Pak. J. Bot., 42(4): 2857-2866, 2010.

EVALUATING SALT TOLERANT COTTON GENOTYPES AT DIFFERENT LEVELS OF NaCl STRESS IN SOLUTION AND SOIL CULTURE

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Abstract

The study was carried out to compare generally used screening methods for salt tolerance: (i) a seedling-based, solution culture method, (ii) plant yield-based, soil method. The physiological and ionic analyses were used for comparisons of methodologies along with yield in soil based systems. The two methods were similar to each other by reproducing similar rankings for genotypes across the methods. In solution culture experiment, genotypes FH-113 and FH-911 produced significantly more (83 and 81% of control respectively) fresh weight compared to the rest of cotton genotypes, while in saline soil conditions the reduction was minimum in tolerant genotype FH-113 with magnitude of 40% in boll weight and 37% in number of bolls plant^{-1} at 21 dS m^{-1} with respect to control. The reduction in yield was much higher in salt sensitive genotype FH-5015 (47-75% of control) at 21 dS m^{-1}. The saline soil experiment demonstrated a higher sensitivity for discriminating Na^+ response among genotypes for yield reduction. It was concluded that solution culture selection approach can be helpful for the screening of cotton genotypes for salt tolerance, if using selective physiological traits and criteria.

Introduction

Salinity is the accumulation of dissolved salts in soil water (Munns, 2005) and a major limiting factor for crop yield in poorly drained soils (Patel *et al*., 2002). Low precipitation and high evapotranspiration are responsible for inadequate leaching and accumulation of salts in the root zone which hinders plant growth. Estimates vary, but approximately 800 million hectares or 7% of the total land surface of the earth is affected by salinity and sodicity (Munns, 2002). The problem is especially critical in semi-arid and arid regions like Pakistan, where hot climate with intensive irrigation using poor quality ground water alongside lack of drainage system is affecting 1/3rd of cultivated land of the country and causing heavy losses in crop yields (Khan *et al*., 2006).

Cotton is considered a moderately to fairly salt tolerant crop with a threshold level of 7.7 dS m^{-1} (Maas, 1986), yet its yield is drastically reduced due to poor germination and subsequent abnormal plant development under saline conditions (Khan *et al*., 2001). There is, however, a substantial variation in tolerance to salinity between cotton cultivars (Choudhary *et al*., 2001; Ashraf, 2002) and it would be necessary to use new cotton accessions as a genetic resource for increasing salt tolerant level of cotton genotypes (Basal *et al*., 2006).

Several screening and selection procedures have been adopted for evaluating salt tolerance in wheat and other crops (Kingsbury & Epstein, 1984; Kelman & Qualset, 1991; Pecetti & Gorham, 1997). Field screening procedures are not commonly used for evaluating salt tolerance in saline soils, because of spatial and temporal variability of soil salinity (Richards, 1993). Hence, screening experiments for salt-tolerant genotypes have been conducted under either *In vitro* or controlled environmental conditions (Kingsbury &

Epstein, 1984; Munns *et al*., 2000) and solution culture screening has been reported as a rapid and reliable method for screening cultivars with improved salt and drought tolerance, but reassessment of selected material under saline conditions has not been reported.

A number of researchers have suggested that screening for salt tolerance could be more effective if the assessment was undertaken under controlled conditions by using physiological markers/traits rather than selecting for yield and yield components under saline soil conditions (Yeo *et al*., 1990; Flowers & Yeo, 1995), for example, low Na^+ uptake and enhanced K^+/Na^+ discrimination (Gorham *et al*., 1984). Also, dry matter had been proposed as a criterion for selecting salt tolerant genotypes in bread wheat (Kingsbury & Epstein, 1984; Meneguzzo *et al*., 2000). However, the genotypic differences observed at early vegetative stage in hydroponic experiments may not necessarily correspond to those observed at the reproductive stage in the field (Zhu, 2001). The purpose of this work was to compare two methods of selection for salt tolerance and to assess the physiological and yield response of cotton genotypes under salt stress.

Materials and Methods

In this study, two concurrent trials were conducted in the wirehouse of Saline Agriculture Research Centre, University of Agriculture, Faisalabad, Pakistan (latitude; 31^o 25‘N, longitude 73^o 90‘E) during the year 2006–2007.

Solution culture experiment

Seed source and growth conditions: The cotton genotypes viz., FH-901, FH-911, FH-5015, FH-5052, FH-113, FH-963, FH-5018, FH-955, FH-954, FH-2529 and FH-6013 were collected form Ayub Agriculture Research Institute, Faisalabad.

Approximately 100 homogenized delinted (with commercial H_2SO_4) seeds were surface-sterilized with 0.04% (w/v) sodium hypochlorite and healthy seeds were taken for sowing in germination trays for nursery establishment. At two leaf stage, uniform seedlings were selected and transplanted to foam plugged holes in polystyrene sheet floating over 100 L iron tubs filled with aerated modified ½ strength Hoagland’s solution (Hoagland & Arnon, 1950). Three levels of salinity; 70, 140 and 210 mol m^{-3} NaCl along with control were developed by stepwise addition of NaCl salt in an increment of 50 mol m^{-3} per day until the desired levels were reached. The pH of the solution was daily maintained at 6.0-6.5. This range of concentrations was chosen to assess the expected wide variation in salt tolerance among the diverse cotton genotypes. The iron tubs were arranged in split plot design with four replications. All nutrient solutions were renewed weekly throughout the experiment. Shoot fresh weight was recorded at the time of harvesting and dry weight was measured after oven drying the samples at 65±5ºC.

Soil culture experiment

This experiment was conducted for further testing of the selected genotypes in saline soil conditions. For this purpose, 4 cotton genotypes viz., FH-113, FH-911 and FH-5018 were selected from solution culture experiment as salt tolerant and FH- 5015 as a salt sensitive genotype. A similar but smaller range of genotypes was used for pot experiment to keep the total pots in a feasible number. The seeds were delinted and imbibed in aerated $CaSO_4.2H_2O$ for 3 h before sowing in pots.

Normal sandy clay loam soil having EC_e = 2.48 dS m^{-1}, SAR=8.65 (mmol $L^{-1})^{1/2}$, Saturation percentage = 26.5%) was passed through 2 mm sieve and salinized with calculated amount of NaCl salt to develop three levels of salinity i.e., 7, 14 and 21 dS m^{-1} while control has the same EC_e as that of original soil. The filled pots were arranged in randomized complete design (CRD) with five replications per treatment and irrigated with tap water having an electrical conductivity (EC) 0.92 dS m^{-1}. At soil water content about half of the field capacity, soil was pulverized and the seeds were sown. After germination, one healthy uniform size seedling was kept in each pot. The recommended basal dose of N, P and K fertilizers was applied @ 100, 50 and 30 mg pot^{-1} in the form of urea, DAP and sulphate of potash, respectively. All the P and K were applied at the time of sowing and nitrogen was split into three doses; at sowing, at first irrigation and at flowering stage.

Extraction of leaf sap and ion analysis: The fully expanded younger leaves were placed in micro centrifuge tube and frozen. Leaf sap was extracted from frozen leaf samples after thawing and crushing with stainless steel rod (Gorham *et al.*, 1984). The sap was centrifuged at 6500 *g* for 10 min (Clandon T-53 centrifuge machine). The supernatant sap was used for Na^+ and K^+ determination by using Sherwood 410 flame photometer.

Results

In experiment-1 the shoot fresh and dry weight (Fig. 1) decreased with the addition of NaCl salt at different concentrations, while the maximum reduction occurred at 210 mol m^{-3} NaCl compared to control or other salinity levels (70 and 140 mol m^{-3} NaCl). The genotypes FH-113 and FH-911 produced significantly more (83 and 81% of control, respectively) fresh weight compared to the rest of the cotton genotypes and found tolerant even at the highest NaCl level. The leaf ionic analysis for Na^+ and K^+ concentration (Fig. 2) showed a successive increase in Na^+ concentration and decrease in K^+ in all genotypes with the gradual increase of salts in the growth medium. The highest level of salinity (210 mol m^{-3} NaCl) increased the Na^+ concentration in the leaf sap drastically compared to the 70 and 140 mol m^{-3} NaCl. On the other hand, a decrease in K^+ concentration at highest level of NaCl was observed; however the reduction was not so sharp. The comparison of different genotypes revealed a low Na^+ and high K^+ concentrations in tolerant genotypes like FH-113 and FH-911, while the concentration of Na^+ was much higher in sensitive genotypes.

In soil culture experiment, the germination percentage (Fig. 3a) salt tolerant genotypes was better compared to FH-5015, however, the reduction was significant in all genotypes and successive decrease with increasing salinity was found. The decrease was more pronounced in the sensitive genotype FH-5015 and 75% reduction was observed at 21 dS m^{-1}. In case of tolerant, the decrease in germination was 40% as compared to control.

The salt tolerant genotypes showed less reduction in leaf area and chlorophyll contents (Figs. 3b & 3c), while reduction was significantly more in case of salt sensitive genotype. FH-901 had the highest and FH-5015 showed the lowest leaf area. The maximum chlorophyll contents were in FH-5018, followed by FH-113 and the minimum were in FH-5015. The reduction in chlorophyll contents with increasing salinity was more pronounced in FH-5015, especially at 21 dS m^{-1} showing 61% reduction compared to respective control while, FH-911 showed 18% reduction in chlorophyll contents at the same level.

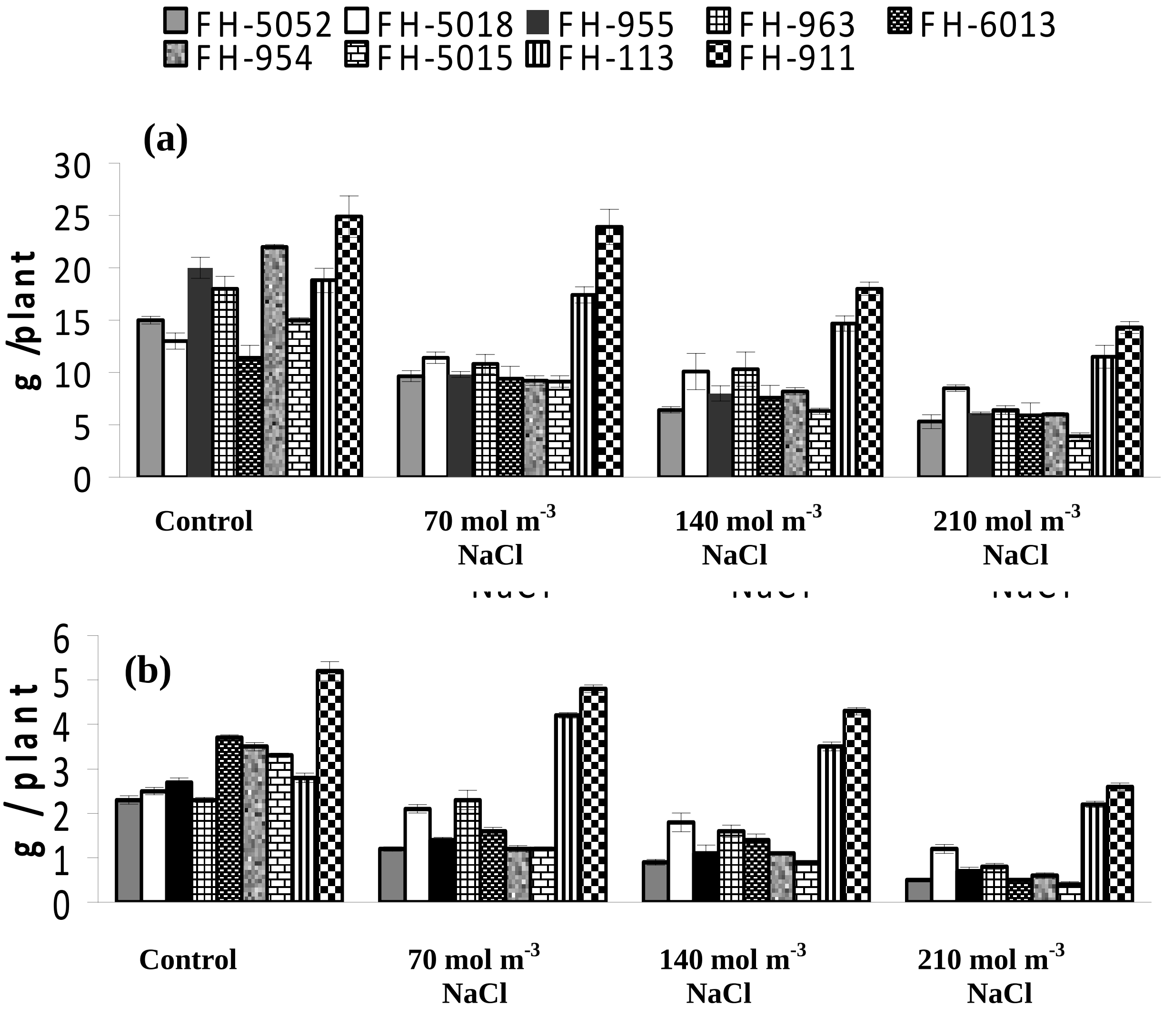

Fig. 1. Shoot fresh (a) and dry weight (b) of cotton genotypes at three levels of salinity after 45 days of salt stress.

The reduction in weight and number of bolls per plant (Fig. 4) was much higher in sensitive genotype FH-5015 (75 and 47% of control, respectively) at 21 dS m^{-1}. The reduction was the minimum in tolerant genotypes and magnitude of reduction was 40% in boll weight and 37% in number of bolls per plant with respect to control at 21 dS m^{-1} in FH-113. However, FH-911 produced the highest number of bolls and boll weight per plant at 21 dS m^{-1} which was non significant to that of FH-5018 and FH-113.

In the present study performance of hydroponically selected salt tolerant cotton genotypes was tested in pot experiment using saline soil to compare the two selection techniques for screening of salt tolerant germplasm. Shoot fresh and dry matter is considered important stress-responsive determinants to evaluate salt tolerance in controlled conditions (Kingsbery & Epstein, 1984; Saqib *et al*., 2002). In solution culture, where yield in term of fruit is not possible, dry matter becomes main indicators along with ionic homeostasis. Cotton genotypes screened on the basis of their high biomass production in solution culture experiment were compared on yield and ionic basis in soil experiment. It was found that solution culture tested genotypes performed equally well regarding chlorophyll contents and ionic homeostasis. FH-911 and FH-113 were screened from solution culture as tolerant along with FH-5015 as sensitive one and these genotypes maintained their respective ranking in soil culture when yield was compared.

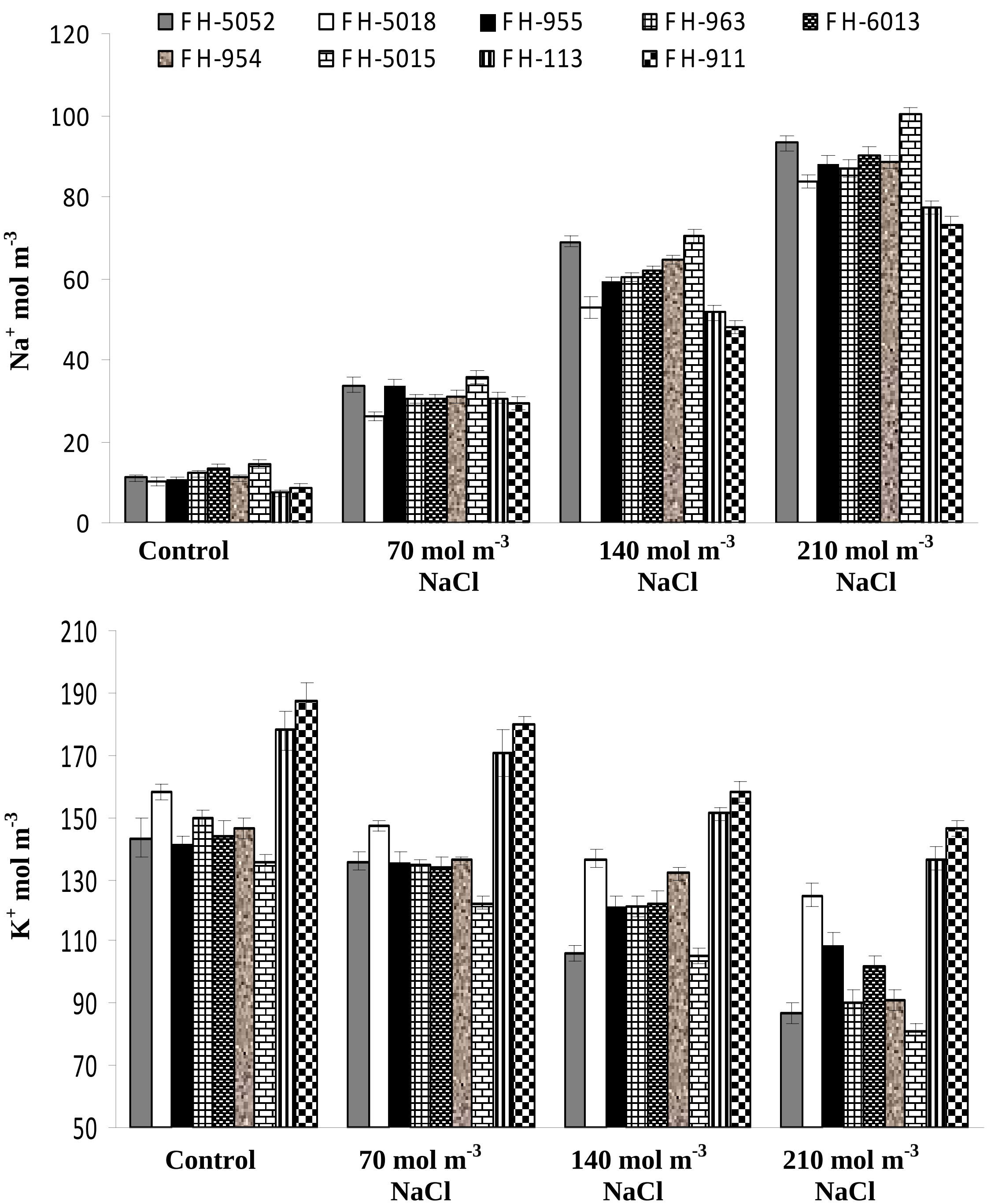

Fig. 2. Sodium and potassium concentrations (mol m^{-3}) in leaf sap of cotton genotypes at different levels of salinity after 45 days of salt stress.

Discussion

During salt stress, salt-sensitive plants clearly showed chlorophyll degradation and growth reduction. Whereas, high chlorophyll content (SPAD value) were observed in salt tolerant genotypes. These higher values of chlorophyll attributed to an increased photosynthetic rate, more dry matter production and higher productivity (Khan *et al*., 2009) and could be a reason for higher yield of salt tolerant genotypes under salt stress (Harinasut *et al*., 2000). The reduction in chlorophyll contents under stress environments was observed in previous studies (Ashraf *et al*., 2005; Iqbal *et al*., 2006; Khan *et al*., 2009) with sever reduction in salt sensitive genotypes.

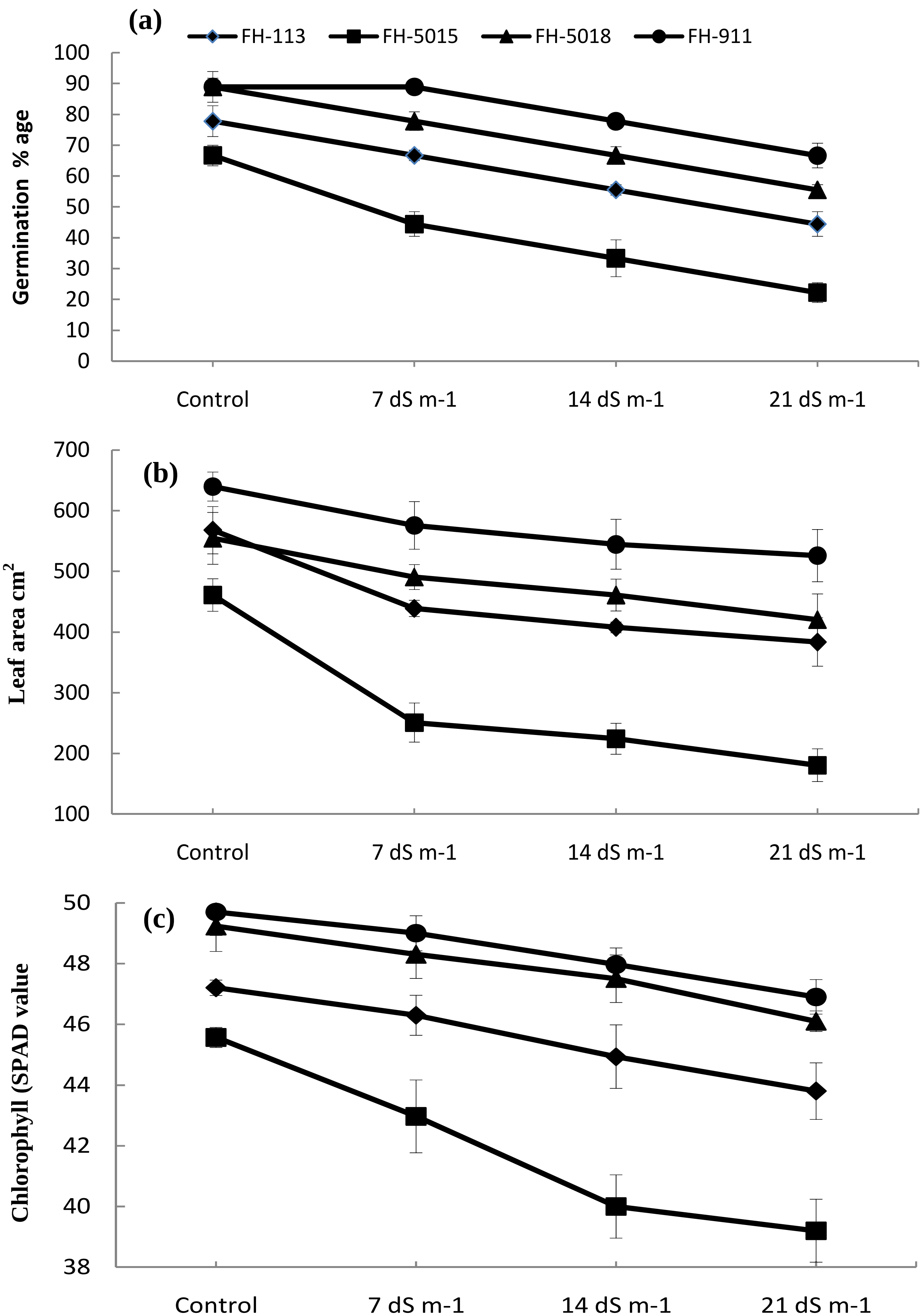

Fig. 3. Germination % age (a), Leaf area plant^{-1} (b) and chlorophyll (SPAD units) content (c) of salt tolerant (FH-113, FH- 5015 and FH-911) and salt sensitive (FH-5018) genotypes at three levels of NaCl salinity.

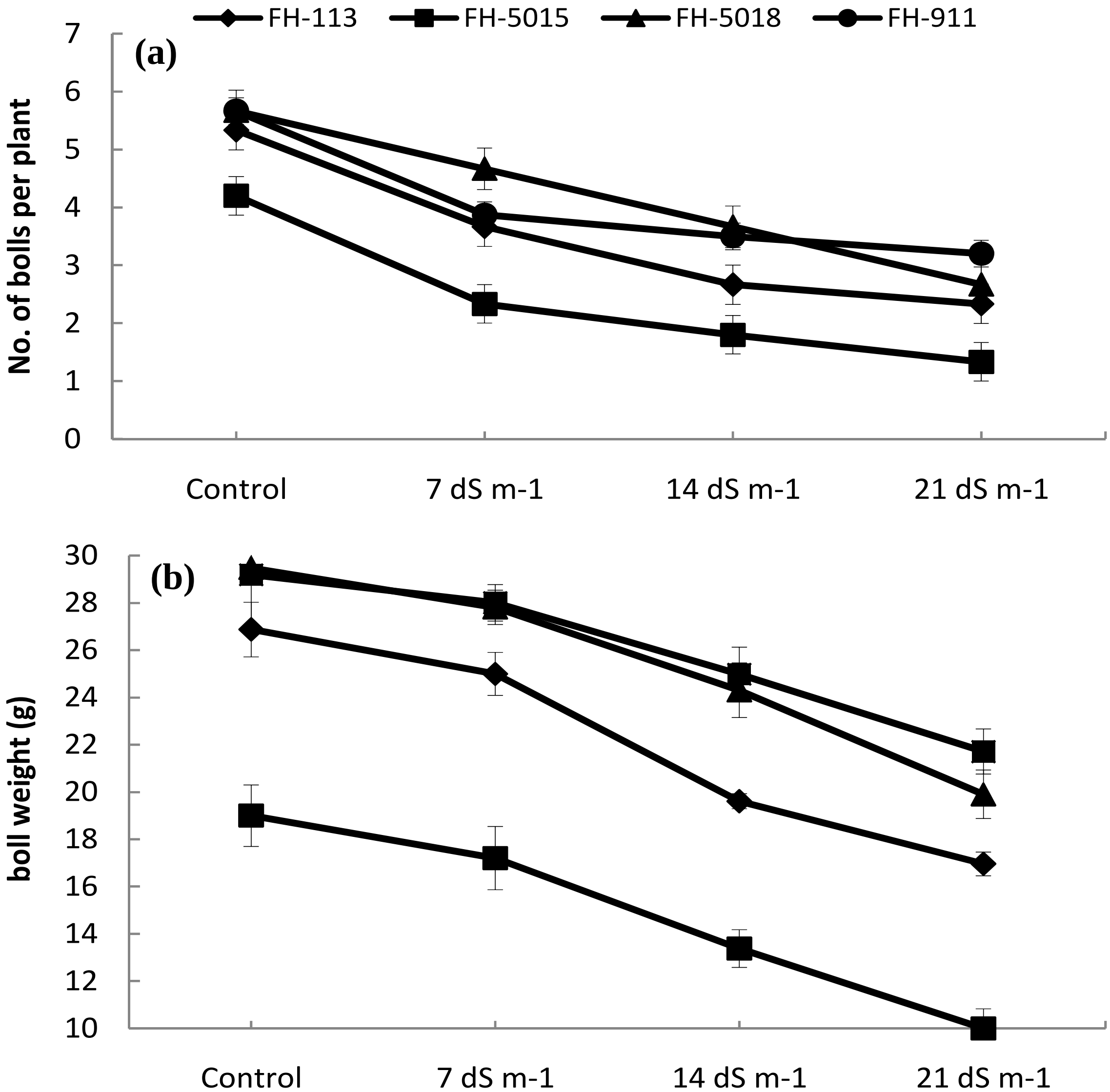

Fig. 4. Number of bolls per plant (a) and weight of bolls plant^{-1}(b) of salt tolerant (FH-113, FH-5018 and FH-911) and salt sensitive (FH-5015) genotypes at three levels of NaCl salinity.

The higher Na^+ concentration in leaf sap with increasing salinity is one of the primary plant responses to salinity stress (Meneguzzo *et al*., 2000) and its higher concentration disturbs the different metabolic activities (Akram *et al*., 2007). The genotypes having ability to retain Na^+ in the root could survive better under stress conditions (Khan *et al.,* 1990; Akram *et al*., 2007). Although genotypic differences were statistically significant in both the experiments but salt tolerant genotypes screened from solution culture showed lower Na^+ accumulation compared to salt sensitive genotypes. This is also evident that genotypes selected from solution culture experiment as salt tolerant due to their lower Na^+ concentration maintained this trait in soil culture as well, while salt sensitive cotton genotype had more Na^+ in leaf sap. It was also suggested that salt tolerance is mostly associated with Na^+ uptake (Santa-Maria & Epstein, 2001), salt exclusion (Colmer *et al*., 1995) and its compartmentation within cell or/and within plant (Ashraf, 1994). The higher amount of Na^+ ion in plant could be due to increased concentration in rooting medium, diffusion through damaged membranes and/or lower efficiency of exclusion mechanism (Akhtar *et al*., 2005).

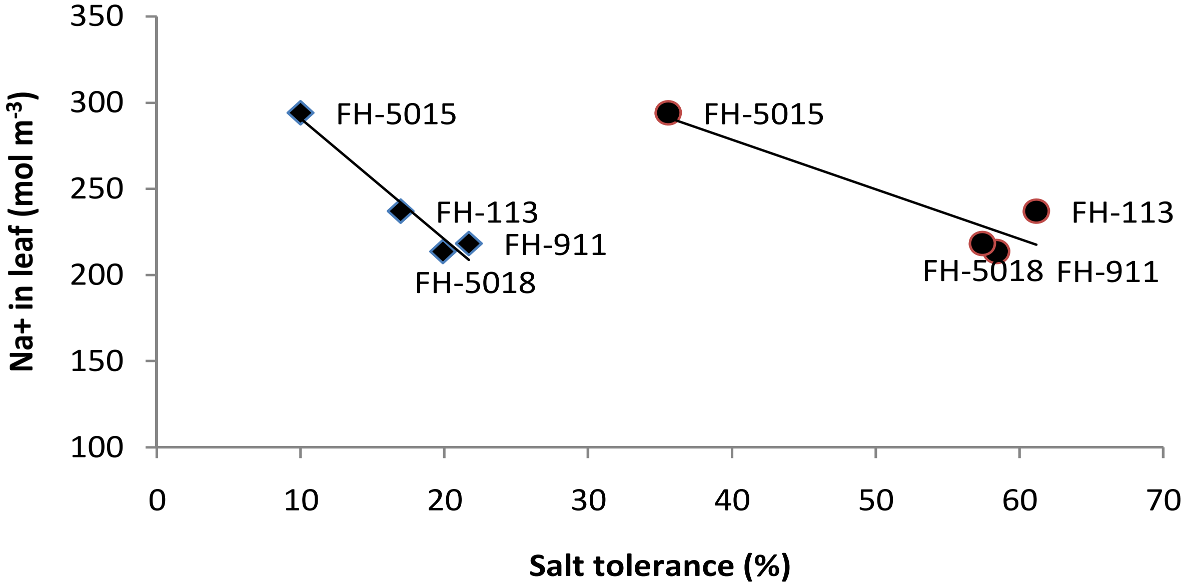

Fig. 5. The correlation between Na^+ concentration (mol m^{-3}) in leaf and salt tolerance % with respect to cotton boll weight (♦R^2 = 0.96) in soil condition and salt tolerance (%) with respect to shoot dry weight (●R^2 = 0.84) in solution culture experiment. The salt tolerance (%) of cotton genotypes is calculated as percent reduction at 210 molm^{-3} NaCl stress with respect to control values.

As Na^+ is the key ion impairing plant growth under salt stress and most of the researchers used shoot dry weight as growth indicator in solution culture experiments along with ionic analysis for salt tolerance assessments. Therefore, salt tolerance (% reduction in salt stress with respect to control) was calculated on the basis of shoot dry weight and cotton yield and its correlation was drawn (Fig. 5) with leaf Na^+ concentration at 210 mol m^{-3} NaCl salt stress. A highly significant negative relationship was observed for salt tolerance (%) with shoot dry weight (r^2 = 0.82) and cotton boll weight (r^2 = 0.96). This relationship of Na^+ accumulation with salt tolerance was previously described by many researchers (Schachtman & Munns, 1992; Saqib *et al*., 2006). Although, the genotypes maintained their respective rankings for salt tolerance in both indicators, yet yield can be observed as more sensitive to Na^+ concentration in leaf as compared to shoot dry weight of the plant.

Conclusion

In conclusion, solution culture screening technique was equally successful for selection and recognition of salt tolerant genotypes in cotton. The both methods experiments propounded similar ranking for genotypes across the methods. It is also suggested that preliminarily selection in solution culture experiments under controlled conditions by using established physiological traits and criteria could be important step in process of breeding/selection of salt tolerant genotypes of field crops.

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(Received for publication 15 October 2009)