Research Article

Identification of Relationship among Exogenous NaCl with Cotton Leaves on Cation Uptake, Nutrient Ratios and Status in Rhizosphere Soil

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Abstract

Soil salinity is the main limitation, plants experience in saline environments. Though cotton is semi-tolerant to soil salinity, high salt concentration adversely affects cotton growth, seed cotton yield and fiber quality. Among the salts, sodium chloride (NaCl) dominates the saline areas. Therefore, an experiment was carried out to understand the exogenous NaCl differential stress over cotton crop nutrient uptake and others. Six cotton cultivars of Central India were tested with four concentrations of NaCl (control, 100,150 and 200 *m*M) in factorial completely randomized design under protected conditions. Artificial NaCl stress was imposed during the crop period in between 21-75 days after sowing (DAS). Cotton leaves were collected before flower initiation @ 45 days after treatment (DAT) for competing nutrient uptake studies. Na^+/K^+, Na^+/Mg^{2+}, and Na^+/Ca^{2+} nutrient ratios were also calculated. Soil nutrient status was also determined postharvest. The results explained that there is no quantitative uptake of cations. Na^+ competes maximum with K^+, Mg^{2+} than Ca^{2+} in the leaves. Low uptake of Na^+ was recorded in Jayadhar compared to others ions to increase in the NaCl concentration. Higher K^+ in Roja and higher Ca^{2+}, Mg^{2+} present in the leaves of G-Cot 25 and Jayadhar. Furthermore, accumulation of Na^+ and Cl^- reduced the uptake of water and other nutrients (Ca^{2+}, Mg^{2+} and K^+). Overall, an inverse relationship was observed between the increase in salt concentration and uptake of K^+, Mg^{2+} and Ca^{2+} in cotton leaves over control. Similarly, nutrient ratios were found to be increased with increasing the salt concentration over control irrespective of *Gossypium species* tested. A high accumulation of nutrient ions was recorded in soil. Based on the above results and established relationship, the order of adaptability can be concluded as in sequence of *G. herbaceum* > *G. arboreum* > *G. hirsutum* under NaCl stress

Keywords: Cotton species, nutrient uptake, nutrient ratios, sodium chloride, soil status

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Introduction

India is the largest cotton producer globally, but per hectare yields are low because the bulk of the crop area suffers due to one or more abiotic factors, namely, drought, heat, water logging and salinity. Among these abiotic factors, soil salinity affects about 6.73 Mha of cultivated land in India [1]. Soil salinity has been reported to result in significant loss in cotton production [2]. Yield reduction in cotton varies between 5 to 9 % due to salinity stress. However, above the threshold salinity level (7.7 dS m^{-1}) 50% yield reductions have been reported [3]. *G. herbaceum*, an Asiatic cotton species, is preferred to be cultivated in most of salt affected areas [4].

Though cotton is known to be moderately tolerant to salinity [5], tolerance level varies with different species and cultivars. Furthermore, different stages in the plant life cycle respond differently to soil salinity stress. For instance, germination and seedling stages are more sensitive than the vegetative stage, which is relatively tolerant to salinity stress [6]. Salt stress induces morphological, phenotypic changes in cotton. In salt affected areas the concentration of soluble salts such as NaCl, $NaHCO_3$, $MgSO_4$, $CaCl_2$, which dissociate into anions: chloride (Cl^-), sulphate (SO_4^-), carbonate (CO_3^-) and bicarbonate (HCO_3^-) as well as cations: calcium (Ca), magnesium (Mg), and sodium (Na). Ion toxicity inhibits plant water and nutrient absorption pattern as well as the transpiration [7]. As a result, it leads to physiological drought and decreased availability of phosphorus and micronutrients [8]. It is also reported to cause imbalance of cellular ions resulting in ion toxicity [9], osmotic stress and production of reactive oxygen species [10]. Consequently, plant growth and morphology is adversely affected [11]. Ion toxicity also results in membrane damage in root and other tissues, sub-optimal level of growth hormones, low or high enzyme activities, modified metabolic processes and finally cause the death of plant and crop failure [12].

Drought and salt stress are also responsible for plant oxidative stress [10]. The root cell membranes are the first one getting affected by the salinity because roots are the primary factor which play role in water and nutrient absorption. Which intern results in stunted root and shoot growth [13]. Varied salt concentration root zone determines the root growth and development. The effects of soil salinity stress on cotton leaves and their position had classified [14]. The subtending leaf of cotton boll (LSCB) is more sensitive than monopodia stem leaf (MSL) and salinity promoted sucrose export from the MSL, conversely inhibited sucrose export from the LSCB to cotton bolls. The leaf glandular trichome secrete more salts in salt tolerant than sensitive genotypes [14]. Ion distribution was low in salt tolerant genotypes and they have greater capacity for ion compartmentalization. An improvement in nutrient uptake potential of cultivars may enhance the salt tolerance of plants in saline environments [15]. Under saline conditions, NaCl accumulation proved to changes in soil properties [34]. With this background and available knowledge, this study was aimed to evaluate the effect of exogenous application of NaCl on the cotton nutrient uptake and changes in their ratios in the leaves along with status of rhizosphere soil.

Materials and Methods

Green house studies using two cultivars of the cultivated species namely, (*G. arboreum, G. herbaceum and G. hirsutum*) were conducted during 2017-18 at ICAR-Central Institute for Cotton Research, Nagpur. Commercial cultivars recommended for Central India viz., G-Cot 25 and Jayadhar (*G. herbaceum*), Phule Dhanwantary and Roja (*G. arboreum*), Suraj and LRA-5166 (*G. hirsutum*) were chosen for the study. The salt concentration included four levels (control, 100, 150 and 200 *mM* NaCl). The study was conducted in the factorial completely randomized design (FCRD). Each treatment had three replicates.

Soil was collected from the nearby cotton fields. The soil belonged to the fine, smectitic, hyperthermic, Typic Haplustert with clay textured, low in organic C, total N, available P and high in exchangeable K. The initial soil chemical properties estimated with internationally accepted standard protocols [33] and data have been presented in **Table 1.** The soil was air dried and sieved to pass through a 2 mm mesh sieve. About 10 kg air-dried soil was filled per pot. Four seeds were sown per pot. Two plants per pot were retained after 10 days of emergence. A common package of practices was followed in a net house. After the establishment of seedlings, NaCl stress was given for a 54 day period (21-75 DAS). NaCl solution was prepared and applied periodically on every third day up to flower initiation.

Table 1 Initial soil chemical properties

pH	EC	$CaCO_3$	OC	N	P	K	S	B	Cu	Mn	Zn	Fe	Ca	Mg	Na	Cl
	dSm^{-1}	%		$kg\ ha^{-1}$			$mg\ kg^{-1}$						$g\ kg^{-1}$			
7.80	0.26	4.30	0.40	120	15	900	15	02	3.5	13	0.6	07	0.04	0.5	2.2	0.06

Plant sampling and analysis

Fourth node leaf samples were collected 45 days after imposing the treatment. Leaf samples were cleaned with distilled water and placed in a hot air oven at 60 ˚C for 24 h. About 0.2 g dried leaves were taken in 50 ml volumetric flask, and digested with 4 ml of concentrated sulphuric acid (H_2SO_4) and 1ml of hydrogen peroxide digestion. Digested clear samples were made up to 50 ml volumetric flask followed by analysis and estimation of mineral nutrients. Total Na^+ and total K^+ in leaves were determined with flame photometer following standard method [16]. Leaf Cl^- content was estimated by Vohlard's method [17]. Leaf Mg^{2+} and Ca^{2+} concentration was determined using an atomic absorption spectrophotometer [16]. The ions present in cotton leaves and rhizosphere soil are expressed in g kg^{-1}. All competing nutrients and Na^+/K^+, Na^+/Mg^{2+}, and Na^+/Ca^{2+} nutrient ratios were also calculated. At harvest stage, soils were collected from the cotton rhizosphere. The soil samples were air dried and processed for determination of K, Na, Ca, and Mg. Exchangeable soil K and Na was determined using flame photometer [18]. The saturated soil paste was used for estimation of chlorine (Cl^-) and calcium (Ca). The silver nitrate titration method was performed for Soil Cl^- [19]. Ca and Mg were estimated by Versenate method [20].

Results

Artificial differential NaCl stress negatively affects the cotton cation nutrient uptake. In the present study, four NaCl treatments (up to 200 mM) with six commercial cotton cultivars and their interactions were examined.

Leaf Na, K, Mg and Ca content

The relative Na^+ ion concentration of cotton leaves was highly significant among the cultivars (C), applied NaCl (N) and interactions(C x N) (**Figure 1**). In general, plants in the control treatment had the lowest level of Na^+ ion.

Similarly with an increasing NaCl concentration (*m*M), Na^+ ion concentration in the leaves was increased. Averaged over cultivars, higher leaf Na^+ ion was present in 200*mM*, followed by 150*mM* over the control. Averaged across the salinity levels (NaCl concentrations) cultivars Na^+ ion uptake was varied. At 200 *m*M, Jayadhar showed low leaf Na^+ ions compared to control. It shows a higher salt tolerance than others. Among the six cultivars studied, leaf Na^+ ion was in the order of Jayadhar < Suraj < Phule Dhanwantary < Roja < G-Cot 25 < LRA-5166. Among the species the following sequence was observed in leaf Na of *G. herbaceum* < *G. arboreum* < *G. hirsutum.*

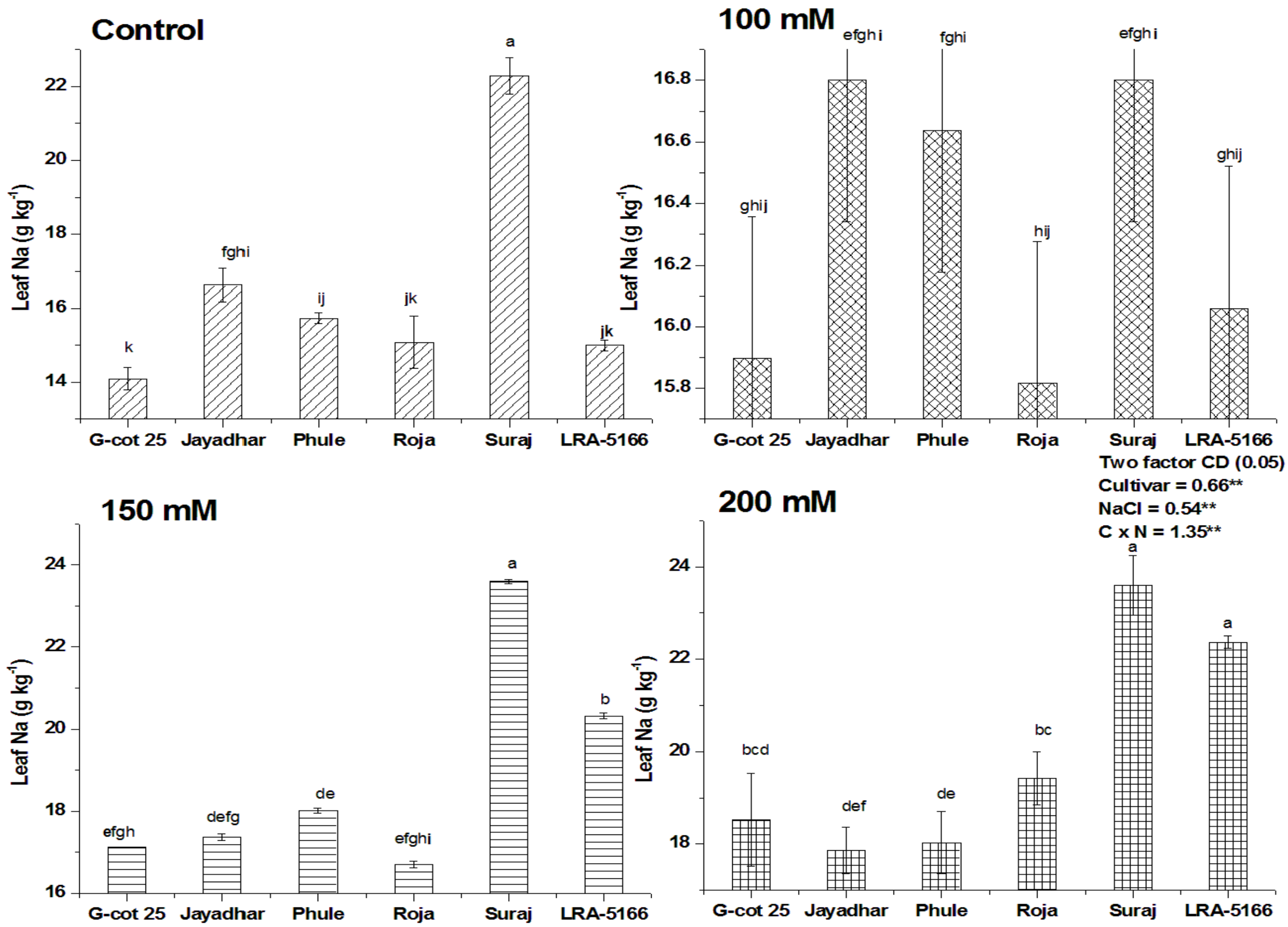

Figure 1 Differential salt stress on uptake of sodium in cotton leaves

Potassium (K) content in leaves differed significantly among cotton cultivars and NaCl treatments (**Figure 2**). In the present study, the K^+ concentration in leaf decreased with an increasing NaCl concentration. Magnitude of the reduction was greater at 200 *mM* followed by 150 *mM* over the control. However, minimum reduction was found in G-Cot 25 followed by Suraj at 200 mM. Among the cultivars tested, leaf K^+ ion reduction was in the order of G-Cot 25 < Suraj < LRA-5166 < Phule Dhanwantary < Jayadhar < Roja. However, the interactions (C x N) were non-significant. In Roja, higher leaf K^+ ion recorded, while it was varied response recorded to Phule Dhanwantary.

The leaf Mg^{2+}ion content of the cotton cultivars was significantly affected by NaCl content (**Figure 3**). However, interaction (C x N) effect was non-significant. The Mg^{2+} content in leaves decreased with increasing NaCl concentration up to 150 mM. At 200mM, the leaf Mg^{2+}ion content found increased than 150mM. Leaf Mg^{2+} ion content was the highest in all the cultivars of the control treatment. Lower leaf Mg^{2+} ion content was recorded with 200 *mM* NaCl, except Phule Dhanwantary, Suraj and LRA-5166. Overall, the average across the NaCl concentration (mM) the following trend was observed Phule Dhanwantary < Jayadhar < LRA-5166 < Roja < G-Cot 25 < Suraj.

NaCl treatments had a significant effect on the leaf Ca^{2+} content (**Figure 4**). However, interaction (C x N) effect was not significant. Similar to K and Ca, leaf Ca content decreased with increase inNaCl concentration. The reduction was greater at 200 *mM* followed by 150*mM* and 100 mM. Among cultivars, reduction of leaf $Ca^{2+}$was in order of Suraj < LRA-5166 <Phule Dhanwantary < Roja < Jayadhar < G-Cot 25.

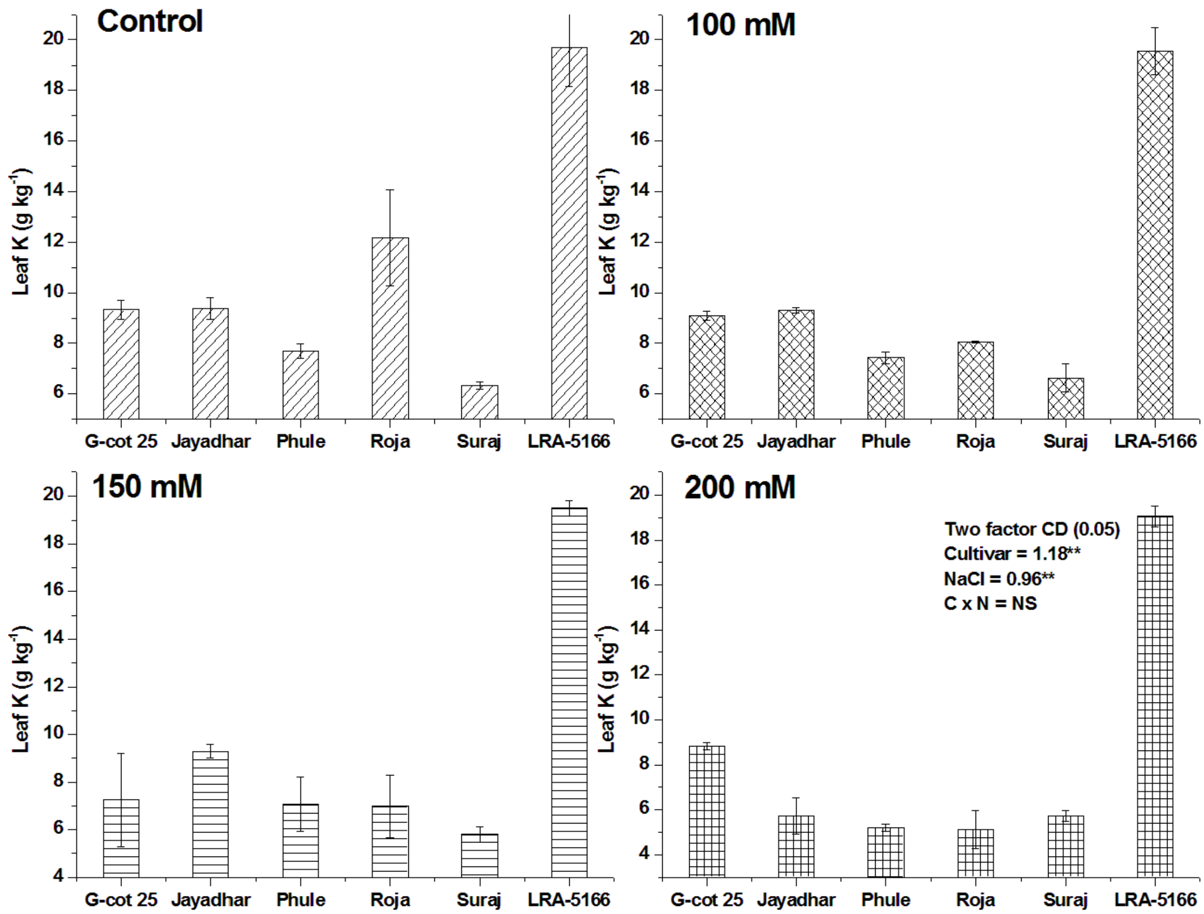

Figure 2 Differential salt stress on uptake of potassium in cotton leaves

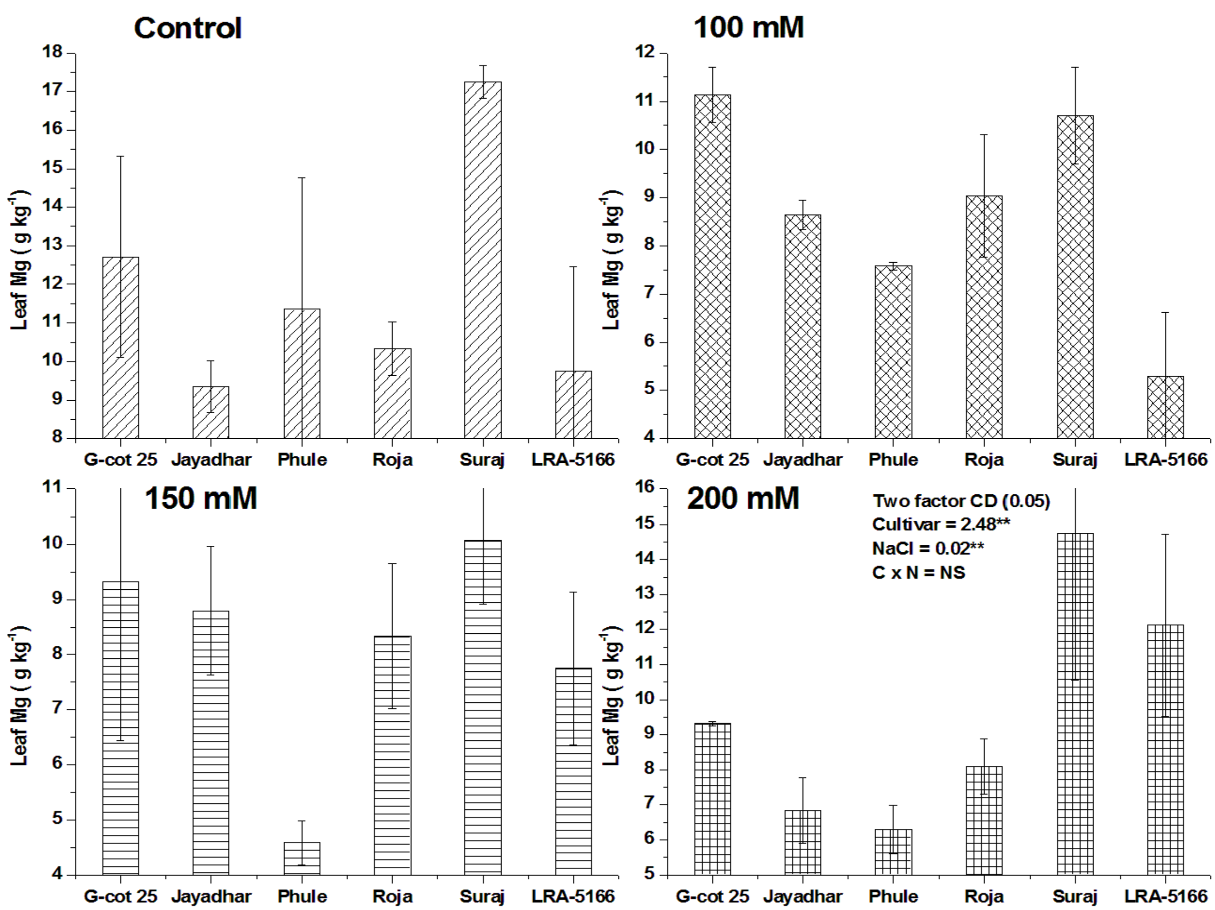

Figure 3 Differential salt stress on uptake of magnesium in cotton leaves

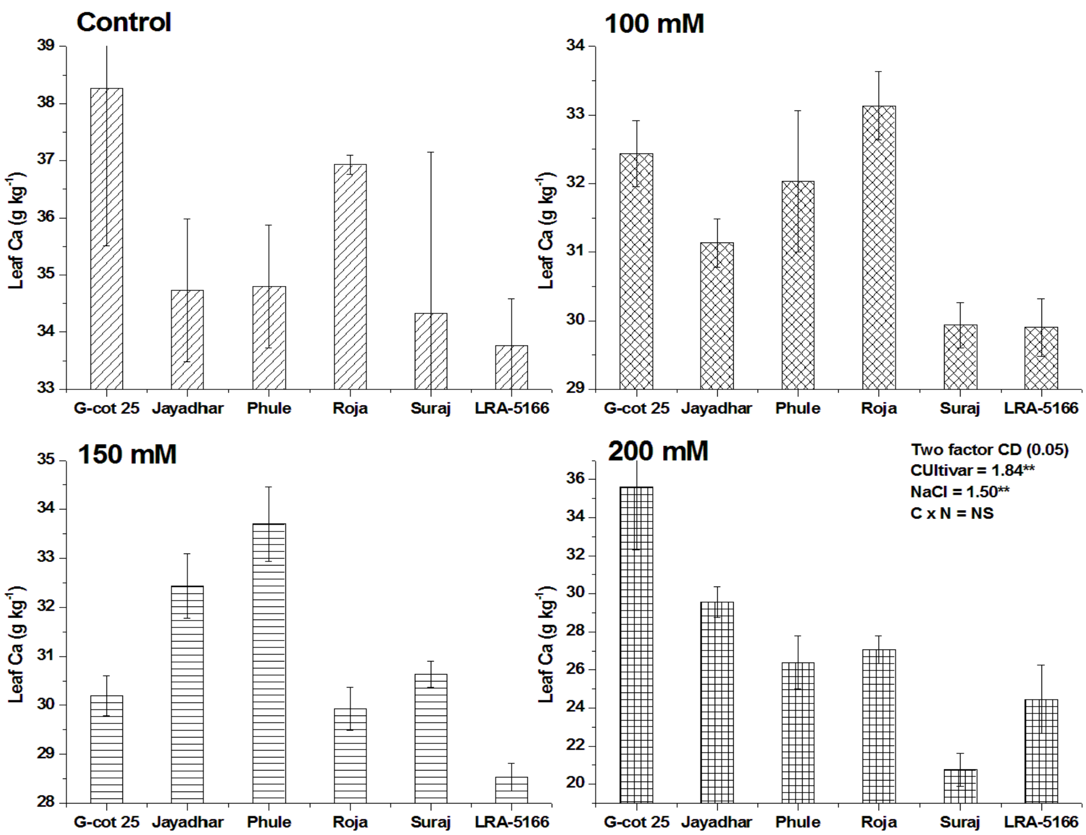

Figure 4 Differential salt stress on uptake of calcium in cotton leaves

Soil Na, K, Ca and Mg status

Soil Na content found to be increased in response to NaCl treatments (**Figure 5**). In case of LRA-5166 at 150 *mM*, Na content decreased, followed by 200 *mM* over control. At 100 *mM* increase in soil Na was more in Phule Dhanwantary (27%) followed by G-Cot 25 (21%); while low soil Na was found in Suraj (1%) followed by LRA-5166 (20%). At 150 *mM* higher soil Na was found in Phule Dhanwantary (34%) followed by G-Cot 25 (26%), while low Na content was observed in Suraj (13%) followed by Jayadhar (17%). At 200 *mM* high Na content found in Phule Dhanwantary (40%) followed by Roja (26%); while low Na found in LRA-5166 (10%) followed by Suraj (19%). Watering of artificial NaCl salt solution didn't influence the cotton rhizosphere sodium level and there were minimum amount of variation recorded at flowering. However, high salt accumulated in rhizosphere soil.

With respect to soil, exchangeable potassium differed significantly with NaCl treatments, but it was non-significant for cultivars and their interactions (**Figure 6**). Rhizosphere soil potassium content was higher and it got increased with increasing NaCl concentration in case of G-Cot 25, Phule Dhanwantary and Roja, while in case of Jayadhar, Suraj and LRA-5166, soil potassium was decreased by NaCl. At 100 mM, the content of potassium in the soil was higher in Roja (59%) followed by Phule Dhanwantary (28%); while the least potassium content was found in Jayadhar (12%) followed by Suraj (38%) compared to controls. At 150 mM NaCl high potassium content found in Phule Dhanwantary (158%**)** followed by G-Cot 25 (15%); while low K content in the soil found in Roja (26%) followed by Suraj (30%) over the control. At 200 mM high content of K was found in Phule Dhanwantary (196%) followed by Roja (89 %); while low content of Mg in the soil found in Suraj (15%) over control. These results ensure that the higher NaCl salt concentration competes with soil exchangeable potassium.

Cultivars and their interactions were significant. However, NaCl treatments were non-significant for soil magnesium (**Figure 7**). The relative soil magnesium varied among the treatments. After imposing NaCl stress, no similar pattern of reduction and increment was recorded in case of soil magnesium. In G-Cot 25 the higher reduction (2%) was recorded at 200 mM while high increment (3%) in soil magnesium was recorded at 150 mM over the control. In Jayadhar, higher reduction (1%) was recorded at 100 mM, while high increment (2%) was recorded at 150 mM concentration over the control. In Phule Dhanwantary higher reduction (5%) was recorded at 200 mM and increment (1%) was higher at 150mM over the control. In Roja, higher reduction was (2%) recorded in 100 mM and increment (5%) found at 100 mM over the control. In Suraj higher reduction (1%) was recorded in 200 mM and increment (2%) was recorded at 200 mM as compared to control. In LRA-5166 reduction (12%) was higher at 100 mM while increment was recorded (11%) in 200 mM.

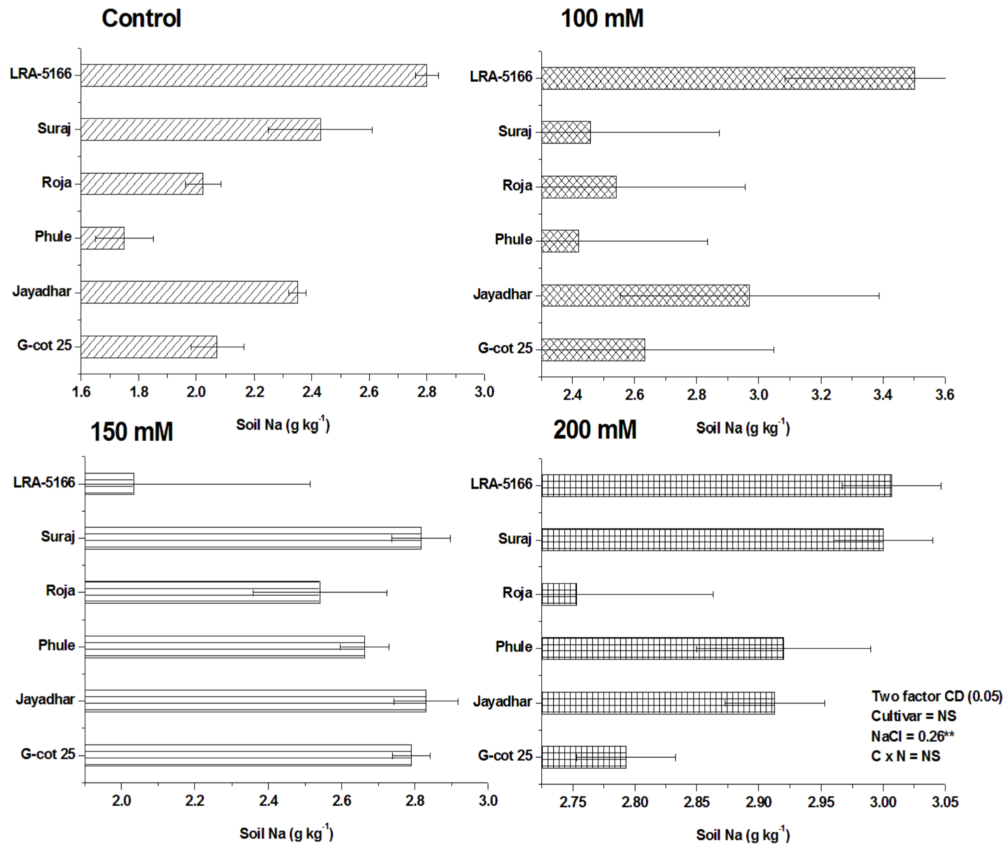

Figure 5 Effect of NaCl stress on sodium status in cotton rhizosphere soil

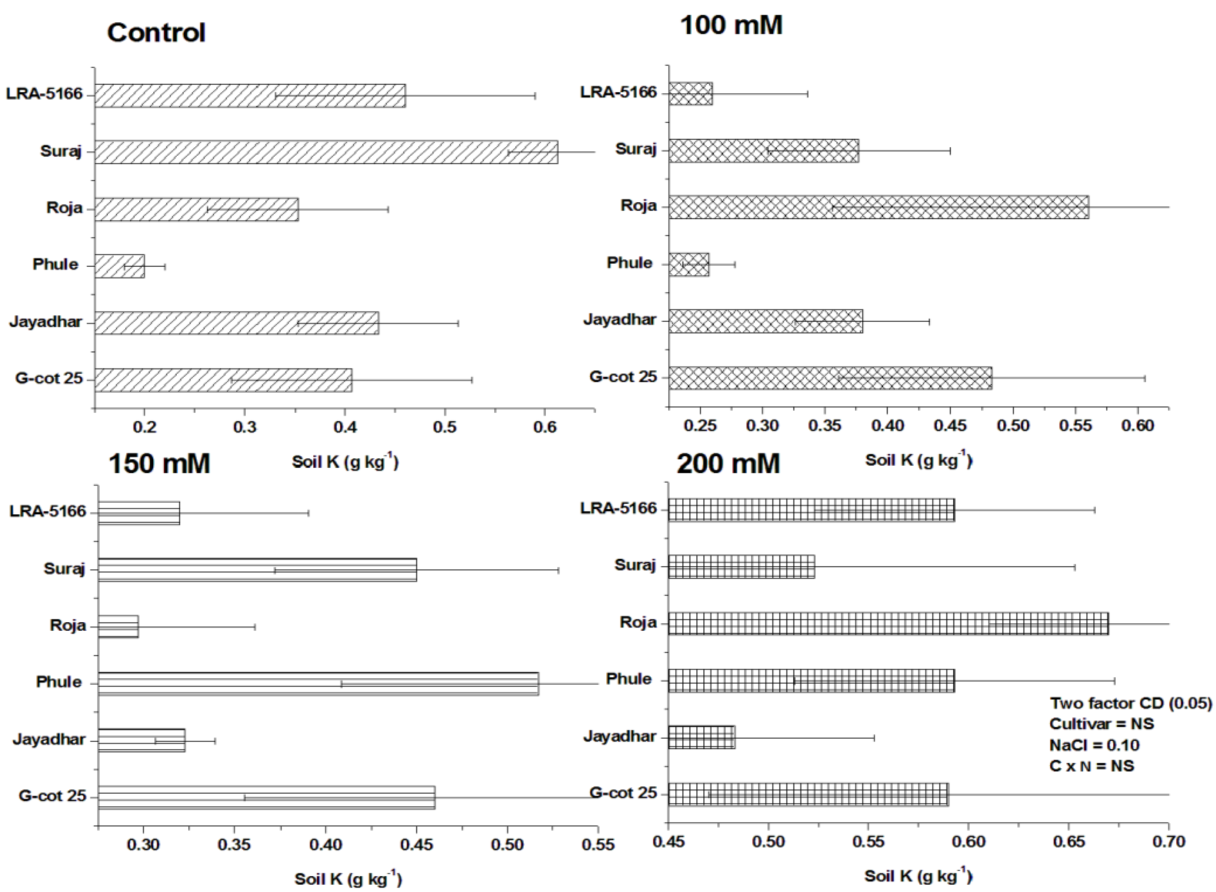

Figure 6 Effect of NaCl stress on potassium status in cotton rhizosphere soil

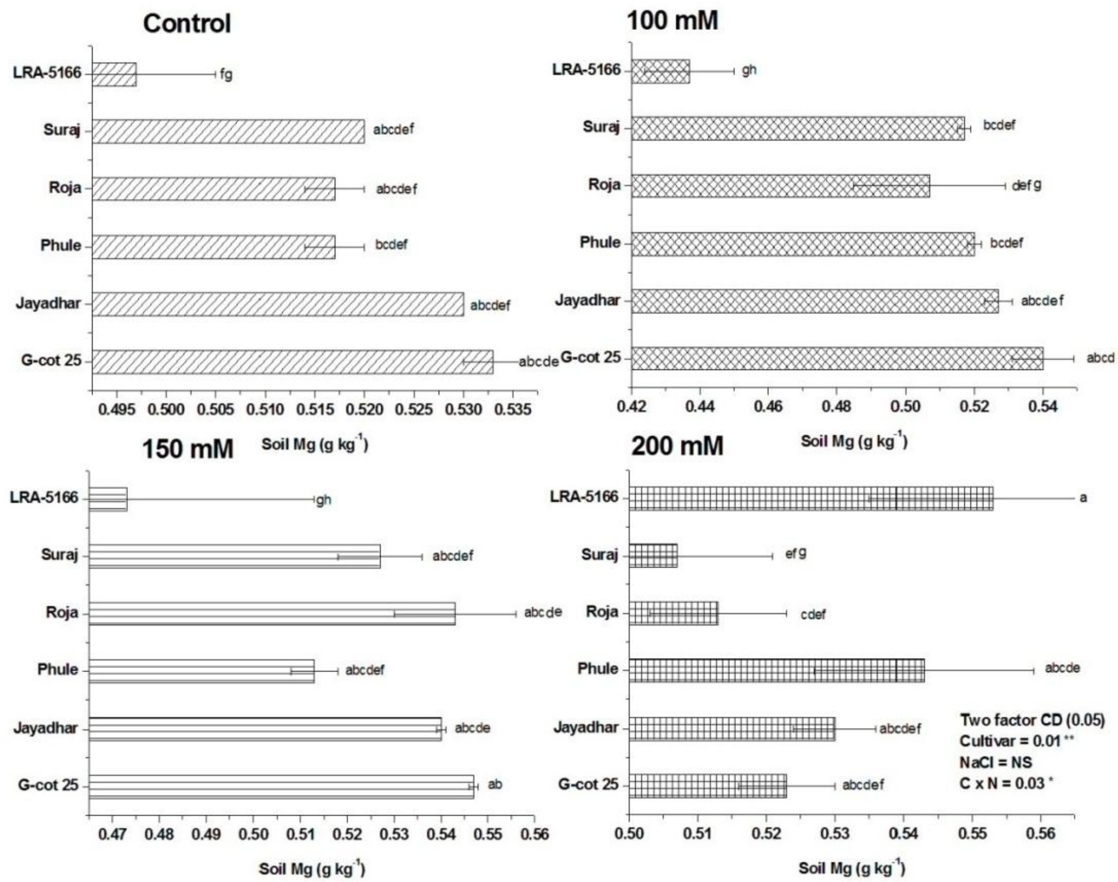

Figure 7 Effect of NaCl stress on magnesium status in cotton rhizosphere soil

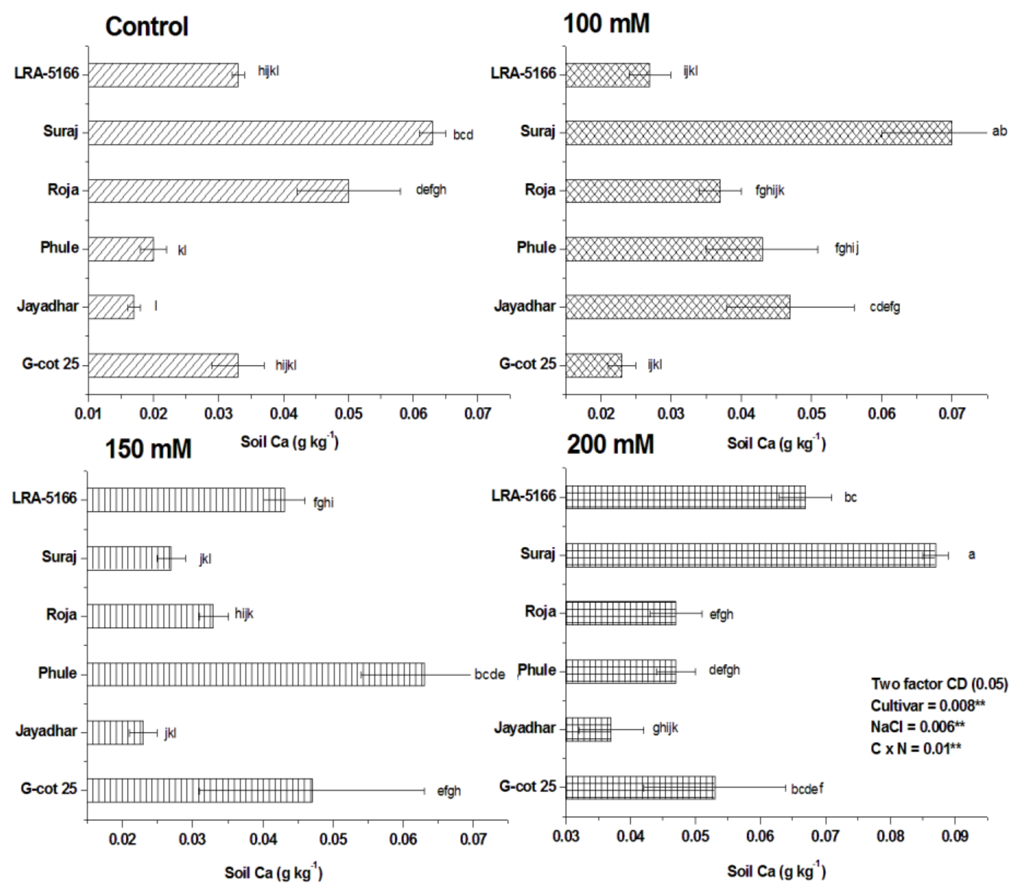

Figure 8 Effect of NaCl stress on calcium status in cotton rhizosphere soil

Soil calcium differed significantly and found to be increased with increasing NaCl concentration (**Figure 8**). The addition of NaCl changed the content of calcium in the soil, but not in the same manner. In G-Cot 25, high content (60%) of Ca was recorded at 200 mM and low content of Ca (30%) was recorded at 100 mM over the control. In Jayadhar, low content of soil Ca (35%) and high content of soil Ca (176%) was found at 100 mM. In Phule Dhanwantary, high content of soil Ca (115%) at 100*mM* and low content of soil Ca (215%) at 150 mM over the control. In Roja, high content of soil Ca (6%) was found at 200 mM and low content (26%) at 100 mM over the control. In Suraj, low content of soil Ca (57%) was recorded at 150 mM and high content (38%) was recorded at 200 mM over the control.

Nutrient ratios

Salt concentration significantly influenced the leaf nutrient ratios of the Na^+ / K^+, Na^+ / Mg^{2+}, and Na^+ / Ca^{2+}cotton cultivars (**Table 2**). It explained that the positive relationship with different nutrient ratios. Similarly, the increasing trend of the Na^+ / K^+, Na^+ / Mg^{2+}, and Na^+ / Ca^{2+} nutrient ratios was found. Across the NaCl applied, the average lowest leaf Na^+/K^+ ratio were recorded in LRA-5166 followed by G-Cot 25 and Jaydhar. The lowest leaf Na^+/Mg^{2+} ratio was recorded in G-Cot 25 followed by Suraj and Roja. The lowest leaf Na^+/Ca^{2+} ratio was recorded in G-Cot 25 followed by Jaydhar and Roja. Overall the Na^+ / K^+, Na^+ / Mg^{2+}, and Na^+ / Ca^{2+} nutrient ratios, among the species *G. herbaceum* exhibited lower values than others.

Table 2 Effect of NaCl on uptake nutrient ratio in cotton leaves

Cultivars	***Concentration (mM)***											
	Na^+/K^+				**Na^+/Mg^{2+}**				**Na^+/Ca^{2+}**			
	Control	**100**	**150**	**200**	**Control**	**100**	**150**	**200**	**Control**	**100**	**150**	**200**
G-Cot 25	1.51	1.75	2.36	2.10	1.11	1.43	1.84	1.99	0.37	0.49	0.57	0.52
Jayadhar	1.77	1.80	1.87	3.12	1.78	1.94	1.98	2.61	0.48	0.54	0.54	0.60
Phule Dhanwantary	2.04	2.24	2.55	3.47	1.38	2.20	3.93	2.87	0.45	0.52	0.53	0.68
Roja	1.24	1.97	2.39	3.80	1.46	1.75	2.01	2.40	0.41	0.48	0.56	0.72
Suraj	3.52	2.53	4.07	4.13	1.29	1.57	2.34	1.60	0.65	0.56	0.77	1.14
LRA-5166	0.76	0.82	1.04	1.17	1.54	3.04	2.62	1.84	0.44	0.54	0.71	0.91
Factors	$LSD_{0.05}$				$LSD_{0.05}$				$LSD_{0.05}$			
Cultivar	0.44**				0.63*				0.03**			
NaCl	0.36**				0.52**				0.03**			
Cultivar X NaCl	0.89*				NS				0.07**			

Discussion

Salinity impairs acquisition of mineral ions and consequently affects the nutrient content in the different tissues of cotton [21-22]. In the present study, we have evaluated the response of different cultivars to varying levels of NaCl concentrations. We observed a significant increase in the Na content of the leaves with increasing NaCl concentration (Figure 1). These results are in agreement [23-24]. Influx of Na^+ increased with increasing the level of salinity, whereas K^+ influx declined with increasing salinity. The uptake of K decreased with soil salinity and the concentration of Na, Cl and Ca increased in plant leaves as well as in other tissues [25]. But Ca^{2+} influx exhibits two different responses. High concentrations of NaCl disturb the $Ca^{2+}$and K^+ transport, which reduces the cotton growth. Ca^{2+}counteracts with NaCl stress and protects the plant. Salt tolerant plants leaves had lowest rate of Na^+ and Cl^- with the high compartmentalization of ions, which avoids the toxicity of salts [26]. Salinity decreases the Ca^{2+} and Mg^{2+} concentration in cotton under salt stress, without any change of K in leaves, but K got decreased in roots as a result of osmotic adjustment in the leaves due to Na^+ and Cl^- [27]. The supplement of Ca^{2+} under salt stressed cotton seedlings enhanced root elongation with interactions of Na^+ and Ca^{2+} in the cell wall, plasmalemma and cytoskeleton[9; 28-30]. The salinity decreases K content inside the tissue, which makes the plant sensitive to diseases [31]. Competing nutrient ratios play a major role in cotton growth and yield. Furthermore, maximum accumulation of ions (Na^+ and Cl^-) reduces water and uptake of other cations (Ca^{2+}, Mg^{2+} and K^+). Nutrient ratios were found to be increased with increasing salt concentration over control irrespective of *Gossypium species* tested. Similarly, Na^+ ions compete with K^+, Mg^{2+} than Ca^{2+} ions in leaves. Among the cultivars, that the order of adaptability can be concluded in sequence of *G. herbaceum* > *G. arboreum* > *G. hirsutum*. The absorption of Ca^{2+} and translocation of Mg^{2+}were limited by higher K^+ uptake.

Cotton cultivars had a differential tolerance to the levels of NaCl with regard to Na, K, Ca and Mg (Figure 1- 4). In general, leaf Na content increased with an increase in the NaCl concentration. The increased salt concentration

(Na^+, Cl^-) under cotton rhizosphere inhibits root, shoot and nutrient uptake,(Ca^+, Mg^+, K^+), flow and influx pattern. Similarly, cotton is highly vulnerable to yield reduction (30%) in salt affected soils [32, 35].

Conclusion

In this study, lowest uptake of Na^+ was observed with cultivar Jayadhar compared to the other cultivars. However, all cultivars showed an increase in Cl^- with increasing the NaCl concentration. Higher K^+ in Roja, however, higher $Mg^{2+}$and Ca^{2+}present in the leaves of G-Cot 25 and Jayadhar. Similarly Na^+ ions compete with K+, Ca^{2+} and Mg^{2+} ions. *G. herbaceum* recorded the lowest nutrient ratios and which reflected their salt tolerance potential than others.

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