares (48 million acres). The salt affected soils are mainly situated in this plain. In Pakistan, about 6.30 million hectares of land are salt-affected and of which 1.89 hectare is saline, 1.85 million hectare is permeable saline-sodic, 1.02 million hectare is impermeable saline-sodic and 0.028 million hectare is sodic in nature. It is estimated that out of 1.89 million hectares saline patches, 0.45 million hectares present in Punjab, 0.94 million hectares in Sindh and 0.5 million hectares in NWFP. Out of 19.3 mha area available for farming, irrigated agriculture is practiced on about 16 mha. The irrigation water is mainly supplied through the world's largest canal system arranged through dams. Intensive and continuous use of surface irrigation has altered the hydrological balance of the irrigated areas, especially Indus basin. The substantial rise in the water table has caused salinity and water logging in large areas of Sindh, Punjab, NWFP and Baluchistan. The magnitude of the

problem can be gauged from the fact that the area of productive land was being damaged by salinity at a rate of about 40000 hectares annually. Salinity is estimated to affect 20 percent irrigated area of Pakistan, ultimately making land production less (AWD 2013). The report states that there are signs of growing water scarcity and environmental stress in large parts of important cultivated areas in Asia. Groundwater levels are falling in northern India, Pakistan, and the northern plains of the People's Republic of China (PRC). With significant lack of water, deterioration of water quality will limit water use, threatening the ecosystems, and creating a drag on socio-economic development. Productive economies are sensitive to water quality. Irrigation-induced Salinization reduces productivity and can ultimately take land out of production. The salinity across the globe has also affected 7 percent of total world land and 33 percent of irrigated land. This was stated by speakers at a seminar titled "Soil Quality for Food Security" arranged by the Institute of Soil and Environment Sciences, University of Agriculture, Faisalabad, at New Senate Hall.

II. EFFECTS OF SALINITY STRESS ON PLANT GROWTH AND DEVELOPMENT

Understanding the effects of salinity stress on cotton growth and physiology is a prerequisite to successful salinity management and control. Salinity effects on cotton plant may vary with the growth stage and extent and time of salinity stress. Biological or economic yield reduction is the main effect of salinity at the whole-plant level, and is usually attributed to various physiological and biochemical processes at the cellular or molecular levels (Meloniet *et al.*, 2003; Nawaz *et al.*, 2010; Munns and Tester, 2008).

III. PHYSIOLOGICAL RESPONSE TO SALINITY STRESS

Soil salinity stress reduces plant growth mainly through three mechanisms osmotically induced water stress, specific ion toxicity due to high concentration of sodium and chloride ions, and nutrient ion imbalance due to high level of Na⁺ and Cl⁻ which reduces the uptake of K⁺, NO₃⁻, PO₄³⁻, etc (Greenway and Munns, 1980). Alteration of cell wall extensibility (Pritchard *et al.*, 1991) and accumulation of salt in the apoplast (Flowers *et al.*, 1991) were also reported as possible causes. In saline soils (≥ 4 dS/m at 25°C) (Richards, 1954), too much salt in the soil environment makes the soil solution maintain such a low osmotic potential that cotton cannot take up enough water, which leads to osmotic or water-deficit effect of salinity stress (Khan *et al.*, 2004). Cotton plants, however, can accumulate compatible solutes or osmoprotectants, which serve as nontoxic solutes for cytoplasmic osmoregulation to cope with the low osmotic potential in the soil (Zhang *et al.*, 2009). Both cellular and metabolic processes involved in osmotic stress due to salinity are similar to those due to drought. The rate at which new leaves are produced depends largely on the water potential of the soil solution, in the same way as for a drought-stressed plant (Nawaz *et al.*, 2010). Reductions in the rate of leaf and root growth are probably due to factors associated with water stress (Munns, 2002). The degree of growth inhibition due to osmotic stress depends on the time and scale of the response, the particular tissue and species in question, and whether the stress treatments are imposed abruptly or slowly (Ashraf, 1994; Munns *et al.*, 2002). There were considerable similarities and moderate differences in mechanisms of photosynthetic inhibition between the two kinds of stress (Tang *et al.*, 2007). Transcript-profiling of plants subjected to drought and salt suggest that plants perceive and respond to these stresses by quickly altering gene expression in parallel with physiological and biochemical alterations; this occurs even under mild to moderate stress. It is apparent that both stresses lead to down-regulation of some photosynthetic genes, with most of the changes being small, possibly reflecting the mild stress imposed. When compared with drought, salt stress affected more genes and more intensely, possibly reflecting the combined effects of dehydration and osmotic stress in salt-stressed plants (Chaves *et al.*, 2009). Toxicity occurs as a result of uptake and accumulation of certain toxic ions from the saline soil or irrigation water. These toxic constituents include mainly sodium, chloride and sulphate. Although chloride and sulphate are essential elements, their content in the saline soil is far more than required for normal growth of plants. They can reduce crop productivity and eventually cause crop failures (Nawaz *et al.*, 2010). The salt taken up by plants concentrates in the old leaves (Munns, 2002); continued transport of salt into transpiring leaves over a long period of time eventually results in very high Na⁺ and Cl⁻ concentrations, and the leaves die.

IV. CAUSES OF SALT ACCUMULATION

The location of Pakistan is in arid and semi-arid climatic zones. Generally high evapo-transpiration in semi-arid and arid zones is the basic cause for salt accumulation on the soil surface. The average summer temperature is about 40°C and the minimum winter temperature remains between 2°C to 5°C. The annual rainfall varies between 100 mm to 700 mm throughout the country. The evaporation rate is generally very high and exceeds that of precipitation. Thus, the insufficient rainfall followed by high evaporative demand and shallow ground water depth, enhances the movement of salts towards soil surface. Salinity is an important problem affecting irrigated agriculture of Pakistan. Improper irrigation practices and lack of drainage have generally led to accumulation of salts in the soil in concentrations, which are harmful to the crops. There is a major imbalance in the amount of salt entering and leaving the soil in Pakistan. Each year about 120 million

tonnes of salts are added to the land in canal water and brackish underground water. Only about 1/5th of this salt finds its ways to the sea. The remainder accumulates in the soil; it continues to decrease the growth and survival of crops. The soils with electrical conductivity of less than 4 dSm⁻¹ are generally considered as salts free, where almost all crops can be grown. As the salt concentration increases, the choice becomes limited and one has to go for tolerant plants suited for specific conditions. Soil salinity has been a major concern to global agriculture throughout human history (Lobell *et al.*, 2007). Currently over 800 million hectares of land throughout the world are salt-affected (FAO, 2005). This is over 6% of the world's total land area (Munns, 2005). In recent times, it has become even more prevalent as the intensity of land use increases globally (Egamberdieva *et al.*, 2010). Cotton is an important cash crop worldwide. Although it is classified as one of the most salt-tolerant major crops and considered a pioneer crop in reclamation of saline soils (Maas, 1990), its growth and development as well as yield and fiber quality are negatively affected by excessive salts in the soil (Maas and Hoffman, 1977; Qadir and Shams, 1997; Higbie *et al.*, 2010). Soil salinity is usually expressed by EC_e (electrical conductivity of a saturated-paste extract), and EC_es around 7.7, 12, and 17 dS m⁻¹ are referred to as low, moderate and high salinity level, respectively (Chen *et al.*, 2010; Maas and Grattan, 1999).

Classification of Soils on the basis of Salinity

Salinity/Sodicity Class	Criteria
Non-Saline Non-Sodic (NS-NS)	EC _e < 4; SAR < 13.
Saline Non-Sodic (S-NS)	EC _e > 4; SAR < 13.
Saline Sodic (S-S)	EC _e > 4; SAR > 13.
Non-Saline Sodic (NS-S)	EC _e < 4; SAR > 13.

V. SELECTION FOR SALT TOLERANCE

The use of cotton varieties tolerant to higher soil salinity levels is one of the approaches to control salinity stress effects on cotton. Therefore, identification and screening of salt-tolerant cotton cultivars or germplasm is of great importance (Asharf, 1994). There are two common screening methods for salt tolerance, one being the seedling-based, solution culture method, the other being plant yield-based, soil culture method (Khan *et al.*, 1995). By comparing the two methods, Akhtar *et al.* (2010) concluded that solution culture based on seedling was as accurate as but more simple than soil culture. However, identification and evaluation of salt tolerant plants based on plant biomass or yield, whether in laboratory or field conditions, is a cumbersome work. Therefore, a number of physiological indicators have been recommended for identification and evaluation of salt tolerance. The K⁺/Na⁺ ratio has been used as a successful criterion for selection of salt tolerance in cotton (Akhtar *et al.*, 2010). Muniset *et al.* (2010) indicated that Na⁺ and K⁺ content, decrease in leaf area, seed germination and seedling growth were the best indicators of salinity for cotton cultivar selection, but proline and chlorophyll concentrations were not useful for accurate assessment of salinity tolerance. Sexton and Gerard (1982) measured the emergence force exerted by germinating cotton seedlings using a transducer. Increasing salinity reduced the emergence force and increased the time required to develop the maximum force. Gorham *et al.* (2009) suggested that such tests could be used to determine the salt tolerance of emerging seedlings. Germination of pollen grains has also been used to screen for salt tolerance (Shen *et al.*, 1997). Salt tolerance varies with cotton genotypes. Some wild upland cotton and wild species have relatively strong salt tolerance (Ye and Liu, 1998). Development of salt-tolerant cotton varieties was initially attempted through direct selection from the existing cotton varieties, strains, and germplasms. After extensive research, however, it was considered that the probability of success by direct selection or screening was very low, particularly in the current commercial cotton varieties (Ye and Liu, 1998).

VI. MOLECULAR BREEDING FOR SALT TOLERANCE

During the 1980s and 90s, the use of tissue culture to screen salt-tolerant mutants held great promise (Das *et al.*, 1990, 1992; Misra *et al.*, 1990, 1995). It was once considered an effective way to develop salt-tolerant varieties and lines directly (Das *et al.*, 1990, 1992; Zhang *et al.*, 1995; Misra *et al.*, 1990, 1995). Although molecular breeding approaches, including transgenic modification and quantitative trait mapping with marker-assisted selection, have shown some success and will continue to increase our understanding of the complexity of plant's physiological pathways (Lubbers *et al.*, 2007), current transgenic cotton with improved salt-tolerance ability is still far behind the requirements for commercial production (Zhang *et al.*, 2009). However, studies on salt-tolerance screening stagnated only at the salt tolerant callus stage (Das *et al.*, 1990, 1992; Misra *et al.*, 1990, 1995), and did not yield somatic embryos and plantlets with increased salt tolerance. This is due to the great difficulty in plant regeneration in tissue culture under salinity stress. The regenerative capacity of callus may be decreased or even lost, and the embryogenic would also be transformed to non-embryogenic callus due to salt stress. Thus, even if the salt-tolerant cell line or salt-tolerant callus was obtained, it is still difficult to obtain salt-

tolerant plantlets. At the same time, direct introduction of exogenous DNA technology also received great attention for salt-tolerance breeding of cotton. It was reported that exogenous dogbane (*Apocynum venetum*) DNA with salt resistance were introduced into the cotton ovaries after self-pollination by pollen tube pathway (Shen *et al.*, 1995).

VII. GENES CONTROLLING SALT TOLERANCE

A number of genes are involved in regulation of plant response to salinity stress in cotton. Rodriguez-Urabea *et al.* (2011) identified 720 salt-responsive genes in an intraspecific backcross population of cotton, of which 695 were down-regulated and only 25 were up-regulated in the salt tolerant bulk. Gene ontology of annotated genes revealed that at least some of the identified salt responsive transcripts belong to pathways known to be associated with salt stress, including osmolyte and lipid metabolism, cell wall structure, and membrane synthesis. Rodriguez-Urabea *et al.* (2011) suggested that further analysis of these salt-responsive genes might assist in laying a foundation for molecular manipulation in development of new cotton cultivars with improved salt tolerance. A cotton cDNA clone, *GhNHX1*, which showed high sequence identity with plant vacuolar-type Na⁺/H⁺ antiporters was isolated via differential hybridization in response to salinity stress in cotton seedlings by Wu *et al.* (2004). Northern blot analysis showed that mRNA accumulation of *GhNHX1* in cotton seedlings was strongly induced by salt stress. *GhNHX1* activity in a mutant for yeast tonoplast Na⁺/H⁺ antiporter showed function complementation, thereby proving that the antiporter is in the vacuolar membrane. Transgenic tobacco plants over-expressing *GhNHX1* had higher salt tolerance than the wild-type plants. Xue *et al.* (2009) isolated a cDNA clone (*GhMT3a*) encoding a 64-amino acid type 3 metallothionein protein from cotton (*Gossypium hirsutum*). Northern blot analysis indicated that mRNA accumulation of *GhMT3a* was upregulated by high salinity.

Quantitative RT-PCR and Northern blot analyses revealed that mRNA accumulation in both *GhDi19-1* and *GhDi19-2* was significantly promoted by salinity. Overexpression of *GhDi19-1* and *GhDi19-2* in Arabidopsis resulted in seedlings exhibiting hypersensitivity to high salinity. Seed germination and seedling growth of the transgenic Arabidopsis were dramatically inhibited by salinity and ABA, compared with the wild type. Both *GhDi19-1* and *GhDi19-2* may be involved in response to salt/drought stress and ABA signaling during early stages of plant development (Li *et al.*, 2010). The research that has been conducted in Pakistan over the last decade shows that there is a wide range of plant species with varying levels of salt tolerance that can be used in saline agriculture. Furthermore, at least some of these plants are able to lower local water-tables, improving the condition of the land, and acting as 'biological pumps'.

Salinity affects the germination, growth and ultimately the yield of cotton (*Gossypium hirsutum* L.) which demands reliable traits for the evaluation and selection of salt tolerant cultivars. Cotton is a major crop widely grown in more than 80 countries for fiber and oil purposes. It is placed in moderately salt tolerant group of plant species (Ashraf, 2002). Under field conditions, saline soils can induce a stunted growth of cotton. Leaves of salt affected cotton plants are smaller and dark blue green in color than the normal leaves and the plants exhibit appearances similar to moisture stress conditions. According to a current survey, more than 800 million hectares of land throughout the world are salt affected (Anon., 2008). Salinity affects plant metabolism by disturbing physiological and biochemical processes of plants due to ionic and osmotic imbalances which results in the reduction of plant growth and productivity (Munns, 2005). Plants can protect themselves from salt toxicity by maintaining higher K⁺ content and K⁺/Na⁺ ratio (Khan *et al.*, 2009) and/or organic solutes (Rodriguez *et al.*, 1997). Salt tolerance has also been reported to be directly related with the accumulation of proline (Munns and Tester, 2007). The impact of salt stress has also been correlated with some morphological and physiological traits like reduction in fresh and dry weight (Chartzoulakis and Klapaki, 2000), chlorophyll contents (Ziafet *et al.*, 2009) and stomatal closure (Zhu, 2001). Soil salinity has been a major concern to global agriculture throughout human history (Lobell *et al.*, 2007). Soil salinity are usually expressed by ECe (electrical conductivity of a saturated-paste extract), and ECes around 7.7, 12 and 17 dS/m are referred to as low, moderate and high salinity levels, respectively (Chen *et al.*, 2009; Maas and Grattan, 1999). In general, soil salinity delays and reduces germination and emergence, decreases cotton shoot growth, and may finally lead to reduced seed cotton yield and fiber quality at moderate to high salinity levels (Khorsandi and Anaghali, 2009).

VIII. GENES IDENTIFIED IN COTTON AGAINST SALT TOLERANCE

AtNHX1 Arabidopsis Encoding a vacuolar Na⁺/H⁺ antiporter that increases sodium uptake in vacuoles, leading to increased vacuolar solute concentration Improved salt tolerance as well as increased plant biomass and fiber in greenhouse and field under salinity stress (He *et al.* 2005, 2007).

TsVPT hellungiallahalophila A H⁺-PPase gene that causes accumulation of Na⁺ and Cl⁻ in vacuoles. Improved salt tolerance as well as increased plant biomass in greenhouse under salinity stresses (Lv *et al.* 2008).

AVPI Arabidopsis

Encoding a vacuolar pyrophosphatase that functions as a proton pump on the vacuolar membrane improved tolerance of both drought and salt stresses in greenhouse, and increased fibre yield under dry-land conditions in the field (Pasapula *et al.* 2011).

AhCMO*Atriplex hortensis* Encoding Choline monoxygenase (CMO) that increases synthesis of glycine betaine Improved salt tolerance as well as increased plant biomass in greenhouse and yield in field (Zhang *et al.* 2007, 2009).

AnnBj1 Mustard Ca²⁺ dependent, phospholipid and cytoskeleton binding protein Improved tolerance as indicated by enhanced fresh weight, relative water content, and dry weight (Divya *et al.* 2010).

IX. CONCLUSION

Although cotton is classified as a salt tolerant crop, its tolerance of salinity stress is far from that of halophytes. Thus, effective management practices in combating soil salinity stress should be taken to increase cotton yield and fiber quality in saline fields. Since salinity effects on cotton are mainly attributed to osmotic stress, ion toxicity and nutritional imbalance, these effects can be alleviated through two key strategies. One is to improve salinity tolerance of cotton plants, the other is to reduce or avoid high Cl⁻ and/or Na⁺ concentration in the soil medium, at least part of the root zone. Improved salinity tolerance of cotton may be achieved through genetic improvement or enhancing the salt tolerance of seeds or plants by chemical, biological or physical treatments. A decrease in salinity stress can be achieved through the reduction of the salt content in the vadoze zone and creating a more suitable soil environment for cotton growth either with engineering or agronomic practices.

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