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Screening of salt tolerant transgenic and non-transgenic cotton varieties under various levels of NaCl induced salinity stress

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Abstract

Salinity induced stress is one of major hurdle for crop production that significantly decreased crops yield. Higher levels of salts reduced the uptake of water, osmotic imbalance, poor germination and restriction of cell division. However, crops varieties that are tolerant towards salts accumulation can grow better under salinity stress. As cotton is moderately salt tolerant and major cash crop, therefore current study was conducted to screen salt tolerant cotton BT and non-BT varieties. For experiment, five BT (CIM616, CIM598, CIM179, CIM602 and GH-Mubarik) and non-BT cotton varieties (Cyto124, CIM554, CIM573, CIM620 and Lalazar) were grown under various levels (0, 50, 100, 150, 200 and 250 mM) of artificially NaCl induced salt stress. NaCl induced salinity stress, 250 mM level was more severe, that reduced shoot and root length, shoot and root fresh and dry weight, owing to decrease in chlorophyll a, chlorophyll b and total chlorophyll. A significant improvement in shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight and root dry weight confirmed the potential of BT CIM598, CIM602, non-BT Cyto124 and CIM620 varieties to withstand against salt induced stress. Furthermore, an improvement in chlorophyll a, chlorophyll b and total chlorophyll and less electrolyte leakage validated the potential of BT CIM598, CIM602, non-BT Cyto124 and CIM620 as salt tolerant cotton varieties. In conclusion, BT CIM598, CIM602 and non-BT Cyto124, CIM620 cotton varieties have the potential to grow better at seedling stage under salinity stress.

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Introduction

Salt induced stress is one of major abiotic plant growth limiting factor that significantly decreased the productivity of crops in all over the world (Bohnert, 1995; Wei *et al.*, 2017). Now a days, out of 230 million hectares, 45 million hectares of irrigated land is salt affected (Parihar *et al.*, 2015). It is well documented that in various countries of the world, 9-34% irrigated soils are saline in nature. High concentration of salts can cause severe damage to cellular metabolism and restrict the process of photosynthesis in plants (Chinnusamy *et al.,* 2006).

It also decreased the uptake of water that plays a crucial role in growth restriction(Parihar *et al.*, 2015). Disorganization of membrane, osmotic imbalance and inhibition of cell division are other common responses that a plant showed under salinity stress (Parida and Das, 2005). Furthermore, an increase in salinity stress resulted in the generation of reactive oxygen species (ROS) that caused oxidative damage and sever injury to the plants (Asada, 2006). Generation of ROS boosts the mechanism of proteins, lipids and nucleic acid oxidation that induced severe cellular toxicity (Apel and Hirt, 2004; Ahmad *et al.,* 2010 a, b). As plants are unable to move from one place to another, that's why they have to adopt a mechanism for the mitigation of salinity stress (Wei *et al.*, 2017).

Besides all the damages caused by salinity, the bioavailability of macronutrients (N, P and K) also become limited under the higher concentration of sodium, chloride and sulphate ions (Zhu*,* 2001). The interaction of Na^+ with the NH_4^+ significantly decreased the availability of nitrogen to the plants (Rozeff, 1995). Similarly, Cl^- and NO_3^- also showed an antagonistic relationship (Bar *et al.,* 1997). Less solubility of Ca-P in salt affected soils due to high pH that restricted the P availability to the plants (Qadir & Schubert*,* 2002). However, alleviated Na in salt affected soils imbalance the uptake of K in plants (Keutgen & Pawelzik*,* 2009). High soil pH due to salinity also restricted the mobilization and phytoavailability of micronutrients (Zhu *et al.,* 2004).

Although cotton is moderately salinity tolerant crop, yet in salt affected soils its poor rate of germination significantly decreased its yield (Ashraf *et al.,* 2002). Presently the area under cotton crop is pointed highly with transgenic (BT) cotton varieties occupying more than 80 percent area. The remaining area is planted with conventional (non-BT) cotton varieties. BT cotton is a variety of cotton genetically modified to contain gene (cry 1AC) of *Bacillus thuringiensis* (BT), which is foreign to its genome and is naturally occurring soil bacterium used to control lepidopteron insects because of toxin it produces (Lalitha *et al.,* 2011). There are various schools of thought that whether transgenic cotton would prevail over a longer period for sustained production under the current scenario (Arshad *et al.,* 2009). The main debate is centred on whether BT cotton consistently performs better than non-BT varieties. Moreover, the adoption of BT varieties results in an economic benefit to producers (Bennett *et al.,* 2006). There are reports that studies on the comparative advantage of BT-cotton and non-BT cotton are needed and revisited on a large area (Centad, 2006). Keeping in mind the importance of cotton as an economic and fiber crop a screening experiment was conducted by cultivating 5 BT and 5 non-BT cotton varieties. It is hypothesized that BT cotton might be more salinity tolerant than non-BT cotton varieties.

Materials and methods

Seeds collection and delinting

The certified BT and non-BT cotton varieties were collected from the Central Cotton Research Institute (CCRI) Multan. Seeds of BT (CIM616, CIM598, CIM179, CIM602 and GH-Mubarik) and non-BT cotton varieties (Cyto124, CIM554, CIM573, CIM620 and Lalazar) were delinted by using 98% pure H_2SO_4 as described by Heydari (2015).

Sand collection and pots preparation

For cultivation of cotton seedlings sand was collected from an experimental area of Department of Soil Science, Faculty of Agricultural Sciences & Technology Bahauddin Zakariya University, Multan. Initially, the required amount of sand was washed 4 times with deionized water to remove all the water soluble salts (Kan *et al.,* 2013). After that in plastic cups having area of 10 × 10 cm, 100 g of sand was filled for the sowing of cotton seeds.

Salinity stress and seeds sowing

For the introduction of various levels (0, 50, 100, 150, 200 and 250 mM) of salinity, salt of NaCl was added in the sand at the time of sowing (Witzel *et al.,* 2009). In each plastic cup, 6 seeds of cotton were sown. When seeds get germinated after 7 days, three healthy seedlings were maintained in each cup by thinning.

Harvesting and growth attributes

All the seedlings were harvested from each plastic cup after 21 days of sowing. The morphological growth attributes i.e. shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight and root dry weight were determined soon after harvesting by following standard protocol.

Chlorophyll contents

The chlorophyll a, b and total contents were determined in cotton seedlings fresh leaves according to the method of Arnon (1949). Extract of leaves was taken in acetone (80%) solution. For determination of chlorophyll a, chlorophyll b and total chlorophyll absorbances were taken at 663 and 645nm wavelength on spectrophotometer. Final estimations were done by using formulas:

$$\text{Chlorophyll a (mg g-1)} = \frac{12.7\,(\text{OD } 663) - 2.69\,(\text{OD } 645)\ V}{1000\,(W)}$$

$$\text{Cholorophyll b (mg g-1)} = \frac{22.9\,(\text{OD } 645) - 4.68\,(\text{OD } 663)\ V}{1000\,(W)}$$

$$\text{Total Chlorophyll (mg g-1)} = \text{Chlorophyll a} + \text{Chlorophyll b}$$

Where,

OD = Optical density (wavelength)

V = Final volume made

W = Fresh leaf weight (*g*)

Electrolyte leakage

Lutts *et al.* (1996) method was followed for the estimation of electrolyte leakage (EL). Initially, all dust particles were washed from leaf surfaces, bydeionized (DI) water and then discs were cut with 1 cm diameter steel cylinder of.

After that one-gram uniform size leaves discs were immersed in a 20 ml DI water containing test tube and incubated at 25 ºC for 24h. The EC (EC1) was determined using pre-calibrated EC meter. The second EC (EC2) was noted after heating test tubes at 120 ºC for 20 min on a water bath. The final value of EL was calculated using the equation as follows:

$$\text{Electrolyte Leakage (\%)} = EC1 / EC2 \times 100$$

Results

Shoot length

Main effect of various levels of salinity (S) and cotton BT and non-BT varieties (V) was significant on the shoot length of cotton seedlings but their interaction S × V remained non-significant (Table 1).

In case of shoot length, among all cotton BT and non-BT varieties CIM602, Cyto124, CIM598, CIM573 and CIM620 performance was better and were statistically alike to each other under artificially induced salinity stress. CIM602 showed maximum increase (39.4%) in shoot length as compared to Lalazar under salinity stress.

Root length

Main effect of various levels of salinity (S) and cotton BT and non-BT varieties (V) was significant on the root length of cotton seedlings but their interaction S × V was non-significant (Table 2). For root length, CIM602 performed the best as compared to all other cotton BT and non-BT under salinity stress. CIM602 showed maximum increase (84.7%) in root length as compared to GH-Mubarik under salinity stress.

Shoot fresh weight

Main effect of various levels of salinity (S) and cotton BT and non-BT varieties (V) was significant on the shoot fresh weight of cotton seedlings. However, the interaction of S × V did not significantly (Table 3).

It was noted that among all the BT and non-BT varieties, CIM598, CIM179, CIM602, Cyto124, CIM554, CIM573 and CIM620 remained significantly better under artificially induced salinity stress for shoot fresh weight. BT CIM598, CIM602 and non-BT CIM620 showed maximum increase (25.5%) in shoot fresh weight as compared to BT GH-Mubarik and non-BT Lalazar under salinity stress.

Table 1. Effect of various levels of salinity (S) on shoot length (cm) of cotton seedlings of various BT and non-BT varieties (V).

Cotton	Varieties	Shoot Length (cm) Salinity Levels (NaCl mM) 0	50	100	150	200	250	ME (V)
		IE (S × V) (Means of 3 replicates ± standard error)						
Bt	CIM616	12.3 ± 0.88	13.7 ± 1.45	12.0 ± 1.53	8.70 ± 0.33	6.70 ± 0.33	6.00 ± 0.58	9.90 BC
	CIM598	14.7 ± 0.88	13.7 ± 0.88	13.3 ± 0.33	11.0 ± 0.00	9.70 ± 0.33	7.70 ± 0.33	11.7 AB
	CIM179	14.0 ± 0.58	12.3 ± 0.88	11.0 ± 0.58	8.70 ± 0.33	7.00 ± 0.58	6.00 ± 0.58	9.80 BC
	CIM602	14.7 ± 0.33	14.0 ± 0.58	13.3 ± 0.88	13.3 ± 0.88	11.0 ± 1.00	9.70 ± 1.20	12.7 A
	GH-Mubarik	12.0 ± 0.58	11.3 ± 0.67	9.70 ± 0.33	9.30 ± 0.33	8.30 ± 0.33	8.00 ± 0.58	9.80 C
Non-Bt	Cyto124	18.3 ± 0.67	15.7 ± 1.45	14.7 ± 1.45	12.3 ± 1.86	10.0 ± 1.53	7.70 ± 1.20	13.1 A
	CIM554	14.0 ± 1.53	12.3 ± 1.86	10.7 ± 1.67	9.00 ± 1.53	7.70 ± 1.76	6.00 ± 1.53	9.90 BC
	CIM573	14.3 ± 0.67	15.0 ± 1.15	13.3 ± 1.86	10.3 ± 0.33	10.3 ± 0.88	8.00 ± 1.73	11.9 A
	CIM620	15.7 ± 0.88	16.0 ± 0.58	15.0 ± 0.58	13.0 ± 1.15	9.30 ± 1.20	7.00 ± 0.58	12.7 A
	Lalazar	13.7 ± 0.33	10.7 ± 0.67	10.3 ± 0.33	8.70 ± 0.67	7.00 ± 0.58	6.00 ± 1.00	9.40 C
	ME (S)	14.4 A	13.5 AB	12.3 B	10.4 C	8.70 D	7.20 E	

Means sharing different letters are significantly different ($p \leq 0.05$). Non-significant interactive effect (S × V) did not have any letter.

ME = indicates main effect; IE = indicates interactive effect.

Root fresh weight

Main effect of various levels of salinity (S) and cotton BT and non-BT varieties (V) was significant on the root fresh weight of cotton seedlings. However, the interaction of S × V did not significantly (Table 4). For root fresh weight, all BT and non-BT varieties were statistically alike to each other except non-BT CIM554 under salinity stress. Non-BT CIM573 and CIM620 showed 31.5% more root fresh weight as compared to non-BT CIM554 under salinity stress.

Table 2. Effect of various levels of salinity (S) on root length (cm) of cotton seedlings of various BT and non-BT varieties (V).

Cotton	Varieties	Root Length (cm) Salinity Levels (NaCl mM) 0	50	100	150	200	250	ME (V)
		IE (S × V) (Means of 3 replicates± standard error)						
Bt	CIM616	5.33 ± 0.33	5.33 ± 0.88	4.33 ± 0.33	3.67 ± 0.33	3.00 ± 0.00	2.67 ± 0.33	4.06 CD
	CIM598	7.67 ± 0.67	5.67 ± 0.33	4.67 ± 0.67	4.00 ± 0.58	3.67 ± 0.33	3.67 ± 0.33	4.89 BC
	CIM179	6.33 ± 0.33	6.33 ± 0.67	5.33 ± 0.33	4.33 ± 0.33	3.33 ± 0.33	3.00 ± 0.00	4.78 BC
	CIM602	9.00 ± 0.58	7.33 ± 0.33	6.67 ± 0.67	6.33 ± 0.33	5.33 ± 0.33	5.33 ± 0.67	6.67 A
	GH-Mubarik	5.67 ± 0.33	4.00 ± 0.58	4.00 ± 0.58	3.67 ± 0.33	2.67 ± 0.33	1.67 ± 0.33	3.61 D
Non-Bt	Cyto124	7.00 ± 0.58	6.33 ± 0.33	5.67 ± 0.67	4.33 ± 0.33	3.00 ± 0.58	2.67 ± 0.33	4.83 BC
	CIM554	7.67 ± 0.88	7.33 ± 0.33	6.00 ± 0.58	4.00 ± 0.00	3.00 ± 0.00	1.67 ± 0.33	4.94 BC
	CIM573	6.33 ± 0.33	5.33 ± 0.33	5.33 ± 0.33	4.67 ± 0.33	4.33 ± 0.67	3.00 ± 0.58	4.83 BC
	CIM620	8.00 ± 0.58	6.67 ± 0.88	6.33 ± 0.67	4.67 ± 0.33	4.00 ± 0.00	4.00 ± 1.00	5.61 B
	Lalazar	6.33 ± 0.67	5.67 ± 0.67	4.67 ± 0.67	3.67 ± 0.67	3.33 ± 0.33	2.33 ± 0.33	4.33 CD
	ME (S)	6.93 A	6.00 B	5.30 C	4.33 D	3.57 E	3.00 E	

Means sharing different letters are significantly different ($p \leq 0.05$). Non-significant interactive effect (S × V) did not have any letter.

ME = indicates main effect; IE = indicates interactive effect.

Shoot dry weight

Main effect of various levels of salinity (S) and cotton BT and non-BT varieties (V) was significant on the shoot dry weight of cotton seedlings while the interaction of S × V did not differ significantly (Table 5). The BT CIM598, CIM602 and non-BT CIM620varieties performed the best among all varieties and were statistically alike with each other better under artificially induced salinity stress. The non-BT CIM620 showed maximum increase (25.5%) shoot dry weight as compared to GH-Mubarik under salinity stress.

Table 3. Effect of various levels of salinity (S) on shoot fresh weight (g) of cotton seedlings of various BT and non-BT varieties (V).

Cotton	Shoot Fresh Weight (g)							
		Salinity Levels (NaCl mM)						
	Varieties	0	50	100	150	200	250	ME (V)
		IE (S × V) (Means of 3 replicates ± standard error)						
Bt	CIM616	0.75 ± 0.09	0.74 ± 0.08	0.66 ± 0.02	0.53 ± 0.02	0.46 ± 0.04	0.47 ± 0.16	0.60 AB
	CIM598	0.81 ± 0.03	0.77 ± 0.06	0.74 ± 0.10	0.60 ± 0.03	0.50 ± 0.05	0.41 ± 0.05	0.64 A
	CIM179	0.74 ± 0.04	0.70 ± 0.04	0.60 ± 0.06	0.46 ± 0.02	0.42 ± 0.02	0.31 ± 0.03	0.54 AB
	CIM602	0.75 ± 0.03	0.71 ± 0.04	0.81 ± 0.04	0.63 ± 0.03	0.53 ± 0.02	0.41 ± 0.01	0.64 A
	GH-Mubarik	0.63 ± 0.01	0.71 ± 0.09	0.57 ± 0.03	0.47 ± 0.03	0.40 ± 0.02	0.29 ± 0.03	0.51 B
Non-Bt	Cyto124	0.74 ± 0.04	0.72 ± 0.06	0.63 ± 0.02	0.55 ± 0.01	0.47 ± 0.01	0.37 ± 0.04	0.58 AB
	CIM554	0.73 ± 0.09	0.73 ± 0.12	0.73 ± 0.16	0.56 ± 0.10	0.41 ± 0.05	0.28 ± 0.05	0.57 AB
	CIM573	0.70 ± 0.04	0.53 ± 0.01	0.63 ± 0.07	0.63 ± 0.12	0.51 ± 0.02	0.48 ± 0.08	0.58 AB
	CIM620	0.83 ± 0.11	0.76 ± 0.04	0.78 ± 0.03	0.62 ± 0.05	0.46 ± 0.04	0.39 ± 0.05	0.64 A
	Lalazar	0.65 ± 0.00	0.69 ± 0.10	0.56 ± 0.01	0.47 ± 0.04	0.38 ± 0.01	0.28 ± 0.03	0.51 B
	ME (S)	0.73 A	0.71 A	0.67 A	0.55 B	0.45 C	0.37 D	

Means sharing different letters are significantly different ($p \leq 0.05$). Non-significant interactive effect (S × V) did not have any letter.

ME = indicates main effect; IE = indicates interactive effect.

Table 4. Effect of various levels of salinity (S) on root fresh weight (g) of cotton seedlings of various BT and non-BT varieties (V).

Cotton	Root Fresh Weight (g)							
		Salinity Levels (NaCl mM)						
	Varieties	0	50	100	150	200	250	ME (V)
		IE (S × V) (Means of 3 replicates ± standard error)						
Bt	CIM616	0.24 ± 0.01	0.23 ± 0.03	0.24 ± 0.03	0.20 ± 0.02	0.15 ± 0.01	0.12 ± 0.01	0.20 AB
	CIM598	0.24 ± 0.03	0.26 ± 0.02	0.25 ± 0.01	0.17 ± 0.01	0.19 ± 0.03	0.16 ± 0.03	0.21 AB
	CIM179	0.24 ± 0.02	0.22 ± 0.01	0.23 ± 0.03	0.20 ± 0.02	0.17 ± 0.01	0.13 ± 0.02	0.20 AB
	CIM602	0.29 ± 0.04	0.25 ± 0.03	0.22 ± 0.05	0.18 ± 0.01	0.13 ± 0.01	0.11 ± 0.01	0.20 AB
	GH-Mubarik	0.29 ± 0.02	0.25 ± 0.02	0.25 ± 0.02	0.20 ± 0.02	0.18 ± 0.02	0.14 ± 0.02	0.22 AB
Non-Bt	Cyto124	0.24 ± 0.02	0.22 ± 0.01	0.23 ± 0.03	0.20 ± 0.02	0.17 ± 0.01	0.13 ± 0.02	0.20 AB
	CIM554	0.26 ± 0.01	0.22 ± 0.02	0.21 ±0.02	0.18 ± 0.02	0.14 ± 0.01	0.12 ± 0.01	0.19 B
	CIM573	0.33 ± 0.08	0.32 ± 0.07	0.28 ± 0.04	0.23 ±0.06	0.19 ± 0.04	0.14 ± 0.04	0.25 A
	CIM620	0.33 ± 0.08	0.32 ± 0.07	0.28 ± 0.04	0.23 ± 0.06	0.19 ± 0.04	0.14 ± 0.04	0.25 A
	Lalazar	0.24 ± 0.03	0.28 ± 0.02	0.26 ± 0.02	0.20 ± 0.01	0.16 ± 0.01	0.13 ± 0.01	0.21 AB
	ME (S)	0.27 A	0.26 A	0.24 A	0.20 B	0.17 BC	0.13 C	

Means sharing different letters are significantly different ($p \leq 0.05$). Non-significant interactive effect (S × V) did not have any letter.

ME = indicates main effect; IE = indicates interactive effect.

Root dry weight

Main effect of various levels of salinity (S) and cotton BT and non-BT varieties (V) was significant on the root dry weight of cotton seedlings while the interaction of S × V did not differ significantly (Table 6). In case of root dry weight, BT CIM598, CIM602 and non-BT Cyto124 performance remained better as compared to all other varieties under artificially induced salinity stress. The non-BT Cyto124 showed the highest increase (85.7%) in root dry weight as compared to BT GH-Mubarik under salinity stress.

Table 5. Effect of various levels of salinity (S) on shoot dry weight (g) of cotton seedlings of various BT and non-BT varieties (V).

Cotton	Varieties	Shoot Dry Weight (g) — Salinity Levels (NaCl mM) — IE (S × V) (Means of 3 replicates ± standard error): 0	50	100	150	200	250	ME (V)
Bt	CIM616	0.16 ± 0.02	0.17 ± 0.01	0.17 ± 0.02	0.11 ± 0.01	0.11 ± 0.01	0.11 ± 0.02	0.14 CD
	CIM598	0.25 ± 0.02	0.23 ± 0.02	0.22 ± 0.01	0.18 ± 0.04	0.15 ± 0.03	0.16 ± 0.02	0.20 AB
	CIM179	0.17 ± 0.03	0.19 ± 0.03	0.16 ± 0.01	0.12 ± 0.02	0.12 ± 0.01	0.14 ± 0.03	0.15 CD
	CIM602	0.23 ± 0.03	0.23 ± 0.01	0.21 ± 0.01	0.18 ± 0.03	0.16 ± 0.03	0.15 ± 0.02	0.19 AB
	GH-Mubarik	0.19 ± 0.02	0.15 ± 0.02	0.13 ± 0.01	0.10 ± 0.03	0.07 ± 0.02	0.05 ± 0.02	0.11 D
Non-Bt	Cyto124	0.23 ± 0.03	0.19 ± 0.02	0.18 ± 0.05	0.15 ± 0.01	0.12 ± 0.01	0.08 ± 0.02	0.16 BC
	CIM554	0.13 ± 0.04	0.17 ± 0.02	0.14 ± 0.03	0.13 ± 0.01	0.10 ± 0.00	0.08 ± 0.00	0.12 CD
	CIM573	0.15 ± 0.03	0.21 ± 0.05	0.19 ± 0.04	0.16 ± 0.03	0.11 ± 0.02	0.07 ± 0.02	0.15 CD
	CIM620	0.25 ± 0.02	0.26 ± 0.01	0.26 ± 0.03	0.21 ± 0.01	0.16 ± 0.02	0.12 ± 0.02	0.21 A
	Lalazar	0.16 ± 0.02	0.16 ± 0.01	0.17 ± 0.03	0.11 ± 0.01	0.10 ± 0.01	0.07 ± 0.02	0.13 CD
	ME (S)	0.19 A	0.20 A	0.18 A	0.15 B	0.12 BC	0.10 C	

Means sharing different letters are significantly different ($p \leq 0.05$). Non-significant interactive effect (S × V) did not have any letter.

ME = indicates main effect; IE = indicates interactive effect.

Table 6. Effect of various levels of salinity (S) on root dry weight (g) of cotton seedlings of various BT and non-BT varieties (V).

Cotton	Varieties	Root Dry Weight (g) — Salinity Levels (NaCl mM) — IE (S × V) (Means of 3 replicates ± standard error): 0	50	100	150	200	250	ME (V)
Bt	CIM616	0.010 ± 0.002	0.011 ± 0.002	0.010 ± 0.000	0.007 ± 0.001	0.007 ± 0.001	0.008 ± 0.002	0.009 CD
	CIM598	0.014 ± 0.002	0.014 ± 0.001	0.013 ± 0.000	0.011 ± 0.002	0.010 ± 0.002	0.009 ± 0.001	0.012 AB
	CIM179	0.009 ± 0.001	0.010 ± 0.001	0.010 ± 0.001	0.006 ± 0.001	0.007 ± 0.001	0.006 ± 0.001	0.008 CD
	CIM602	0.015 ± 0.001	0.014 ± 0.001	0.013 ± 0.001	0.011 ± 0.002	0.009 ± 0.002	0.009 ± 0.001	0.012 AB
	GH-Mubarik	0.011 ± 0.001	0.009 ± 0.001	0.008 ± 0.001	0.006 ± 0.002	0.004 ± 0.001	0.003 ± 0.001	0.007 D
Non-Bt	Cyto124	0.015 ± 0.001	0.016 ± 0.001	0.016 ± 0.002	0.013 ± 0.001	0.010 ± 0.001	0.007 ± 0.001	0.013 A
	CIM554	0.008 ± 0.002	0.010 ± 0.001	0.008 ± 0.002	0.008 ± 0.001	0.006 ± 0.000	0.005 ± 0.000	0.007 CD
	CIM573	0.009 ± 0.002	0.012 ± 0.003	0.012 ± 0.003	0.010 ± 0.002	0.007 ± 0.001	0.004 ± 0.001	0.009 CD
	CIM620	0.014 ± 0.002	0.012 ± 0.001	0.011 ± 0.003	0.009 ± 0.001	0.007 ± 0.001	0.005 ± 0.001	0.009 BC
	Lalazar	0.010 ± 0.001	0.010 ± 0.001	0.010 ± 0.002	0.007 ± 0.001	0.006 ± 0.001	0.004 ± 0.001	0.008 CD
	ME (S)	0.011 A	0.012 A	0.011 A	0.009 B	0.007 BC	0.006 C	

Means sharing different letters are significantly different ($p \leq 0.05$). Non-significant interactive effect (S × V) did not have any letter.

ME = indicates main effect; IE = indicates interactive effect.

Photosynthetic pigments

Main effect of various levels of salinity (S) and cotton BT and non-BT varieties (V) was significant for chlorophyll a, chlorophyll b and total chlorophyll in leaves of cotton seedlings while the interaction of S × V did not differ significantly (Tables 7, 8 and 9). For chlorophyll a, chlorophyll b and total chlorophyll contents, verities BT CIM598, CIM602, non-BT Cyto124 and CIM620 performed significantly best under salinity stress. Verities CIM616, CIM179, CIM602, Cyto124 and CIM620 remained statistically alike to each other for chlorophyll a content under salinity stress. Similarly, for chlorophyll b, verities CIM616, CIM179 and CIM554 remained statistically alike to each other. Maximum increase of 85%, 123% and 94% in chlorophyll a, b and total was noted in verities of BT CIM598, CIM602 and Cyto124 as compared to Lalazar respectively.

Table 7. Effect of various levels of salinity (S) on chlorophyll a (mg g^{-1}) of cotton seedlings of various BT and non-BT varieties (V).

Cotton	Chlorophyll a (mg g^{-1})							
	Salinity Levels (NaCl mM)							
	Varieties	0	50	100	150	200	250	ME (V)
		IE (S × V) (Means of 3 replicates ± standard error)						
Bt	CIM616	0.753 ± 0.03	0.593 ± 0.03	0.580 ± 0.02	0.423 ± 0.06	0.310 ± 0.01	0.237 ± 0.01	0.483 ^{BC}
	CIM598	0.770 ± 0.03	0.693 ± 0.03	0.600 ± 0.05	0.523 ± 0.03	0.497 ± 0.03	0.370 ± 0.04	0.576 ^{A}
	CIM179	0.690 ± 0.08	0.523 ± 0.04	0.487 ± 0.01	0.427 ± 0.03	0.293 ± 0.03	0.223 ± 0.01	0.441 ^{CD}
	CIM602	0.730 ± 0.06	0.580 ±0.04	0.603 ± 0.03	0.467 ± 0.03	0.380 ± 0.05	0.360 ± 0.07	0.520 $^{A-C}$
	GH-Mubarik	0.530 ± 0.03	0.453 ± 0.02	0.400 ± 0.06	0.313 ± 0.02	0.243 ± 0.02	0.193 ± 0.04	0.356 ^{DE}
Non-Bt	Cyto124	0.770 ± 0.06	0.677 ± 0.09	0.577 ± 0.04	0.527 ± 0.06	0.393 ± 0.04	0.400 ± 0.06	0.557 ^{AB}
	CIM554	0.590 ± 0.03	0.460 ± 0.03	0.380 ± 0.04	0.357 ± 0.06	0.283 ± 0.04	0.267 ± 0.03	0.389 ^{DE}
	CIM573	0.600 ± 0.03	0.463 ± 0.03	0.360 ± 0.04	0.300 ± 0.03	0.253 ± 0.02	0.253 ±0.03	0.372 ^{DE}
	CIM620	0.700 ± 0.10	0.613 ± 0.05	0.593 ± 0.03	0.460 ± 0.04	0.387 ± 0.06	0.353 ± 0.11	0.518 $^{A-C}$
	Lalazar	0.430 ± 0.04	0.383 ± 0.10	0.333 ± 0.04	0.233 ± 0.01	0.253 ± 0.02	0.237 ± 0.01	0.312 ^{E}
	ME (S)	0.656 ^{A}	0.544 ^{B}	0.491 ^{B}	0.403 ^{C}	0.329 ^{D}	0.289 ^{D}	

Means sharing different letters are significantly different ($p \leq 0.05$). Non-significant interactive effect (S × V) did not have any letter.

ME = indicates main effect; IE = indicates interactive effect.

Electrolyte leakage

Main effect of various levels of salinity (S) and cotton BT and non-BT varieties (V) was significant for electrolyte leakage in leaves of cotton seedlings while the interaction of S × V remained non-significantly (Table 10). The verities BT CIM598, CIM602, non-BT Cyto124 and CIM620 performed best regarding less electrolyte leakage under salinity stress. A significant reduction of 33% in electrolyte leakage was noted in non-BT Cyto124 as compared to Lalazar.

Discussion

It is well documented fact that better shoot and root elongation is one of solid indication of salinity tolerance in crops when cultivated in salt affected soils (Bhute *et al.*, 2012). According to Leidi and Saiz (1997), poor shoot and root growth are one of the salientfeatures of salinity susceptible varieties of various crops. Higher uptake of salts by roots elevates the potential toxic effects of unbalance ions (Na) in the seedlings that are cultivated in salt affected soils (Hajibagheri *et al.,* 1989; Bhute *et al.,* 2012).

In the current experiment on an average, some of BT and non-BT cotton varieties (CIM602, Cyto124, CIM598, CIM573 and CIM620) showed significantly better shoot and root length under saline conditions.

This improvement in the shoot and root length is a solid indication of salinity tolerance character in CIM602, Cyto124, CIM598, CIM573 and CIM620. Bhute *et al.* (2012) observed a significant improvement in the shoot and root fresh weight of salt tolerant varieties. Datta *et al.* (2009) also noted a significant reduction in fresh and dry weight of shoot and root when wheat was cultivated under salinity stress. They suggested that the slow uptake of water is a key factor responsible for the reduction in shoot and root fresh and dry weight under salinity stress.

Table 8. Effect of various levels of salinity (S) on chlorophyll b (mg g-1) of cotton seedlings of various BT and non-BT varieties (V).

Cotton	Varieties	Chlorophyll b (mg g-1) Salinity Levels (NaCl mM) 0	50	100	150	200	250	ME (V)
		IE (S × V) (Means of 3 replicates ± standard error)						
Bt	CIM616	0.407 ± 0.02	0.320 ± 0.02	0.313 ± 0.01	0.229 ± 0.03	0.167 ± 0.01	0.128 ± 0.01	0.261 B
	CIM598	0.470 ± 0.02	0.423 ± 0.02	0.366 ± 0.03	0.319 ± 0.02	0.303 ± 0.02	0.226 ± 0.03	0.351 A
	CIM179	0.373 ± 0.04	0.283 ± 0.02	0.263 ± 0.01	0.230 ± 0.01	0.158 ± 0.01	0.121 ± 0.01	0.238 BC
	CIM602	0.504 ± 0.04	0.400 ± 0.03	0.416 ± 0.02	0.322 ± 0.02	0.262 ± 0.04	0.248 ± 0.05	0.359 A
	GH-Mubarik	0.286 ± 0.02	0.245 ± 0.01	0.216 ± 0.03	0.169 ± 0.01	0.131 ± 0.01	0.104 ± 0.02	0.192 CD
Non-Bt	Cyto124	0.524 ± 0.04	0.460 ± 0.06	0.392 ± 0.03	0.358 ± 0.04	0.267 ± 0.03	0.272 ± 0.04	0.379 A
	CIM554	0.319 ± 0.02	0.248 ± 0.01	0.205 ± 0.02	0.193 ± 0.03	0.153 ± 0.02	0.144 ± 0.01	0.210 B-D
	CIM573	0.324 ± 0.02	0.250 ± 0.02	0.194 ± 0.02	0.162 ± 0.02	0.137 ± 0.01	0.137 ± 0.02	0.201 CD
	CIM620	0.518 ± 0.07	0.454 ± 0.03	0.439 ± 0.02	0.340 ± 0.03	0.286 ± 0.04	0.261 ± 0.08	0.383 A
	Lalazar	0.232 ± 0.02	0.207 ± 0.05	0.180 ± 0.02	0.126 ± 0.01	0.137 ± 0.01	0.144 ± 0.02	0.171 D
	ME (S)	0.396 A	0.329 B	0.299 B	0.245 C	0.200 D	0.179 D	

Means sharing different letters are significantly different (p ≤ 0.05). Non-significant interactive effect (S × V) did not have any letter.

ME = indicates main effect; IE = indicates interactive effect.

Table 9. Effect of various levels of salinity (S) on total chlorophyll (mg g-1) of cotton seedlings of various BT and non-BT varieties (V).

Cotton	Varieties	Total Chlorophyll (mg g-1) Salinity Levels (NaCl mM) 0	50	100	150	200	250	ME (V)
		IE (S × V) (Means of 3 replicates ± standard error)						
Bt	CIM616	1.160 ± 0.05	0.914 ± 0.05	0.893 ± 0.03	0.652 ± 0.10	0.477 ± 0.02	0.364 ± 0.02	0.743 BC
	CIM598	1.240 ± 0.06	1.116 ± 0.04	0.966 ± 0.08	0.843 ± 0.05	0.800 ± 0.05	0.596 ± 0.07	0.927 A
	CIM179	1.063 ± 0.12	0.806 ± 0.05	0.749 ± 0.02	0.657 ± 0.04	0.452 ± 0.04	0.344 ± 0.02	0.678 CD
	CIM602	1.234 ± 0.11	0.980 ± 0.06	1.020 ± 0.05	0.789 ± 0.05	0.642 ± 0.09	0.608 ± 0.12	0.879 AB
	GH-Mubarik	0.816 ± 0.05	0.698 ± 0.03	0.616 ± 0.09	0.483 ± 0.03	0.375 ± 0.03	0.298 ± 0.06	0.548 DE
Non-Bt	Cyto124	1.294 ± 0.10	1.137 ± 0.15	0.969 ± 0.06	0.885 ± 0.11	0.661 ± 0.06	0.672 ± 0.10	0.936 A
	CIM554	0.909 ± 0.05	0.708 ± 0.04	0.585 ± 0.06	0.549 ± 0.10	0.436 ± 0.06	0.411 ± 0.04	0.600 DE
	CIM573	0.924 ± 0.05	0.714 ± 0.04	0.554 ± 0.06	0.462 ± 0.05	0.390 ± 0.03	0.390 ± 0.05	0.572 DE
	CIM620	1.218 ± 0.17	1.067 ± 0.08	1.032 ± 0.05	0.800 ± 0.06	0.673 ± 0.10	0.615 ± 0.18	0.901 A
	Lalazar	0.662 ± 0.06	0.590 ± 0.15	0.513 ± 0.06	0.359 ± 0.02	0.390 ± 0.04	0.381 ± 0.03	0.483 E
	ME (S)	1.052 A	0.873 B	0.790 B	0.648 C	0.530 D	0.468 D	

Means sharing different letters are significantly different (p ≤ 0.05). Non-significant interactive effect (S × V) did not have any letter.

ME = indicates main effect; IE = indicates interactive effect.

The findings of Bhute *et al.* (2012) regarding the decrease in fresh and dry weight of cotton root and shoot also supported the above argument. In the current study, a significant increase in shoot dry weight of cotton varieties BT CIM598, CIM602 and non-BT CIM620 confirmed their ability to tolerate the salt stress. Similarly, significant improvement in root dry weight confirmed the salinity tolerance ability of cotton varieties BT CIM598, CIM602 and non-BT Cyto124. According to Senaratna and McKersie (1983), increasing abiotic stress damaged the integrity of the cell membrane due to which the leakage of the electrolyte is increased.

Table 10. Effect of various levels of salinity (S) on electrolyte leakage (%) of cotton seedlings of various BT and non-BT varieties (V).

Cotton	Varieties	Electrolyte Leakage (%) — Salinity Levels (NaCl mM) — IE (S × V) (Means of 3 replicates ± standard error): 0	50	100	150	200	250	ME (V)
Bt	CIM616	24.0 ± 4.4	24.3 ± 5.5	31.7 ± 2.3	37.3 ± 1.9	48.3 ± 4.3	56.0 ± 3.5	36.9 BC
	CIM598	17.7 ± 1.8	23.7 ± 1.3	30.3 ± 3.8	33.0 ± 1.2	34.0 ± 6.2	45.3 ± 1.8	30.7 C
	CIM179	28.3 ± 3.8	31.0 ± 1.7	38.0 ± 1.5	47.7 ± 1.8	55.0 ± 3.5	57.0 ± 4.6	42.8 AB
	CIM602	21.0 ± 3.1	28.0 ± 1.5	25.7 ± 1.2	39.3 ± 2.8	50.0 ± 4.0	56.3 ± 3.4	36.7 BC
	GH-Mubarik	27.0 ± 4.6	31.3 ± 3.8	39.3 ± 2.6	51.3 ± 3.8	53.7 ± 5.5	56.7 ± 2.2	43.2 AB
Non-Bt	Cyto124	16.3 ± 5.0	20.0 ± 2.6	25.7 ± 6.1	37.7 ± 2.6	40.3 ± 2.8	43.3 ± 4.4	30.6 C
	CIM554	28.0 ± 2.1	30.7 ± 2.6	34.7 ± 3.8	44.3 ± 4.1	50.3 ± 3.5	59.3 ± 2.3	41.2 AB
	CIM573	26.0 ± 1.7	34.0 ± 1.5	34.0 ± 4.7	42.7 ± 4.1	57.0 ± 3.2	60.0 ± 3.2	42.3 AB
	CIM620	17.7 ± 2.7	25.0 ± 2.3	31.7 ± 3.5	34.0 ± 3.2	37.3 ± 1.9	43.7 ± 4.7	31.6 C
	Lalazar	29.7 ± 3.3	36.7 ± 4.6	41.7 ± 4.5	51.3 ± 2.6	55.3 ± 5.4	60.0 ± 2.6	45.8 A
	ME (S)	23.6 F	28.5 E	33.3 D	41.9 C	48.1 B	53.8 A	

Means sharing different letters are significantly different ($p \leq 0.05$). Non-significant interactive effect (S × V) did not have any letter.

ME = indicates main effect; IE = indicates interactive effect.

The results of the current study also showed a similar effect where electrolyte leakage was highest among those varieties (GH-Mubarik, Lalazar, CIM179 and CIM554) that were susceptible against salinity stress. Better synthesis of chlorophyll a, chlorophyll b and total chlorophyll also validated the fact of salinity tolerance among some BT and non-BT cotton varieties. Less synthesis of chlorophyll in salinity sensitive varieties was might be due to higher biosynthesis of ethylene in cotton seedlings.

The findings of Matile *et al.* (1997) justified our argument of low chlorophyll synthesis as they observed severe damage to chlorophyll due to activation of activation of chlorophyllase (chlase) due to degradation of lipid in the cell membrane.

Conclusion

It is concluded that BT CIM598, CIM602, non-BT Cyto124 and CIM620 has potential to grow under salinity induced stress. GH-Mubarik and Lalazarhave no or minimum potential to withstand against salt induced stress.

However, for better performance further pot and field investigation is need to introduce BT CIM598, CIM602, non-BT Cyto124 and CIM620 as salt tolerant varieties.

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