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Research Article
Morpho-Biological Traits of Upland Cotton at the Germination Stage under Optimal and Salinity Soil Conditions

[1]Ilyos S. Normamatov, [1]Abdusalom Kh. Makamov, [1]Umidjon A. Boykobilov, [1]Sanjar G. Achilov, [1]Naim N. Khusenov, [1]Jurabek K. Norbekov, [1]Maftuna M. Kholmuradova, [1]Zebo Z. Yuldashova, [1]Mukhammadjon Kh. Mirzakhmedov, [1]Shuhrat O. Kushakov, [2]Otabek G. Karabekov, [1]Mukhtor M. Darmanov, [1,3]Akmal M. Asrorov and [1]Zabardast Buriev

[1]Department of Marker Association Mapping, Center of Genomics and Bioinformatics, Uzbekistan Academy of Sciences, University Street 2, Kibray, Tashkent 111215, Uzbekistan
[2]Scientific Institute of Soil Science and Agrochemistry, Kamariso street 3, Almazar, Tashkent 100179, Uzbekistan
[3]International Agricultural University, University Street 2, Kibray, Tashkent 111215, Uzbekistan

Abstract
Background and Objective: Soil salinity is a major abiotic stress factor affecting cotton production worldwide. This study aimed to deal with the morpho-biological traits of cotton affected by chloride-sulfate salt during the germination stage. **Materials and Methods:** About 21 cotton genotypes were obtained from Uzbekistan germplasm. Plant materials were grown in a greenhouse under optimum and soil salinity (mainly strong sulfate salinity) conditions. Data on plant morpho-physiological traits including germination rate, plant length and plant fresh and dry weight were collected at the seedling stage. The greenhouse experiments lasted 30 days. **Results:** Namangan-77, considered to be susceptible to salinity, was highly damaged by salinity stress. Salt-resistant KK-1795, Hapicala 19, Zangi Ota and An-Boyovut-2 varieties showed better morpho-biological characteristics than other varieties. A PCR test with the salinity-associated BNL-3140 marker proved that these varieties are molecularly resistant to salinity. **Conclusion:** Results on the plants' germination rate and biomass as well as salinity-associated primer testing resulting from highly sulfated saline soil serve to identify genes that respond to salinity.

Key words: Morpho-physiological traits, salt stress, Polymerase Chain Reaction (PCR), Simple Sequence Repeats (SSRs), Quantitative Trait Locus (QTL), alkaline, soil salinity

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Corresponding Author: Ilyos S. Normamatov, Department of Marker Association Mapping, Center of Genomics and Bioinformatics, Uzbekistan Academy of Sciences, University Street 2, Kibray, Tashkent 111215, Uzbekistan

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Soil salinity is one of the significant abiotic stresses that reduce plant productivity and damage quality worldwide[1]. Globally, approximately 800 million hectare of agricultural land is used for human consumption and 6% of it is represented as high salinity[2]. Due to climate change, enhanced sea levels and disproportionate irrigation systems, salinity-affected areas have already enlarged[3]. Owing to soil salinity, crop areas around the world are reducing, furthermore, competition between grain and fibre crops is growing. As a result, cotton fields are becoming more saline and alkaline[4]. It is supposed, if this increase in salinity level continues to grow, 50% of the total existing cultivated field might face salinity stress by 2050[5]. Besides, owing to environmental degradation disposed of by climate change, the salinity problem may also become more serious in some areas with low and mid-latitudes. Moreover, the global population is assumed to attain approximately 9 billion by 2050[6]. Thus, investigating salt-tolerant plant genotypes has been becoming a vital issue. Developing and breeding salinity-tolerant crop genotypes is one of the efficient approaches to struggle against salinity stress. Worldwide, there are various plants including cotton genotypes that are tolerant to soil salinity. The cotton plant is a source of seed oil, feed for livestock and heat energy. The fibre crop is an essential industrial raw material, required in huge amounts by the world population. The requirement for cotton fibre is increasing because of the growing world population. The main percentage of cotton fibre is generated by *Gossypium hirsutum* (90%)[7] and 8% belongs to *Gossypium barbadense*[8]. An adequate assessment of cotton genotypes is an important factor for salinity tolerance from a genetic aspect[9].

In general, salt tolerance is a multifaceted phenomenon and plant cells have some mechanisms, to counteract this form of stress levels. The main effect of salinity stress is osmotic stress, which begins with an increase in the concentration of Na^+ and Cl^- in the root of a plant[10]. The root system formation at the seedling stage is extremely important for the development and performance of cotton[11]. As the concentration of NaCl in the soil increases, it delays the emergence stage of plant seeds. So, at very high concentrations of NaCl (6.0 dS m^{-1}), emergence percentage (EP), emergence speed index (ESI) and mean time to emergence (MTE) were adversely affected[12]. An increase in the amount of Na^+ in the cell reduces the process of cellular assimilation of K^+ and Ca^{2+}, which disrupts the effective order of stomatal regulation and slows down the growth and photosynthesis of plants[13]. Na^+ ion excess accumulation has a toxic effect that adversely affects the physiological process of the plant and reduces the amount of water[14].

Plants naturally overcome salt stress by enhancing osmotic adaptation, maintaining ionic balance and maintaining the water content of tissues[15]. Salinity also affects the photosynthetic apparatus in plants. Salinity stress was shown to reduce the number of photosynthetic pigments and carbohydrates in plants[16].

India, China, United States, Pakistan, Brazil, Australia and Uzbekistan are among the top 10 cotton-producing countries[17]. Salinity affects the cotton crop yield possibly resulting from the damaged germination rate of the plant. The germination stage of cotton is considered the most salt-sensitive period[18].

This study evaluated the morpho-biological traits resulting from the effects of chloride-sulfate salt on the germination stage of cotton and screened with BNL-3140 SSR (single sequence repeat) marker, associated with salinity.

MATERIALS AND METHODS

Experimental design: On August 6, 2021 seeds were sown based on a randomized complete design in a mixture of sterilized 40% sand and 60% strong sulfated saline and non-saline soil in plastic containers at a depth of 3 cm ($10\times10\times10$ cm) each pot contained 7- 9 plants, 450 g mixture. Young saplings were grown in a greenhouse under 16 hrs photoperiod, the temperature was between 25-30°C. Phenotypic changes were observed for 30 days. During this period, cotton genotypes growing in optimal conditions were irrigated 12 times with 50 mL of water and cotton samples growing in saline soil were watered six times with 50 mL. Due to the possibility of root damage of young seedlings under saline conditions, the plants growing in saline soil were watered less frequently.

Soil ion analysis: The salt composition in the soil samples of various depths was studied at The Scientific Research Institute of Soil Science and Agrochemistry. The salinity level of the soil samples was determined as follows. Chlorine was quantified by titration with nitric acid in the presence of silver nitrate ($AgNO_3$). Quantification of calcium and magnesium was performed with Trilon B. Sulfate ion was determined by barium chloride. Potassium and sodium were quantified by using a flame photometer. The dry residue was determined based on constant weighting at 105°C in a thermostat.

Plant materials and growth parameters: The main goal of the study was to identify cotton genotypes resistant to salinity.

Table 1: List of upland cotton cultivars used in the study

Local names	Origin
An-Boyovut-2*	Uzbekistan
Namangan-77**	Uzbekistan
KK-1795***	Uzbekistan
Hapicala 19***	Australia
Zangi Ota***	Uzbekistan
KK-1796	Uzbekistan
L-1000	Uzbekistan
C-9006	Uzbekistan
KK-1086	Uzbekistan
Catamarca 811	Argentina
C-9008	Uzbekistan
L-N1	Uzbekistan
L-141	Uzbekistan
0-30	Uzbekistan
C-4769	Uzbekistan
L-45	Uzbekistan
Saenr Pena 85	Argentina
C-2025	Uzbekistan
KK-602	Uzbekistan
SAD-3511	Mexico
C-417	Mexico

*An-Boyovut-2 is the resistant variety to salinity, **Namangan-77 is the susceptible variety to salinity and ***KK-1795, Hapicala 19 and Zangi Ota varieties were found resistant in this work

In total, 21 cotton genotypes were studied for salinity resistance, The Namangan-77 and An-Boyovut-2 varieties were used as susceptible and resistant controls, respectively. The remaining 19 cotton genotypes were chosen due to their high fibre quality. The seeds used in this work were obtained from the germplasm of the Center of Genomics and Bioinformatics: 16 varieties were local and 5 were from Argentina, Australia and Mexico, diverse regions of the world (Table 1).

Biomass determination: Greenhouse experiments were terminated when 30 days-old cotton seedlings, grown under optimal and salt stress, were collected. Their length and weight were determined. Further, the plants were divided into roots and shoots that were dried at 65°C (E028-230V-T) for 3 hrs to establish the dry weight. Average mean value data on shoot and root weight of fresh and dried samples were recorded. The dry weight of the dismembered shoot and roots of the plants was measured by Digital Precision Analytical Balance (FA2204).

DNA isolation: DNA was isolated from young leaves of randomly selected samples using the CTAB method to identify genetic diversity among cotton genotypes[19].

Statistical analysis: The experiments were set up as a completely randomized design, including 21 cotton cultivars and two different conditions. The collected data were statistically assessed by the GraphPad program.

RESULTS AND DISCUSSION

Soil sample composition: The emergence of young seedlings is a stage of extreme sensitivity to salt stress[15]. The fresh weight of cotton seedlings is greatly influenced by soil salinity[20]. Salinity harms the growth and development of cotton, plant height, fresh and dry weight, plant, root shoot ratio, leaf area and development and other physiological indicators[21,22]. Thus, in this study, a comparative study was conducted on morpho-biological indicators during the germination stage of cotton varieties under optimum and salt-affected conditions in a greenhouse. Before starting the research, soil samples were taken at the 0-30 cm depth in the Mirzaobod District of the Syrdarya Region. According to recent reports, saline soil in this region mainly comprises NaCl and Na_2SO_4. The detrimental effects of NaCl on plants were due to its accumulation in soil that enhances the osmotic pressure[23]. The percentage levels of indicators in soil samples obtained from depth were given in Table 2.

The concentrations of ions that differ at least four-fold between the soil samples of 0-30 cm depth were indicated in bald. In accordance results of the analysis of Syrdarya Region soil samples, the proportion of water-soluble salts in the soil constituted from 0.480-2.040% of total soil, in particular, chlorine content was represented 0.017-0.045% and sulfate ions were determined at the range of 0.232-1.113%. Moreover, the amount of calcium cation was 0.045-0.250%, the amount of magnesium was 0.015-0.055% and the amount of sodium was 0.030-0.179% in the Tashkent area. The soil layers at a depth from 0-60 cm were strongly and moderately sulfate saline while the middle and lower layers were found to be less sulfate and chloride saline. Concerning the soil sample of the Tashkent region, the percentage of water-soluble salts in the soil constituted 0.140-0.290% of the total soil. This, in turn, chlorine salt content represented 0.010-0.014% and sulfate salt content was found between 0.060-0.152% and mainly found as medium sulfate saline. Furthermore, the amount of calcium cation was 0.015-0.030%, the amount of magnesium was 0.009-0.018% and the amount of sodium was 0.006-0.020% in the Syrdarya Region. From the results of the above analysis, it can be seen that these soils were various saline soils.

Plant germination: The germination rate of KK-1795, L-141, Hapicala 19, Zangi Ota and resistant control (An-Boyovut) were $\geq$40% of the total sowed seeds (Fig. 1).

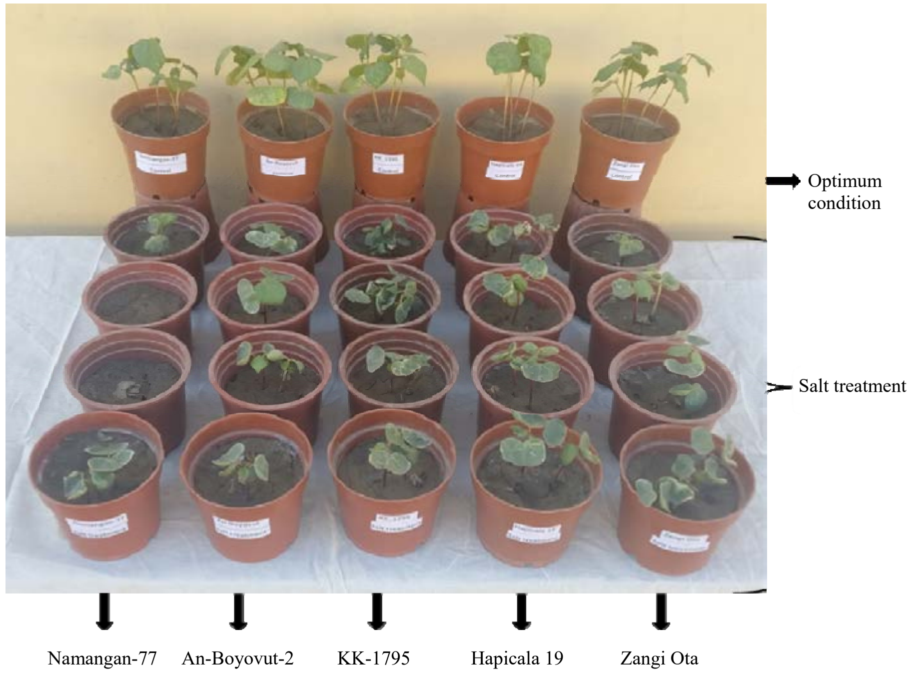

Fig. 1: Germination rate of cotton seeds in optimal and salinity soil in the laboratory condition

Table 2: Amount of water-soluble salts in soils (%)

Depth (cm)	Dry residue (%)	Cl^-	SO_4^{-2}	Ca^{+2}	Mg^{+2}	Na^+	Salinity type	Salinity level
Tashkent Region								
0-30	0.290	**0.010**	**0.152**	**0.030**	0.018	**0.020**	Sulfate	Slightly
30-70	0.140	0.014	0.060	0.025	0.003	0.011	Chloride-sulfate	Slightly
70-100	0.170	0.010	0.074	0.020	0.012	0.006	Sulfate	Slightly
100-150	0.150	0.010	0.068	0.015	0.009	0.014	Sulfate	Slightly
Syrdarya Region								
0-30	2.040	**0.045**	**1.113**	**0.250**	0.055	**0.179**	Sulfate	Strongly
30-70	0.710	0.063	0.325	0.060	0.027	0.085	Sulfate	Moderately
70-100	0.500	0.017	0.251	0.070	0.015	0.030	Chloride-sulfate	Slightly
100-150	0.480	0.031	0.232	0.045	0.018	0.052	Sulfate	Slightly

Highlighted raw of the 0-30 cm depth was used to grow the plants in the experiments

According to the GraphPad analysis, it was illustrated in Fig. 2 that the germination rate of cotton samples grown in optimal conditions accounted for 90-100% of approximately all lines while samples grown in saline environments had performed different percentages due to their salt resistance. Among the donor genotypes, KK-1795, Hapicala 19 and Zangi-Ota lines showed higher tolerance to salinity than both controls An-Boyovut-2 and Namangan-77 as resistant and susceptible variety, in order.

Plant growth: Salt stress has detrimental effects on the production of cotton biomass decrease in leaf area and numbers, stem thickness, shoot and root weight and cotton yield[24]. In Fig. 3, it is apparent that there was no significant difference in the height of plants grown under optimal conditions between 20-25 cm to others. However, An-Boyovut-2, KK-1795, Hapicala 19 and Zangi Ota varieties grown in saline soils differed significantly from the control cotton genotype (between 13-15 cm). Low levels of salts can be beneficial for plant growth. However, higher concentration always causes a toxic effect. In *Arabidopsis*, high concentrations of both sulfate ions and NaCl were demonstrated to cause a lower level of carbon and nitrogen in the plant[25]. In another work, even medium NaCl concentrations were found to change plant morpho-biological traits[26]. Obtained data in this work are in correspondence with these results that both plant germination rate and plant height were strongly affected by salt stress. It should be noted that the differences in

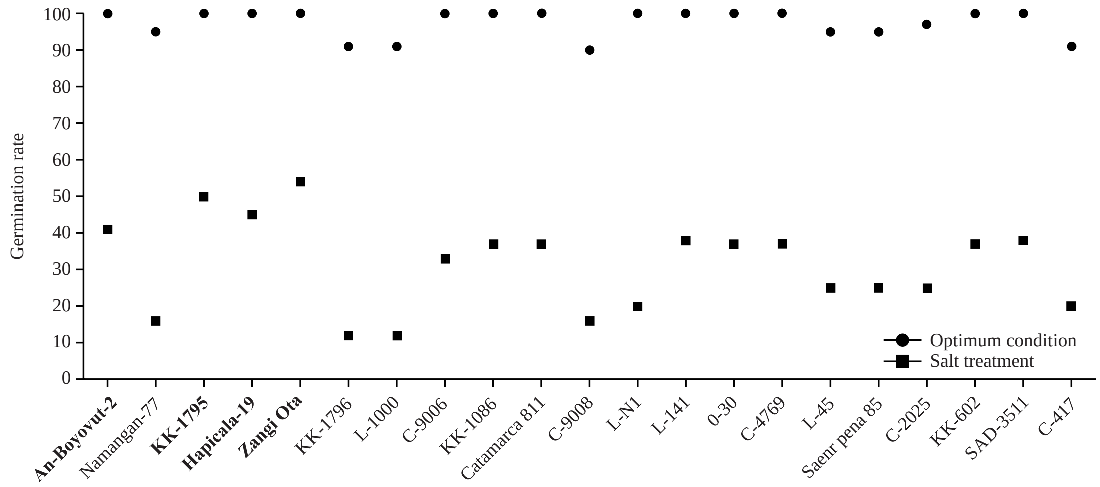

Fig. 2: Germination rate of cotton genotypes planted in naturally strong sulfate salinity and non-salinity soil in laboratory conditions

The Y-axis is the percentage of germination of the study samples, the names of the 21 cotton genotypes used in the experiments are given on the X-axis and Resistant varieties were shown in bold letters

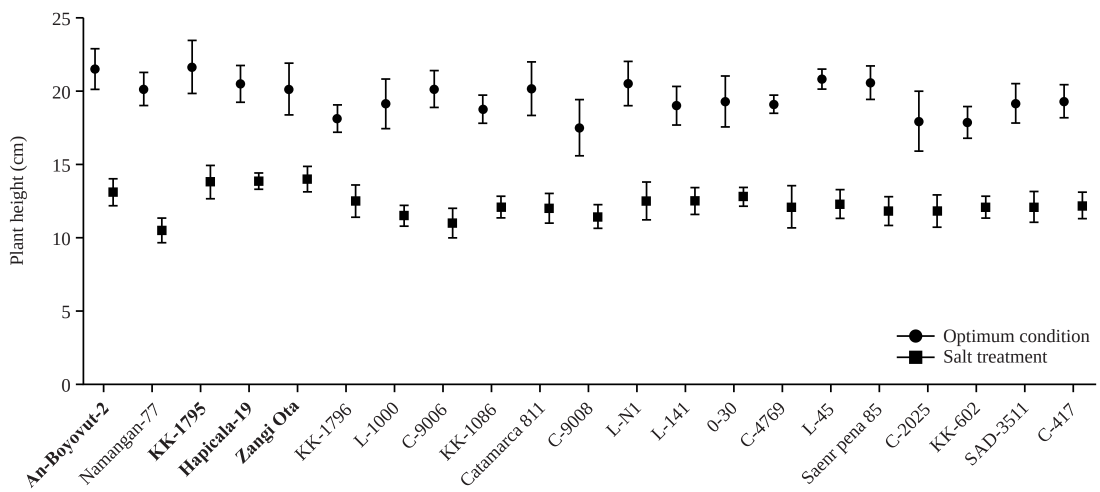

Fig. 3: Height of the sowed cotton samples in naturally strong and non-salinity soil in laboratory conditions

Y-axis is the height of the study samples, the names of the 21 cotton genotypes used in the experiments are given on the X-axis, the variety An-Boyovut-2 and Namangan-77 were used as saline-resistant and -susceptible varieties, respectively, resistant varieties are shown in bold letters and error bars indicate the SD value

germination rate were less significant in resistant genotypes when grown in optimum and salt-stress conditions.

Biomass changes: In Fig. 4, there is a significant difference between the salinity and the optimal condition. As mentioned above, salinity stress hurts the reduction of fresh plant wet biomass, the weight indicator of the Namangan-77 variety fell visibly in comparison to KK-1795, Hapical 19 and Zangi Ota whereas almost no difference was observed with other lines in optimal environmental. Concerning weight of the plants ranged from 1-1.5 grams. The weight of An-Boyovut-2, KK-1795, Hapicala 19 and Zangi Ota cotton genotypes represented (between 1 and 1.2 g) a significantly different than Namangan-77 (0.8 g) in the salinity condition. The plant height was highly inhibited in salt-stressed conditions both in resistant and susceptible genotypes. These results can be attributed to various stressing factors such as sulfate ions and NaCl[27]. Sulfate salts (Na_2SO_4, K_2SO_4) were reported to inhibit plant growth more severely than sodium

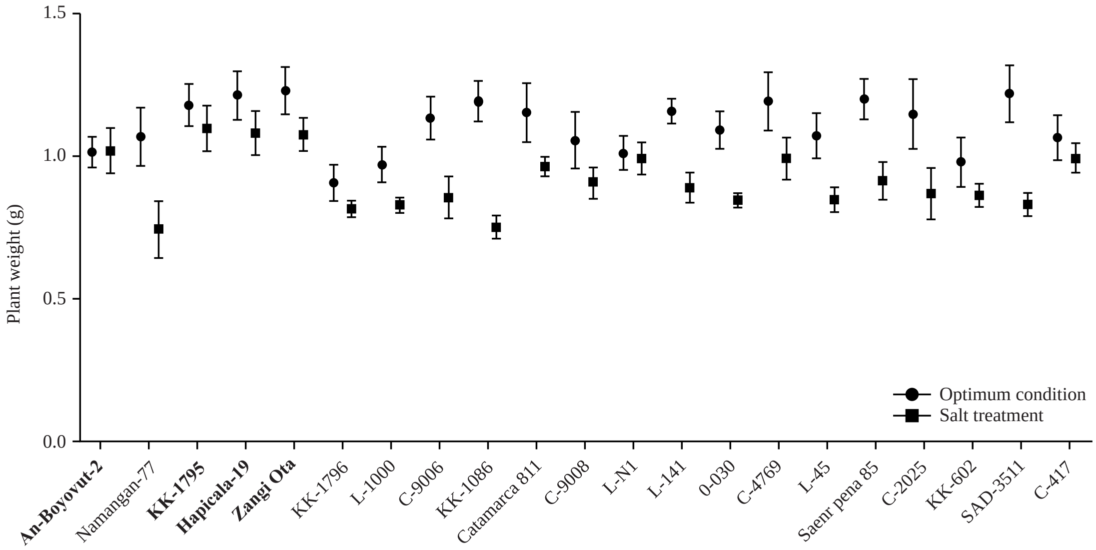

Fig. 4: Weight of the sowed cotton samples in naturally strong and non-salinity soil in laboratory conditions

Y-axis is the weight of the study samples, the names of the 21 cotton genotypes used in the experiments are given on the x-axis, resistant varieties are shown in bold letters and error bars indicate the SD value

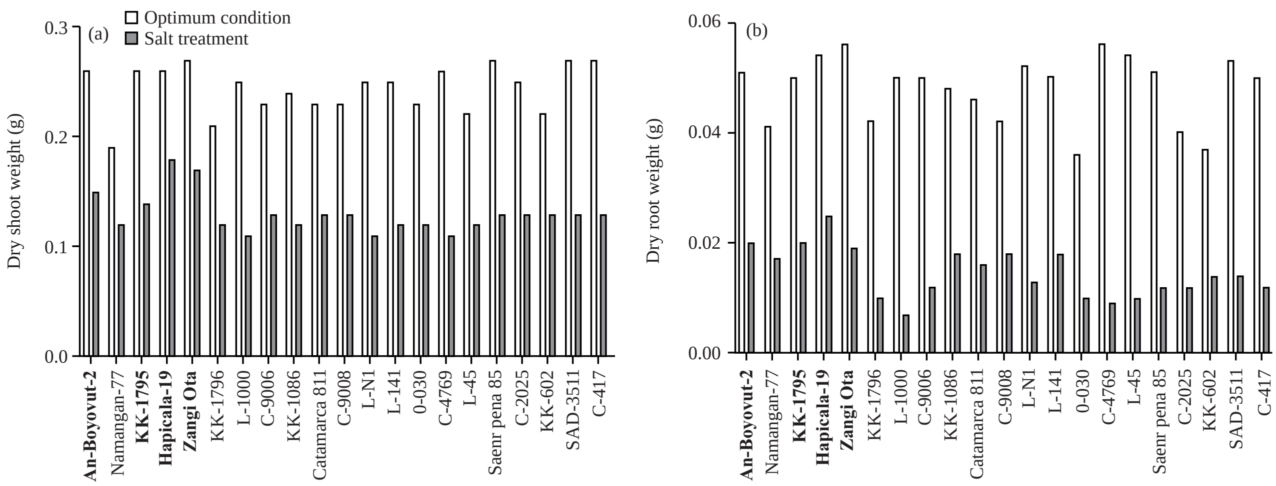

Fig. 5(a-b): Average weight dry shoot (a) left and (b) dry root (right) of cotton genotypes in two conditions

Resistant varieties are shown in bold letters, the names of the 21 cotton genotypes used in the experiments are given on the x-axis and the variety An-Boyovut-2 and Namangan-77 were used as saline-resistant and -susceptible varieties, respectively

chloride (NaCl). Besides, calcium, phosphorus and manganese in plant shoots were lower under the influence of Na_2SO_4 than NaCl[28].

The variety An-Boyovut-2 and Namangan-77 were used as saline-resistant and -susceptible varieties, respectively.

The results obtained from the preliminary analysis of dry plant weight can be compared in Fig. 4. High salinity in soil has reduced the dry weight of plants[15]. In salt treatment plants dry biomass is significantly reduced to optimum conditions. A high level of salinity was found to decrease the fresh and dry weight of the root[29]. The dry biomass of the cotton sample's weight indicators was higher in Zangi Ota, Hapicala 19, KK-1795 and Ab-Boyovut-2 compared to other remaining cotton samples in both conditions (Fig. 5a-b).

PCR analysis: The genomic DNA of cotton genotypes was extracted and carried out by Polymerase chain reaction using BNL-3140. Recent studies showed that 107 base pair fragment length of BNL3140 (D9) was associated with dry root weight and root shoot ratio ($R^2 = 0.06$-0.10) in salt condition[30]. The PCR analysis showed the presence of genetic polymorphism between Namangan-77 and KK-1795, Hapicala 19, Zangi Ota and An-Boyovut-2 cotton varieties. The molecular mass of genotypes was determined using the Gel Analyzer program. Namangan-77 cotton variety represented 100 base pairs while KK-1795, Hapicala 19, Zangi Ota and An-Boyovut-2 cotton varieties accounted for 107-110 base pairs. Molecular analysis showed that KK-1795, Hapicala 19, Zangi Ota and An-Boyovut-2 cotton genotypes have resistance alleles in cotton dry root weight and root shoot ratio.

CONCLUSION

Among experimental cotton samples, KK-1795, Hapicala 19 and Zangi Ota cotton genotypes showed high salt tolerance than the Namangan-77 variety. Moreover, populations taken by these lines were important in identifying Quantitative Trait Loci (QTL) and candidate genes associated with salinity-resistant traits. In the future, high-yielding local varieties can be obtained based on identified QTL loci. In addition, three cotton genotypes, found to be salt-resistant, can be used as a genetic source in cotton breeding.

SIGNIFICANCE STATEMENT

The main goal of the work was to determine salinity-resistant ones among cotton genotypes with good fibre quality indicators. In total, 21 genotypes available in our science centre were used for experiments. Among samples planted in highly sulfated soil, cotton genotypes KK-1795, Hapicala 19 and Zangi Ota were found with a higher index than other samples in terms of all studied morpho-biological characteristics. These results serve to conduct molecular research to identify candidate genes that respond to salinity stress.

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