

REVIEW

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# Impact of salinity stress on cotton and opportunities for improvement through conventional and biotechnological approaches

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## Abstract

Excess salinity can affect the growth and development of all plants. Salinization jeopardizes agroecosystems, induces oxidative reactions in most cultivated plants and reduces biomass which affects crop yield. Some plants are affected more than others, depending upon their ability to endure the effects of salt stress. Cotton is moderately tolerant to salt stress among cultivated crops. The fundamental tenet of plant breeding is genetic heterogeneity in available germplasm for acquired characteristics. Variation for salinity tolerance enhancing parameters (morphological, physiological and biochemical) is a pre-requisite for the development of salt tolerant cotton germplasm followed by indirect selection or hybridization programs. There has been a limited success in the development of salt tolerant genotypes because this trait depends on several factors, and these factors as well as their interactions are not completely understood. However, advances in biochemical and molecular techniques have made it possible to explore the complexity of salt tolerance through transcriptomic profiling. The focus of this article is to discuss the issue of salt stress in crop plants, how it alters the physiology and morphology of the cotton crop, and breeding strategies for the development of salinity tolerance in cotton germplasm.

**Keywords** Biotechnology, Cotton, Development, Morphological, Physiological, Salinity

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## Introduction

Salinity has exerted its impact on the productivity of field crops around the globe. It is regarded as serious threat for nations with economies based on agriculture. According to an estimate by the Food and Agriculture Organization of the United Nations [1], 8.7% of the world's land (833mha) is effected by salinity and sodicity *i.e.*, presence of a high proportion of sodium salt relative to other salts. The major causes of salinity in Pakistan are poor quality underground water, water logging, fertilizer intensive agriculture and sea water intrusion. The major cause in the disintegration and breakdown of arable land is poor irrigation practices. Published reports have indicated that salinity and sodicity reduce the cultivated land area available for agricultural production [2]. In Pakistan, salinity affects ~18% of the land in the Sindh province, 2% of the land in the Khyber Pakhtun Khawa province, and ~3% of the land in the Punjab province. Both primary and secondary cotton growing areas are present in these provinces. When cotton is grown in salinity affected soils in these provinces, cotton yields are very low. Therefore, it is crucial to take this issue seriously and develop sensible solutions to address salinity and sodicity in soils. This article highlights the effect of salt stress on crop plants in general, how it specifically alters the physiology and morphology of the cotton plant, and the strategies needed to develop salinity tolerance in cotton germplasm.

### Salinity: Hampered plant growth and development

The development and growth of the plants can be hampered by excessive salinity. Depending on a plant's capacity to withstand excessive salt, some plants are impacted more than others [3]. Halophytes are plants that can withstand high soil salinity levels without experiencing a decrease in yield. Glycophytes, on the other hand are salt sensitive plants that exhibit poor performance under excess salt concentrations. Different plants have different levels of tolerance for salt stress. For instance, barley has been reported to tolerate salts up to an 8.5 dSm<sup>-1</sup> threshold level, and soybeans have been reported to tolerate salts up to a 6.2 dSm<sup>-1</sup> threshold level. Onions, however, have been found to be more sensitive to salt, only tolerating it up to 1.4 dSm<sup>-1</sup> threshold level, equal to carrots' 1.6 dSm<sup>-1</sup> threshold level [4, 5]. Cotton is considered as moderately salt tolerant crop among the cultivated crops in Pakistan [6]. Yield is unaffected by salt concentrations up to 7.7 dSm<sup>-1</sup>, but when salt concentrations reach 15 dSm<sup>-1</sup>, yield is reduced by 50% [7]. The quantity and quality of irrigation water, the kind of soil, the amount of irrigation water that evaporates, the temperature and density of the irrigation water, the surface geology, even the influx of salt water from the seas all affect salinity concentration. In Pakistan, majority of the soil is alkaline

with little organic matter, and the subterranean water is unfit for irrigation, which leads to an increase in soil salinity. As the plants roots absorb water from the soil, these high salt concentrations cause physiological disruptions in the plant, including water uptake, water balance, accumulation of some solutes, K<sup>+</sup> uptake, disruption of ionic homeostasis, photosynthesis, respiration, gaseous exchange, transpiration, anatomy and morphology of the plant, and growth pattern [8]. The seedling, vegetative, and reproductive growth phases are effected by excessive salinity. Compared to a mature plant, seedlings are more vulnerable to salinity [9]. Salinity has put a drastic effect on cotton seed germination. The poor germination of seeds in the presence of excess salt can be due to two major phenomena: first, lower osmotic pressure leads to poor water imbibition capacity of seed water, and second, abnormal embryo development due to absorption of injurious ions from the soil. Very high concentrations of salt will inhibit germination [7, 10]. Plant growth is inhibited by salt stress for three reasons: 1) osmotic stress, leads to decrease in water potential in root zone and plants are unable to absorb the water they require [11], 2) specific ionic toxicity, where excessive both chloride and sodium ions are present and absorbed by the plant [12] and 3) nutrient deficiency, where plants are unable to absorb crucial nutrients like potassium, calcium, and nitrates [13]. When compared to crucial elements like K<sup>+</sup> and Ca<sup>++</sup> which are found in salt-affected soil at higher concentrations, Na<sup>+</sup>, Mg<sup>++</sup>, Cl<sup>-</sup>, and SO<sup>4-</sup> are scarce and have a poor translocation rate inside the plant [14]. Sometimes too much sodium has an antagonistic effect and interferes the absorption of potassium and calcium. Under conditions of salt stress, calcium and potassium ions are crucial in the exclusion of Na<sup>+</sup> from plant root cells through the plasma membrane [15]. Alfalfa has been shown to have higher salt tolerance in plants with a 20:1 ratio of Na<sup>+</sup> to Ca<sup>++</sup> [16]. Saline soils with high levels of sodium and chloride ions lead plants to absorb more of these ions, which results in ionic toxicity, notably from Cl<sup>-</sup> ions. Plants' cellular metabolic pathways are also disrupted by sodium and chloride ions [17]. Excessive sodium and chloride ions block the absorption of potassium ions, which are necessary for stomata to open and close and activate the cell's metabolic pathways. The growth of the plants is adversely effected due to absence of K<sup>+</sup> that leads to the disruption of opening and closing of stomata. The high internal sodium and chloride concentration has been linked with reduced plant growth and low yield in many crops like mungbean [18], rice [19], wheat [20] and cotton [21]. Plants show reduced expansion of leaves caused by low water uptake [22]. Exclusion of Na<sup>+</sup> ions occurs through anatomical organs in the plant cells [23].

At the beginning of salt stress, it has been documented that hormonal changes and the induction of reactive oxygen species (ROS) occur [24]. When stomata closure occurs to avoid water losses by transpiration, photosynthetic activities and carbon dioxide uptake tend to decrease. Plants exhibit aberrant root cell development, which causes a reduction in growth and development under salt stress and ultimately results in cell death [25]. The impact of salt stress on plants has wide range depending upon their growth stage. For instance, salt has a major impact on wheat plant growth during the early vegetative stage, but less so throughout the flowering and grain-filling stages [26]. Similarly, upland cotton plants suffer the most damage when they are in the seedling stage, whereas plants in the boll formation stage suffer the least damage [27]. Excessive salt affects the vegetative stage in strawberries, delaying flowering and fruit development [28].

#### Damage to cotton due to salinity

Cotton is considered a moderately salt tolerant crop, but its yield is affected by low and abnormal germination in saline conditions. According to researchers, salinity adversely affects the stages of plant development as well as poor and aberrant plant germination [29]. A greenhouse experiment using potted plants was conducted using sodium chloride and sodium carbonate, and results showed that higher salt stress at the seedling stage disturbed plant morpho-physiological and biochemical attributes [30]. There is evidence to suggest that plant germination and yield decrease as salt stress increases. Additionally, the type of soil had a significant impact [31]. Silt and clay-rich soils had lower germination rates than sandy soils, which had higher germination rates. Salinity in silt and clay soils was thought to have a greater negative impact on the germination of cotton seeds because they have a higher capacity to retain harmful ions than sandy soils [32]. Physiological characteristics were used to assess the salinity tolerance of four genotypes of cotton (*Gossypium hirsutum* L.). Further, the ability of a cotton species to selectively absorb  $K^+$  by the activation of salt overly sensitive (SOS1), serine/threonine protein kinases (AKT1) and high affinity  $K^+$  transporter (HAK5) gene families also contributed to lower effects of salt stress [15]. The salt tolerance ability of diploid cotton species *G. klotzschianum* L. was assessed with the application of 300 mM NaCl for 0, 3, 12 and 48 h on roots. According to physiological research, levels of the hormone abscisic acid (ABA) increased significantly at 48 h while those of  $H_2O_2$  did so at 12 h. However, at 48 h, there was a significant drop in glutathione (GSH) concentration. However, 37,278 unigenes were found in this experiment using transcriptome data, and 8,312 and 6,732 genes showed

differential expression, indicating that they are involved in salt tolerance. According to gene function annotation and expression analyses, maintenance of ion homeostasis by ROS, hormone biosynthesis, and SOS1 gene signal transduction pathways ensures that cotton develops salt tolerance [33].

In order to determine the genetic basis of yield component, fiber quality, stomatal conductance, and plant architecture, a genome wide association study (GWAS) was carried out in cotton [34]. Cotton (*G. hirsutum* L.) genotypes were also explored based on  $Na^+/K^+$  transport in leaves and hypocotyls as well as the relative abundance of salinity tolerant genes (GhSOS1, GhNHX1 and GhAKT1) by using physiological and qPCR assays [14]. It was revealed that the concentration of  $K^+$  was higher than  $Na^+$  in leaves of salt tolerant cotton genotypes compared to salt sensitive genotypes. Further, an abundance of transcripts of GhSOS1 in leaves (controlling the  $Na^+$  ion extrusion and its transport from root to shoot) and GhAKT1 in hypocotyls (helping in  $K^+$  ion absorption and transport) were found in salt tolerant cultivars of rice [35]. An increase was also seen in concentration of the GhNHX1 transcript which encodes vacuole localized NHX protein, but no correlation was found between expression level and salt tolerance [36].

In maize and Arabidopsis, the ABP9 gene induces resistance to abiotic stress. It produces a bZIP transcription factor that binds to abscisic response element (ABRE2) motif of the maizecatalase1 gene. The ABP9 gene was transformed into cotton (*G. hirsutum* L.), and 12 transgenic lines were produced [37]. Under salt stress in the greenhouse, these lines were observed to have strong root systems, increased germination, reduced stomatal aperture, and stomatal diameter.

The ABP9 gene was overexpressed, which improved oxidative stress tolerance and reduced oxidative damage. The ABP9 gene increased the transcription of stress-related genes including GhGBP2, GhNCED2, GhZFP1, GhERF1, and GhHB1 within the transcriptome [38]. Using a principal component analysis, three groups of soybean cultivars-salt tolerant, moderately salt tolerant, and salt sensitive were developed against salinity stress. In comparison to sensitive genotypes and moderately salt tolerant genotypes, the salt tolerant genotypes had higher potassium content in the leaves than sodium as well as enhanced leaf area and water usage efficiency [5]. These results suggested that these parameters may also be used effectively for the validation of salt tolerance in other species of crop plants. Wheat varieties were evaluated for salinity tolerance with applications of potassium and zinc to plant potting medium in the greenhouse. According to the findings, potassium and zinc greatly reduced the severity of salt stress, and salt tolerant

cultivars had greater fresh and dry biomass than control cultivars. Antioxidant enzymes (SOD, CAT, and APX) lose activity when exposed to salt stress, although their activity was noticeably increased in pots treated with zinc and potassium [39]. Other studies have shown that potassium applications can assist plants in overcoming salt stress [9].

### **Breeding strategies for salinity tolerance**

#### **Assessment of upland cotton accessions under salt stress conditions**

##### ***Mediums of plant growth***

Various types of media have been used for the screening of plants under salt stress [6]. Solution culture, hydroponic solution, and sand culture with salt solution were frequently employed as media in different studies [40]. In addition, some researchers have used pot culture with application of salt solution; petri dishes were also used with moistened filter paper containing a brine solution (10% NaCl) [41, 42].

##### ***Concentration of NaCl***

Cotton can yield normally under electrical conductivity up to  $7.7 \text{ dSm}^{-1}$ . To measure the limits of growth under higher salt concentrations, applications of salt stress at  $10 \text{ dSm}^{-1}$ ,  $15 \text{ dSm}^{-1}$  and  $20 \text{ dSm}^{-1}$  have been evaluated [43]. Further screening at a higher intensity of salt stress ( $25 \text{ dSm}^{-1}$ ) has been applied by sowing the cotton crop in saline soil having electrical conductivity of  $36.1 \text{ dSm}^{-1}$  [44, 45]. However, some researchers have employed magnesium sulfate, sodium sulfate, and sodium chloride in various combinations to develop salt stress. NaCl has been widely utilized in screening for salt stress [46].

##### ***Estimation of genetic variability***

The basic concept of plant breeding is to collect and explore the genetic variability for traits that a researcher wants to improve in crop plants [47]. Variation from high to low for parameters that enhance salt stress tolerance is pre-requisite for the selection of parent genotypes that are used in indirect selection or hybridization programs [48, 49]. Scientists have explored genetic diversity in tomato, rice, wheat, sorghum and maize which showed the presence of variation for salt tolerance related traits [50]. The stage of the crop when exposed to salt stress, the intensity of the salt stress and the type of germplasm evaluated by conducting screening experiment all affect how much variation is present for salinity tolerance [51]. In one study, the analysis of variance showed that data was non-significant under a lower level of salt stress, but it was significant when a higher level of salt stress was applied [52]. This literature review has identified that intra-specific and inter-specific variation was found

among various lines of cotton under salt stress [53]. After the confirmation of variations within the crop, three approaches may be used to develop improved germplasm [54]. First, examine the presence of salt tolerance in germplasm and directly grow the lines that are tolerant to salinity [55]. The second approach involves the screening of genetic material (developed from cross pollination or self-pollination or already available accessions) under salt stress, and the genotypes that revealed good performance should be selected for future breeding methods [56]. The final strategy involves the identification of wild relatives that can tolerate salt, so that genes from these relatives can be transferred into present genetic stock for the development of salt tolerance through hybridization and transgenic crop breeding [57].

##### ***Gene action in saline environments***

Salt tolerance mechanism is a complex process and involving multiple genes and interactions between genetic, developmental, and physiological factors [58]. However, there is still some ambiguity because while it has been suggested that crop plants' salt tolerance may be caused by minor genes, other research has found that salt tolerance is controlled by a single dominant gene [59]. In a maize salt tolerance study, difference of presence/absence of a single gene (glycine betaine) between the tolerant and susceptible lines were present [60]. The range of variation in germination percentage data in another study revealed the existence of polygenic inheritance [61]. Exclusion of chloride ( $\text{Cl}^-$ ) from progeny of crosses between cultivars of soybean indicated that a single dominant gene controls the inheritance of  $\text{Cl}^-$  exclusion [62]. In contrast, literature also revealed that exclusion of  $\text{Cl}^-$  from shoots of some woody perennial species was due to polygenic inheritance [63]. Research also revealed the existence of non-additive and additive types of genetic behavior for salinity tolerance in soybean [64], wheat [65], tomato [66] and pearl millet [67]. Salt tolerance was governed by non-additive gene action in maize [68]. In contrast, the salt tolerance phenomenon in sorghum was governed by additive genes, partial dominance and dominance [69–71].

##### **Basis of selection for salt tolerance**

###### ***Statistical models***

The capacity of crop plants to withstand against stress was analyzed by using various statistical models like absolute and relative models that are used as selection criterion for identifying salt tolerant genotypes [72, 73]. A salinity tolerance index has also been employed to differentiate between salt tolerant and sensitive genotypes [74].

## Useful traits for plant selection

### Morphological traits

Plant morphologic parameters such as plant height, root length, shoot length, root weight, shoot weight and seedling vigour traits, including root and shoot length [10], fresh root and shoot weight, dry root and shoot weight, seedling vigor and dry mass are used as selection criteria for salt tolerance [75, 76].

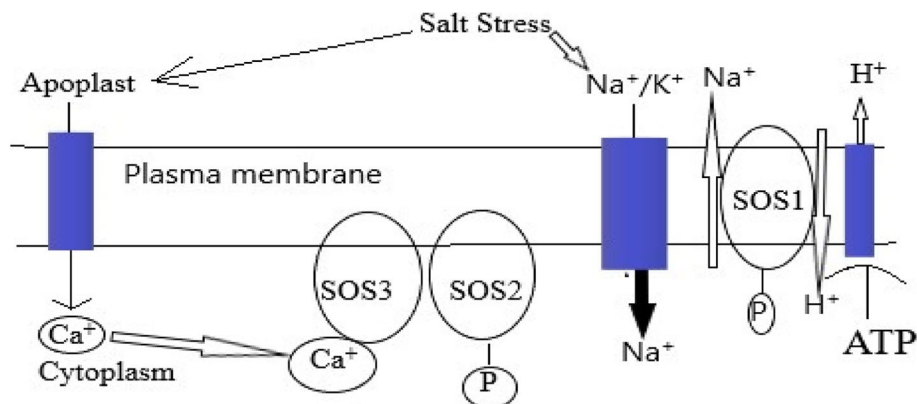
### Physiological and biochemical traits

Numerous physiological markers have been approved for use in genetic screening for salt tolerance. Commonly used parameters in field conditions include stomatal conductance [77], chlorophyll content [78], proline and osmolytes, leakage of electrolytes from leaf discs [20],  $\text{Na}^+$  and  $\text{Cl}^-$  exclusion, and potassium to sodium ratio [17]. Recently, relative leaf water content (RLWC) also was proven to be a good selection criterion for the salt tolerance of cotton [79]. Plants primarily combat oxidative stress using an internal defense system made up of many enzymatic (SOD, CAT, POD, APX, GR, MDHAR, DHAR, GPX, GOPX, GST, AOX, PRXs, TRXs, GRX, etc.) and nonenzymatic antioxidants (AsA, GSH, ascorbic acid, phenolic acids, flavonoids, carotenoids,  $\alpha$ -tocopherol, etc.). Under salt stress conditions,  $\text{H}_2\text{O}_2$  is produced in cells which leads to the formation of ROS. The ROS damages the various organelles of plant cells [80]. The total soluble protein content in plant cells decreases due to the increase in salt concentration.  $\text{H}_2\text{O}_2$  synthesis activates the cell defense system, which results in the production of antioxidants. The production of antioxidants has been used as an effective criterion to identify plants showing salt tolerance. CAT is essential in the detoxification of hydrogen peroxide into water and oxygen [81]. Increased  $\text{H}_2\text{O}_2$  generation initiates a cascade of events that leads to the increased level of SOD within the plant cell. Salt tolerant genotypes showed a higher quantity of SOD under salt stress than sensitive genotypes. It decreased the toxic effect of ROS in plant leaves and root cells. Peroxidase (POD) also served the purpose of detoxifying  $\text{H}_2\text{O}_2$  effects inside of the cytosol, chloroplast, and vacuoles.  $\text{H}_2\text{O}_2$  was likewise transformed into water and molecule oxygen by it [82]. The presence of above mentioned antioxidants are important in regulating plant stress responsive mechanism for scavenging  $\text{H}_2\text{O}_2$  which ultimately reduces the toxic effect of salt in plants. The presence of POD has been found higher in salt tolerant accessions whereas plants susceptible to salt stress have lower quantities of antioxidants [83]. APX is an important enzyme found abundantly in algae and plants [84]. The main function of APX is to scavenge  $\text{H}_2\text{O}_2$  in the cytosol and chloroplasts, while CAT mainly functions in

the peroxisomes. Because APX is more widely distributed and has a significant affinity for  $\text{H}_2\text{O}_2$ , it is more effective as an  $\text{H}_2\text{O}_2$  scavenger under salt stress conditions than CAT. The oxidoreductase class of enzymes includes GR, which requires NADPH as a reducing agent. The cytosol, mitochondria, and chloroplasts contain the majority of the GR isoforms [85]. The GR enzyme protects cells against ROS by converting GSSG to GSH with the complementary oxidation of NADPH and restoration of GSH concentration. GPX is mostly localized in the chloroplast; however, various isoforms are located in the mitochondria, cytosol, and peroxisomes [86]. The GPX enzyme is considered a key enzyme in the removal of  $\text{H}_2\text{O}_2$  as it functions both outside and inside the cell organelles. MDHAR has several isoforms that are present in organelles such as mitochondria, chloroplasts, glyoxysomes, peroxisomes, and the cytosol. Together with other antioxidant enzymes such as SOD, APX, MDAR, and GR, MDHAR ensures the completion of the Foyer-Asada-Halliwell pathway and the removal of ROS in chloroplasts and cytoplasm. The accumulation of non-enzymatic antioxidants like  $\alpha$ -tocopherol [87], carotenoids [88], ascorbic acid [89] and polyphenols [90] has been shown to be higher in halophytes than in glycophytes. Taken together, regulation of enzymatic and nonenzymatic components of the antioxidant system enables halophytes to protect themselves against oxidative damage. Therefore, the role of antioxidant is very important for the selection of salt tolerant genotypes in cotton [91].

### Molecular basis for salt tolerance

It is difficult to understand the genetic basis of complex traits which are controlled by many genes. Because tolerance is based on several parameters and is not completely understood at the molecular level, there has been limited success in the development of salt tolerant genotypes in cotton. However, advancement in biochemical and molecular research approaches has made it possible to explore complex traits through transcriptomic profiling under salt stress. The inclusion of transcriptomic analysis facilitate the scientist to develop salt tolerant accessions in *Arabidopsis* [20] and tobacco [92]. At the molecular level, salt tolerance mechanisms fall into two categories: osmotic homeostasis and ionic homeostasis. When these two systems work together, secondary stresses emerge [93]. The transportation of sodium and potassium ions in plant cells are possible through ionic homeostasis. However, the role of chloride ions is still not clear in homeostasis. One of the mechanisms relies on salt overly sensitive (SOS) stress signaling pathways that leads to tolerance of sodium ions [94] (Fig. 1). The  $\text{H}^+$  pumps play a major role in ionic homeostasis,  $\text{Na}^+$  influx channels and sodium ions compartmentalization in vacuoles [95].



**Fig. 1** Salt overly sensitive (SOS) stress signaling pathways that lead to tolerance of sodium ions in cotton

There are two types of H<sup>+</sup> pumps: ATPase and ATPase in plasma membrane.

Specific transporters facilitates Na<sup>+</sup> to cross cell membrane such as low affinity cation transporters (LCT) and high affinity potassium transporters (HKT) [21]. It has also been suggested that some non-selective cation channels (NSCC) play a role in the long-distance transfer of sodium ions to the shoots in plants. The Na<sup>+</sup>/H<sup>+</sup> (NHX) antiporters, specifically SOS1, facilitate several sodium ion transport pathways that move sodium across the plasma membrane. This antiporter family is thought to aid in the compartmentalization of Na<sup>+</sup> ions in vacuoles, which results in salinity tolerance [96]. The NHX family and high expression of SOS1 genes, combined with a cell-specific promoter and regulators, are used to developed genotypes that are salt tolerant [97]. The saline solution leads to ionic imbalance between apoplast and symplast that results in loss of turgor pressure [98]. When the turgor pressure was below the capacity of the cell wall, plant development ended. Plants accumulate numerous osmolytes and osmoprotectants to maintain osmotic homeostasis [99]. The expansion in vacuolar and cell volume is due to compartmentalization of injurious ions and the accumulation of osmoprotectants in plant cells [31]. The osmoprotectants are organic and consist of glycine betaine, fructose, trehalose, proline and glucose. These play an important role in maintaining plasma membrane and thylakoid integrity, preventing lipid peroxidation and maintaining the structure of various protein molecules through detoxification of ROS (e.g., H<sub>2</sub>O<sub>2</sub>, proline and POD) [100, 101]. The role of calcium ions and H<sub>2</sub>O<sub>2</sub> in the signaling of cytosol is important because they exert stress and induce the secretion of abscisic acid (ABA), leading to stomatal closure and activation of genes for salinity tolerance [102]. The over expression of genes under salt stress facilitated the plants to cope with salt stress conditions. It is also facilitated the development of salt tolerant

accessions, [103]. Similarly, the gene for glycine betaine coda,  $\beta$  glycine betaine, produced more choline oxidase, choline dehydrogenase and betaine aldehyde dehydrogenase [104]. Besides the cited genes, HVA1, Di and DREB gene families are involved in drought and salinity tolerance in crop plants [105]. The role of Cys2/His2-type zinc-finger proteins GhDi 19–1 and GhDi 19–2 is crucial to avoid cell dehydration and integrity [37, 106, 107]. Some of the genes related to salinity tolerance in cotton are given in Table 1.

### Mechanisms of salinity tolerance

Some plants growing in salt stress environments have been observed to adapt to decreased water potential and high Na<sup>+</sup> toxicity in order to sustain normal growth [122]. Furthermore, adjustment of ionic balance for maintaining homeostasis is an important parameter in plants [123, 124]. The exclusion of injurious ions and less movement of Na<sup>+</sup> inside the cell prevent it from a high level of damage [6]. The development of salt tolerance is a phenomenon that also occurs in glycophytes. To develop high salt tolerance, plants use two different strategies: 1) morphological approach like succulence and the presence of structures that control salt stress, and 2) physiological strategies like ion selection, ionic compartmentalization, and osmolyte production [22]. In addition, various metabolic processes that include osmotic potential, nutritional deficiencies and ionic toxicity are occurring inside the cotton plant that negatively affect their growth under salt stress. Among these stress inducing factors, the primary factor is nutritional imbalance, and the secondary factors are osmotic potential and ionic toxicity [125]. Plants undergo physiological drought when their osmotic potential drops due to high salt levels. Furthermore, salinity increases the level of Na<sup>+</sup> in soil which produces injurious ions and additionally causes a lack of essential minerals, resulting in reduced plant

**Table 1** Summary of genes related to salinity tolerance in cotton and their functions

Sr #	Genes	Function	Reference
1	<i>GhDREB</i>	Dehydration responsive element binding protein	[108]
2	<i>GhERF6</i>	ERF encoding genes	[109]
3	<i>GhNHX1</i>	Tonoplast Na/H antiporter	[110]
4	<i>GhMT3a</i>	Type 3 metallothionein protein	[111]
5	<i>GhMPK2</i>	Mitogen-activated protein kinase	[112]
6	<i>GhNAC1</i>	Encode NAC domain	[113]
	<i>GhNAC4</i>		
	<i>GhNAC6</i>		
7	<i>GhSOD1</i>	Superoxide dismutase	[114]
8	<i>GhDi19-1</i>	Drought induced protein which is Cys2/His2 zinc-finger protein	[115]
	<i>GhDi19-1</i>		
9	<i>GhWRKY11</i>	WRKY transcription factor	[116]
	<i>GhWRKY12</i>		
	<i>GhWRKY13</i>		
	<i>GhWRKY14</i>		
	<i>GhWRKY15</i>		
	<i>GhWRKY20</i>		
	<i>GhWRKY21</i>		
	<i>GhWRKY24</i>		
	<i>GhWRKY30</i>		
	<i>GhWRKY32</i>		
	<i>GhWRKY33</i>		
	<i>GhWRKY34</i>		
10	<i>GhAnn1</i>	Annexin gene	[117]
11	<i>GhCCL</i>	Cold circadian rhythm-RNA binding-like protein	[118]
12	<i>GhTPS11</i>	Trehalose-6-phosphate synthase	[119]
13	<i>GhABF2</i>	bZIP-encoding gene	[120]
14	<i>GhZAT34</i>	Genes of zinc finger proteins	[121]
	<i>GhZAT79</i>		

development and production [15]. Figure 2 summarizes the physiological, biochemical and molecular responses of crop plant towards salt stress.

### Physiological mechanisms

#### Succulence

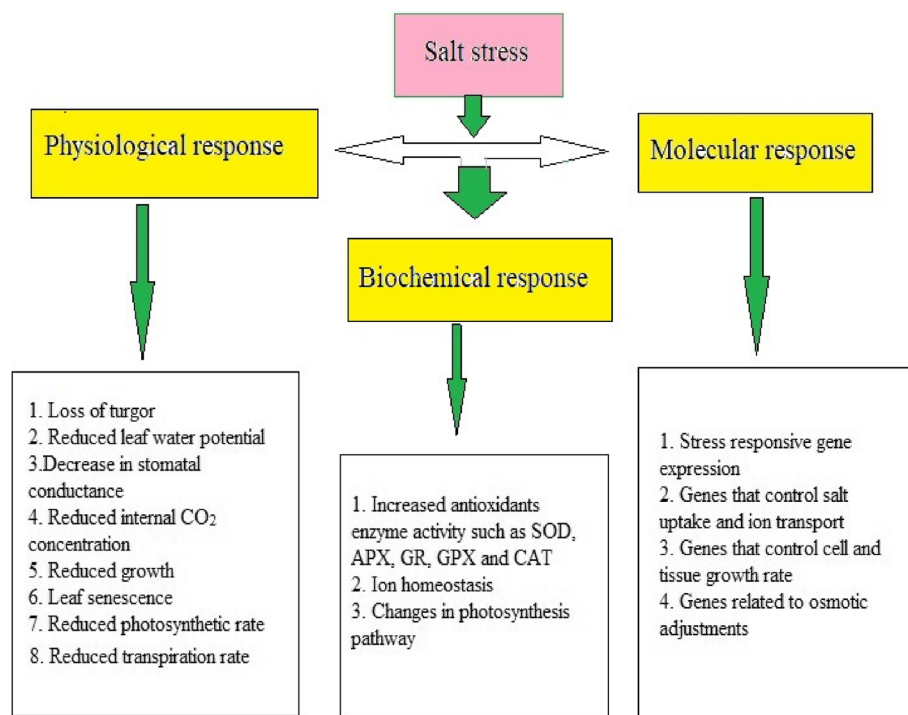
Plants that use a lot of water can balance ionic toxicity in salty environments. These plants are classified as succulents because they absorb a lot of water [126]. Succulence generally involves increased cell size, increased leaf water content per unit area and decreased growth and cell suspension [127]. In terms of physiology, the cotton plant's high water intake helps in diluting the harmful ions, resulting in a high level of salt tolerance. Plants can tolerate salt stress due to the thickness of leaves coupled with increased mesophyll cell size [12, 128]. As a result, there are more mitochondria, which means there is more energy available for salt compartmentalization and Na<sup>+</sup> exclusion [129].

### Specialized structures

Some halophytes have evolved specific structures throughout time that reduce their susceptibility to salt stress [130]. These structures, including bladders, trichomes and salt glands, work as storehouses under saline conditions. Besides succulence, cotton plants have evolved some structural changes like fewer and smaller leaves to cope with salt stress. The salinity tolerant mechanism involved the root lignification and the existence of wax on epidermis in plants [2, 131].

### Ionic compartmentalization

Vacuoles are largely used in ionic compartmentalization in leaves to store salts reducing the number of harmful ions [132]. Salt-tolerant plants may store three times as much salt in their bodies as normal plants. The maintenance of less injurious ions by the salt tolerant cultivars in the cytosol controlled the metabolic activities in chloroplast and phloem, thus the process of photosynthesis is



**Fig. 2** Physiological, biochemical and molecular responses of crop plants against salt stress

not disturb [117, 133]. Furthermore, it has been observed that due to the compartmentalization of salts in vacuoles, significant salt accumulation in cells does not disrupt the photosynthetic activities [134]. Additionally,  $\text{Na}^+$  and  $\text{Cl}^-$  ions can be delivered and accumulate in particular tissues through a variety of distinct channels without interfering with metabolic activities [24].

#### Selective ionic transportation

Cotton plants that are salt tolerant have distinct routes that allow them to absorb certain ions and deliver them to different parts of the plant. When the  $\text{K}^+/\text{Na}^+$  ratio deviates from normal, many metabolic processes and physiological pathways are disrupted [24]. Different types of transporters and proton pumps are used to transfer  $\text{K}^+/\text{Na}^+$  into plants. As a result, a plant's ability to maintain  $\text{K}^+/\text{Na}^+$  inside the cell can be considered as a selection criterion for tolerance to saltwater [25]. Plant antiporters have been identified which are responsible for reducing  $\text{Na}^+$  toxicity within plant cells as well as potassium ion absorption [27]. Some plants have various ion transport systems that assure the existence of a high potassium ratio, which confers salt tolerance [135].

#### Compatible solutes

Certain substances have a negative impact on the soil's water permeability in the root zone, which limits the

amount of water that can reach the plants [136]. To cope with these damages, plants have developed a phenomenon where osmolytes are secreted to adjust osmotic balance [18]. Using this method, cellular sub-organelles remain protected and oxidative damage inside the plants is reduced [137, 138]. Polyols, sugars, mannitol, ammonium and sulfur compounds, and amino acids are examples of osmolytes. Polyamine, glycine, choline and proline have all been found to aid in plants' ability to tolerate salt stress. More than 80 times increased proline level has been found in tobacco exposed to high salt concentrations as compared to the control [139]. When plants are subjected to salt stress, a large production of abscisic acid has been observed to maintain  $\text{K}^+/\text{Na}^+$  equilibrium in cotton [140].

#### Recent advances in cotton for salinity tolerance

The presence of phenotypic variation in response to increasing salt concentrations and subsequently exploring its genetic basis may lead to the development of salt tolerant germplasm in cotton [141]. In this modern era of molecular genetics, various transcription factors were found that are considered important regulators of gene expression. Several salinity tolerant genes have been reported in crop plants. However, in cotton, few genes related to salt tolerance have been identified. Those identified include *ERF* [142], tonoplast  $\text{Na}^+/\text{H}^+$  antiporter



[143], *GhMT3a* [144], *ZFP* [145], *NAC* [146], *DREB* [147], *MPK* [86] and *MKK*. Fan et al. [148] identified 108 *WRKY* genes (*GarWRKYs*) in *G. aridum* using transcriptome sequencing data, and real time-polymerase chain reaction (RT-PCR) analysis confirmed the expression of 26 *GarWRKY* genes in the roots. Overexpression of ROS scavengers like *GhCAT1*, *GhSOD1* and *GhMT3a* was observed in various cotton lines showing tolerance to salt stress. The expression of the novel gene (*GhNHX1*) regulates the  $\text{Na}^+/\text{H}^+$  antiporter, tonoplasts and defense responses against salt stress. Expression analysis showed that mRNA level of *GhNHX1* was higher in salt tolerant genotypes as compared to sensitive genotypes, suggesting its importance in the salt tolerance mechanism. Genome modification through targeted genome editing has revolutionized plant genomics for improving plants' characteristics against biotic and abiotic stressors [149]. Mega nucleases, transcription activator-like effector nucleases (TALENs), zinc finger nucleases (ZFNs), and clustered regularly interspaced short palindromic

repeats (CRISPR/Cas) systems have all been used to alter genomes. CRISPR–Cas9 has been widely utilized to transform plants by inducing mutations via either homology-dependent repair (HDR) or non-homologous end-joining of double-stranded breaks (NHEJ) [150]. The following Fig. 3 shows the importance of modern molecular approaches in the development of salt tolerance cultivars of cotton.

## Conclusion

In conclusion, salinity is a serious problem which poses a threat to ensuring food security as more than half of the countries in the world are facing this problem. Salt stress causes toxicity of specific ions and induces water stress and nutrient imbalance in plant tissues which disturbs plant growth and development. Salt stress affects the metabolic activities of enzymes, impairs nutrient uptake and results in nutritional disorders which leads to yield reduction and fiber quality deterioration in cotton. High-throughput and efficient modern molecular approaches are important for salt stress genetic screening. CRISPR–CAS9 and genomics approaches have been shown to be quick and effective ways for exploring the molecular regulation of plant salt tolerance. Transcriptome sequencing techniques have also been frequently employed to discover new genes involved in the control of plant salt stress responses. Development of salt tolerant cotton cultivars offers a management strategy to control the negative salt stress response in cotton. To develop these cultivars, it is crucial to form collaborations joining the skills and expertise of conventional plant breeding and modern molecular approaches.

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## Authors' contributions

Conceptualization, M.T.C., M.T.A., Z.A., S.M. and L.H.; writing and draft preparation, M.T.C., S.M., Y.J., review and editing, M.T.A., X. D., L.H. and I.A.R. All authors have read and agreed to the published version of the manuscript.

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## Availability of data and materials

Not applicable.

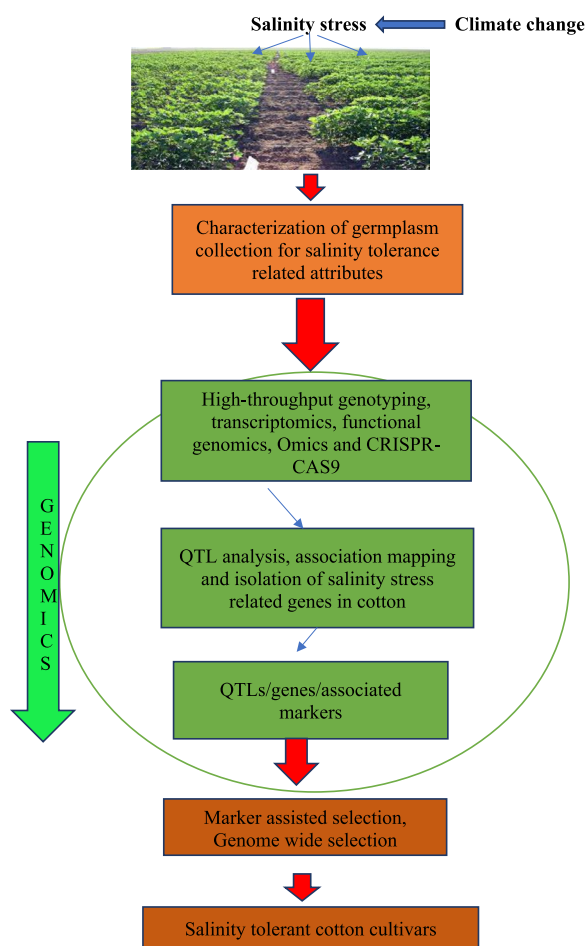
## Declarations

## Ethics approval and consent to participate

Not applicable.

## Consent for publication

Not applicable.



**Fig. 3** Schematic diagram for the development of salinity tolerant cotton cultivars using modern molecular approaches

### Competing interests

The authors declare no competing interests.

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