Soil & Environ. 28(1): 68-74, 2009
www.se.org.pk

Ionic and water relations of cotton (*Gossypium hirsutum* L.) as influenced by various rates of K and Na in soil culture

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Abstract

A pot study was conducted to investigate the growth response, ionic and water relations of two cotton varieties. Four levels of K and Na were developed after considering indigenous K, Na status in soil. The treatments of K + Na in mg kg^{-1} were adjusted as: 105 + 37.5, 135 + 30, 135 + 37.5 and 105 + 30 (control). Control treatment represented indigenous K and Na status of soil. Higher but non significant relative water contents were observed in treatments of 135 + 30 mg kg^{-1} followed by 135 + 37.5 mg kg^{-1}. The beneficial effects of Na with K application were observed greater in NIBGE-2 than in MNH-786. Both varieties varied non-significantly with respect to K:Na ratio in leaf, water potential and total chlorophyll contents. Significant relationship (R^2=0.51, n= 4, average of four replicates) was found between total dry weight and relative water contents in NIBGE-2.

Key words: Cotton (*Gossypium hirsutum* L), ionic ratio, water potential, relative water contents

Introduction

Cotton is our major cash crop. Its economic importance can be visualized from a factual notion that it accounts for 60% of total foreign exchange earnings of Pakistan (Ahmad, 1993). In addition, it is mainstay for domestic needs of fiber and contributes upto 70% to our domestic edible oil production. Average cotton yield of Pakistan (712 kg ha^{-1}) is lower than some other cotton producing countries of the world (Anonymous, 2006). Fertilization of cotton with K is rather a complex issue, because soils vary widely in terms of their K supplying capacity and K fertilizer requirement. Potassium exists as a constituent of some primary minerals from which many soils were originally formed. It is a part of the interlayer of clay minerals such as hydrous mica, and it may become available due to freezing and thawing or wetting and drying. Potassium dissolved in soil solution is in equilibrium with K^+ attached or bound electrostatically to organic matter and the surface of clay particles. Thus, only a portion of total soil K is soluble, in an exchangeable form and readily available to plant roots. However, at other times K held in a non exchangeable form in soil minerals can become exchangeable. When K fertilizer is applied to soil, some fertilizer may be bound or trapped with in soil minerals so that part of it is either not available or slowly available to plants (Reddy *et al.*, 2000). Maser *et al.* (2002) reported that K^+ counteracts Na^+ stress while Na^+ in turn, can alleviate K^+ deficiency to a certain degree. Sodium can replace K^+ to a certain degree, particularly in its osmotic functions in the vacuole. Thus under K^+ starvation, addition of Na^+ may actually promote plant growth. The extent of replacement of K^+ by Na^+ depends largely on the plant species. Several scientists (El-Sheikh and Ulrich, 1970; Marschner, 1971, 1995) have reported a clear positive relationship between the uptake and translocation of Na^+ to the shoot and the extent of replacement of K^+ in the plant species. The accumulation of Na^+ ions inside the vacuoles provides a 2-fold advantage: (a) reducing the toxic levels of sodium in cytosol; and (b) increasing the vacuolar osmotic potential with the concomitant generation of a more negative water potential that favors water uptake by the cell and better tissue water retention under high salt salinity (He *et al*., 2005). Abd-Ella and Shalaby (1993) reported for cotton that lower leaf water potential was associated with higher salinity and $K^+ : Na^+$ ratio. The accumulation of Na^+ ions inside the vacuole generated more negative water potential that favored water uptake by the cell and better tissue water retention under high salinity (He *et al*., 2005).

Under moderate salinity, Na^+ concentration in leaves was modest and K^+ concentration was higher than Na^+ (Thomas, 1980). Khan *et al*. (1998) reported that NIAB-78, the most tolerant cultivar, retained higher Na^+ concentration in the roots than MNH-93. D-9 was the most salt sensitive. Retention of high Na^+ in the roots could be the mechanism of salt tolerance in cotton. A high accumulation of Na^+ in the leaves of salt tolerant cultivars of cotton has also been found (Leidi and Saiz, 1997). $K^+ : Na^+$ ratio attributed to K^+/Na^+ exchange across the plasma lemma of root cortex cells and selective uptake of K^+ (Jeschke and Wolf, 1988). Passive accumulation of Na^+ in the roots and shoots caused the low $K^+:Na^+$ ratios in both the tissues (Greenway and Munns, 1980).

Yield and quality of crop plants can be influenced positively through the effects of mineral nutrition on water

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relations (Mengel and Arneke, 1982; Marschner, 1995). Oosterhuis and Bednarz (1997) reported significant reduction in leaf chlorophyll concentration and in leaf photosynthesis in cotton crop under K deficiency. Under mild salinity, NIAB-78 exhibited increase in root growth but not shoot (Jafri and Ahmad, 1994). Zhang *et al*. (2006) explained that cotton growth, nutrition absorption and yield were improved by adding appropriate amounts of K^+ and Na^+. Dry matter production of crop is mostly dependent on its assimilatory system. Assimilatory system comprises of all green area of plants.

Cotton is one of the salt tolerant crops (Maas, 1990). Increase in growth with low concentration of salts has been observed (Pessarakli, 1995). Exploitation of plant genetic capacity for better nutrient uptake and utilization is a promising tool that has been reported to cope with mineral nutrient stress in soil (Baligar *et al*., 1990). Leaf chlorophyll concentration was not affected in red beet cultivar by Na^+ substitution for K^+ (Subbarao *et al*., 1999). Vacuolar compartmentation and control of water contents in rice determined the differential genotypic response (Flowers *et al*., 1991). Application of higher levels of K^+ improved water relations as well as growth and yield of mung bean under mild level of saline conditions (Kabir *et al*., 2004). Subbarao *et al*. (1999) studied improved growth of red beet (*Beta vulgaris* L.) and determined that Na^+ replaced K^+ without adversely affecting metabolic functions such as water relations. Sodium accumulation in leaf vacuole provides an efficient method of osmotic adjustment under $K^+ + Na^+$ treatment when combined with compartmentation within the cell (Yeo, 1983). The differential ion uptake and distribution indicated several regulation mechanisms acting at different rates (Jeschke, 1984). Selectivity in uptake and transport, vacuolar compartmentation and even more integrated (and complex) responses such as control of water content (Flowers *et al.*, 1991) determined the differential genotypic response.

The present soil culture investigation was conducted to evaluate the ionic and water relations of two cotton varieties under various K^+ and Na^+ rates. It would be helpful in validating the results of solution culture experiments where the varieties behaved differently at varying rates of K^+, Na^+ in the root medium with respect to their ionic distribution pattern /translocation and uptake kinetics.

Materials and Methods

The experiment was carried out in a wire house of Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad to determine ionic and water response of two selected cotton varieties (NIBGE-2 and MNH-786) to K^+ and Na^+ application in soil culture. The absolute maximum and minimum temperature of 47 ^{O}C and 38 ^{O}C were recorded during month of June in year 2007. In a wire house, cotton crop experienced harsh temperatures (day/night) during its growth period. The average day temperature was 45 ± 3 ^{O}C and the night temperature 31 ± 5 ^{O}C, and relative humidity ranged from 25 to 58 percent. The soil samples used in pots were collected from the University Research Farm. It was air-dried, ground, passed through 2 mm sieve and mixed thoroughly. Texture of the soil was sandy clay loam, pH, 8.10; organic matter, 0.71 %; $CaCO_3$, 4.5%; ECe, 1.2 dS m^{-1}; Olsen-P, 6.7 mg kg^{-1}; NH_4OAc-K, 105 mg kg^{-1} and NH_4OAc- Na, 27 mg kg^{-1} based on the methods as described in part-3, Methods of Soil Analysis (Bigham, 1996). It was sandy clay loam hyperthermic Ustalfic Haplargid according to FAO (1990) and Soil Survey Staff (1998). The soil belongs to Hafizabad Soil Series as described by Soil Survey Report (1969).

Twelve kilograms of air-dried, sandy clay loam soil was filled in each of 32 glazed pots (45.5 x 31.0 cm). The pots were lined with polyethylene sheet to avoid accretion of salts by the pot. They were kept inside wire house under natural light. Various pots received a uniform basal dose of one third of 112.5 mg of N and full dose of 37.5 mg of P_2O_5 per kg of soil at the time of sowing. All the nutrients were applied in solution form. At field capacity, four seeds of the two cotton varieties were sown in the pots on June 28, 2006. Each variety was quard-ruplicated according to CRD factorial. Four treatments were adjusted according to initial K^+ and Na^+ level in soil. Initial K^+ and Na^+ level in soil was 105 + 30 mg kg^{-1}, respectively. The four treatments of $K^+ + Na^+$ in mg kg^{-1} in sulphate form were as: 135+ 30, 105+ 30, 135+ 37.5 and 105+ 37.5. After adding Na^+, ECe was noted as 1.31 dS m^{-1}. Treatments were decided to keep ECe level near to normal with a view of keeping uniformity in four treatments. Thinning was done 10 days after emergence, to allow one plant to grow in each pot. Weeding and other plant protection measures were done when it was necessary. Distilled water was used for irrigation purpose during the growth period. Data on relative water content (RWC %) in leaf was calculated as: fresh weight - oven dry weight / turgor weight - oven dry weight (Subbarao *et al*., 1999), a fully expanded second leaf from top was excised to determine leaf water potential (-MPa) with a Scholander type pressure chamber (Scholander *et al*., 1965) and total chlorophyll contents in leaf (mg Chl g^{-1} leaf tissue)) were determined with the method as described by (Arnon, 1949). The fresh leaves were cut into 0.5 cm segments and extracted overnight with 80 % acetone at -10^{O} C. The extract was centrifuged at 14000 x g for 5 minutes and the absorbance of the supernatant was read at 645 and 663 nm using the spectrophotometer. The youngest fully expanded main stem leaf samples were collected from each pot at 80 days after planting. The leaves were stored on ice, carried to the laboratory and dried for 48 h at 72^{O}C to a constant weight for ionic analysis. Well ground samples were digested in 10 mL of (3:1) nitric: perchloric acid mixture following Miller (1998). The digested samples were diluted with distilled water and K^+, Na^+ concentration in samples was determined with flame photometer (Jenway PFP 7). $K^+ : Na^+$ ratio was calculated by dividing K^+ concentration with Na^+ concentration. Before peak flowering, plants were harvested from each treatment. They were partitioned in to shoot and root. K use efficiency in shoot was calculated by dividing shoot dry matter by K^+ concentration in shoot. They were oven dried at 72^{o}C to a constant weight for dry weight (g plant^{-1}) of shoot and root. The data obtained were subjected to statistical analysis using computer software "MSTAT-C" (Russell and Eisensmith, 1983) by following the methods of Gomez and Gomez (1984). Completely randomized factorial design was employed for analysis of variance. Duncan's multiple range test was used for mean separation (Duncan, 1955).

RESULTS

Potassium: sodium ratio in leaf

Main effects of rates of K^+ and Na^+ significantly ($p<0.01$) influenced $K^+ : Na^+$ ratio in leaf. Varieties and their interaction with rates of K^+ and Na^+ influenced $K^+ : Na^+$ ratio in leaf non-significantly (Table 1). Maximum mean K:Na ratios in leaf (2.25) i.e. 149% more than control, was observed at $K^+ + Na^+$ @ 135 +30 mg kg^{-1} due to addition of more K^+ and minimum $K^+ : Na^+$ (0.47) i.e. 48% less than control, was obtained at 105+37.5 mg kg^{-1} in both varieties due to addition of Na^+ in a treatment. Thomas (1980) reported that under moderate salinity, Na^+ concentration in leaves was modest and K^+ concentration was higher than Na^+. Khan *et al*. (1998) reported that NIAB-78, the most tolerant cultivar, retained higher Na^+ concentration in the roots than MNH-93. A high accumulation of Na^+ in the leaves of salt tolerant cultivars of cotton has also been found (Leidi and Saiz, 1997). Jeschke and Wolf (1988) explained that $K^+ : Na^+$ ratio was attributed to K^+/Na^+ exchange across the plasma lemma of root cortex cells and selective uptake of K^+. The results were not in agreement with those of Greenway and Munns (1980).

Water potential (-MPa)

Main and interactive effects of rates of K^++Na^+ application and varieties had non-significant effect on water potential in leaf (Table 1). Abd- Ella and Shalaby (1993) reported for cotton that lower leaf water potential was associated with higher salinity and K^+:Na^+ ratio. The accumulation of Na^+ ions inside the vacuole generated more negative water potential that favored water uptake by the cell and better tissue water retention under high salt salinity (He *et al*., 2005). The results confirm the findings of above researchers.

K concentration (mg g^{-1}) in shoot

Main effects of rates of $K^+ + Na^+$ application and varieties had significant ($p<0.01$) effect on K^+ concentration (mg g^{-1}) in shoot (Table 2). Interaction between rates of $K^+ + Na^+$ application x varieties had a non-significant effect on K^+ concentration (mg g^{-1}) in shoot. Maximum average K^+ concentration in shoot of 22 mg g^{-1} was demonstrated with 135+30 mg kg^{-1} followed by (20 mg g^{-1}) at 135 + 37.5 $K^+ + Na^+$ which were statistically at par with each other as against the minimum K^+ concentration (11 mg g^{-1}) at $K^+ + Na^+$ 105+ 37.5 mg kg^{-1}). Maximum K^+ concentration of 18.05 mg g^{-1} was revealed by NIBGE-2 over MNH-786 (16.45 mg g^{-1}).

K use efficiency (g^2 shoot dry matter (SDM) mg^{-1} K) in shoot

Main effects of rates of $K^+ + Na^+$ application and varieties had significant ($p<0.01$) effect on K use efficiency in shoot (Table 2). Interaction between rates of $K^+ + Na^+$ application x varieties influenced K use efficiency non-significantly. Highest mean K use efficiency in shoot of 4.17 g^2 SDM mg^{-1} K was demonstrated with 105+ 37.5 mg kg^{-1} followed by K use efficiency of 2.86, 2.63 and 2.16 g^2 SDM mg^{-1} K at K + Na @105+ 30, 135+ 37.5 and 135+30 mg kg^{-1} those were statistically at par with one another. Higher K use efficiency of 3.05 g^2 SDM mg^{-1} K was revealed by MNH-786 than that of NIBGE-2 (2.86 g^2 SDM mg^{-1} K).

Total chlorophyll content (mg g^{-1}leaf)

There was significant ($p<0.05$) main effect of K^+ and Na^+ rates on total chlorophyll contents. They were significantly reduced in both varieties when grown under control treatment where no K^+ and Na^+ were applied (Table 3). Varieties differed non-significantly with regard to total chlorophyll content, but interaction between rates of K+Na application x varieties was found significant ($p<0.01$). Maximum chlorophyll content (18.08 mg g^{-1} leaf**)** was recorded when grown at 135+30 mg kg^{-1} K+Na. The results are in consistence with Subbarao *et al*. (1999).

Relative water contents (%)

Main effects of rates of $K^+ + Na^+$ application and varieties had significant ($p<0.01$) effect on relative water contents (Table 3). Interaction between rates of $K^+ + Na^+$ application x varieties had a non-significant effect on relative water contents. Maximum average relative water contents of 30.16% was demonstrated with 135+30 mg kg^{-1}

Table 1. K^+ : Na^+ ratio and Water Potential (-Mpa) in leaf of selected cotton varieties grown with different K^+ and Na^+ rates in soil culture

(Values are means of four replicates)

Treatment	K^+ : Na^+ ratio in leaf			Water Potential (-Mpa) in leaf		
	Cotton Variety			Cotton Variety		
$K^+ + Na^+$ (mg kg^{-1})	NIBGE-2	MNH-786	Mean	NIBGE-2	MNH-786	Mean
135 + 30	2.25 ns	2.25	2.25 a	-1.40 ns	-1.44	-1.42
105 + 30 (control)	0.89	0.92	0.91 b	-1.32	-1.36	-1.34
135 + 37.5	1.01	1.02	1.01 b	-1.31	-1.35	-1.33
105 + 37.5	0.46	0.49	0.47 c	-1.30	-1.32	-1.31
Mean	1.15	1.17	1.16	-1.33	-1.37	-1.35

ns = non-significant

Means with different letter(s) differ significantly according to Duncan's Multiple Range Test ($p<0.05$)

Table 2. K^+ concentration (mg/g) and K^+ use efficiency (g^2 SDM/mg K) in shoot of selected cotton varieties grown with different K^+ and Na^+ rates in soil culture

Treatment	K^+ concentration (mg g^{-1}) in shoot			K use efficiency (g^2 SDM mg^{-1} K)		
	Cotton Variety			Cotton Variety		
$K^+ + Na^+$ (mg kg^{-1})	NIBGE-2	MNH-786	Mean	NIBGE-2	MNH-786	Mean
135 + 30	23.5	20.5	22 a	2.05	2.26	2.16 b
105 + 30 (control)	16.2	15.8	16 b	2.82	2.89	2.86 b
135 + 37.5	20.6	19.4	20 a	2.70	2.55	2.63 b
105 + 37.5	11.9	10.1	11 c	3.86	4.49	4.17 a
Mean	18.05 a	16.45 b		2.86 b	3.05 a	

Table 3. Total chlorophyll contents (mg g^{-1} leaf) and relative water contents (%) in leaf of selected cotton varieties grown with different K^+ and Na^+ rates in soil culture

(Values are means of four replicates)

Treatment	Total chlorophyll contents (mg g^{-1}leaf)			Relative water contents (%) in leaf		
	Cotton Variety			Cotton Variety		
$K^+ + Na^+$ (mg kg^{-1})	NIBGE-2	MNH-786	Mean	NIBGE-2	MNH-786	Mean
135 + 30	20.00 a	16.17 b	18.08 a	32.48 ns	27.84	30.16 a
105 + 30 (control)	13.84 bc	12.67 c	13.25 b	17.45	13.75	15.60 c
135 + 37.5	16.10 b	15.36 bc	15.73 ab	31.13	24.74	27.94 a
105 + 37.5	15.22 bc	14.26 bc	14.74 b	22.28	22.20	22.24 b
Mean	16.29 a	14.62 a		25.84 a	22.13 b	

ns= non-significant

Means with different letter(s) differ significantly according to Duncan's Multiple Range Test ($p<0.05$)

K+Na i.e. 93% more than that of control (15.60%). Substantial increase in relative water contents (25.84%) was revealed by NIBGE-2 over MNH-786. Flowers *et al*. (1991) concluded that control of water contents in rice determined the differential genotypic response. The results are in conformity with Kabir *et al*. (2004) and Subbarao *et al*. (1999) who narrated that application of higher levels of K^+ improved water relations as well as growth and yield of mung bean and red beet (*Beta vulgaris* L.) under mild level of saline conditions.

Dry matter production (g plant^{-1})

Shoot dry matter, root dry matter and total dry matter were measured at maturity. Main effects of varieties and interaction influenced the shoot dry matter production significantly ($p<0.05$) (Table 4). Higher average shoot dry matter (48.91g plant^{-1}) was produced by NIBGE-2 than that of MNH-786. Main effects of K^+, Na^+ rates affected the root dry matter markedly, as against the shoot dry matter. Maximum mean root dry matter (4.78 g plant^{-1}) was accumulated at 135+ 37.5 mg kg^{-1} $K^+ + Na^+$ treatment, which was 26% higher than the control (Table 4). The differences in root dry matter production were significant ($p<0.05$) due to interaction between rates of $K^+ + Na^+$ application and varieties. Significant ($p<0.01$) differences in total dry matter were observed due to K^+, Na^+ rates. Maximum average total dry matter (57.40 g $plant^{-1}$) was produced in plants when grown with 135+37.5 mg kg^{-1} $K^+ + Na^+$ treatment. However, remaining three treatments behaved equally for total dry matter production of the two varieties. The results are in line with the findings of Jafri and Ahmad (1994) and Zhang *et al*. (2006).

Discussion

K:Na ratio in leaf which caused upward movement of water toward leaf from the root zone. Increasing more negative water potential enhanced the chlorophyll contents (Figure 3) for NIBGE-2. Figure 4 explained significant negative relationship between K concentration and K use efficiency in shoot of two selected cotton varieties.

Conclusion

Maximum beneficial effect of Na with deficient K was observed for relative water contents in NIBGE-2 than in MNH-786. Both varieties differed non-significantly with regard to K:Na ratio, water potential and chlorophyll contents. The results suggested that both varieties showed better response to modest application of Na with adding K

Table 4. Total dry matter, shoot dry matter and root dry matter (g $plant^{-1}$) of selected cotton varieties grown with different K^+ and Na^+ rates in soil culture

(Values are means of four replicates)

Treatment	Total dry matter (g $plant^{-1}$)			Shoot dry matter (g $plant^{-1}$)			Root dry matter (g $plant^{-1}$)		
	Cotton Variety			Cotton Variety			Cotton Variety		
$K^+ + Na^+$ (mg kg^{-1})	NIBGE-2	MNH-786	Mean	NIBGE-2	MNH-786	Mean	NIBGE-2	MNH-786	Mean
135 + 30	53.06 ns	50.46	51.75 b	48.28 b	46.33 b	47.30 ns	4.78 ab	4.13 b-d	4.45 ab
105 + 30 (control)	49.71	49.30	49.50 b	45.73 b	45.70 b	45.71	3.98 cd	3.60 d	3.79 c
135 + 37.5	60.83	53.98	57.40 a	55.70 a	49.55 b	52.63	5.13 a	4.43 bc	4.78 a
105 + 37.5	50.40	49.18	49.79 b	45.95 b	45.30 b	45.63	4.45 bc	3.88 cd	4.16 bc
Mean	53.49	50.73		48.91a	46.72 b		4.58 a	4.01 a	

ns = non-significant

Means with different letter(s) differ significantly according to Duncan's Multiple Range Test ($p<0.05$)

Dry matter production of crop is mostly dependent on its assimilatory system. Assimilatory system comprises of all green area of plants. Cotton is one of the salt tolerant crops (Maas, 1990). Increase in growth with low concentration of salts has been observed (Pessarakli, 1995). It was important to note that K, Na treatments did not significantly ($p<0.01$) influence the shoot dry matter at final harvest, though the seed cotton yield enhanced significantly. The increment in total dry matter was more prominent at 135+37.5 mg kg^{-1} $K^+ + Na^+$. Figure 1 indicated some significant relationship (R^2=0.51, n=4 i.e. mean of 4 replicates) between total dry weight and relative water contents for NIBGE-2, as compared to MNH-786. Idowu and Aduayi (2006) explained that a Na:K ratio of 0.45 up to 0.60 for shoot and 1.44 up to 1.80 for root, maintained good shoot water balance, resulted in lower floral abortion, and markedly enhanced the tomato fruit yield. Figure 2 showed significant (R^2 = -0.96, -0.98, n=4) relationship between water potential and K:Na ratio in leaf. It explained that more negative water potential was observed by increasing

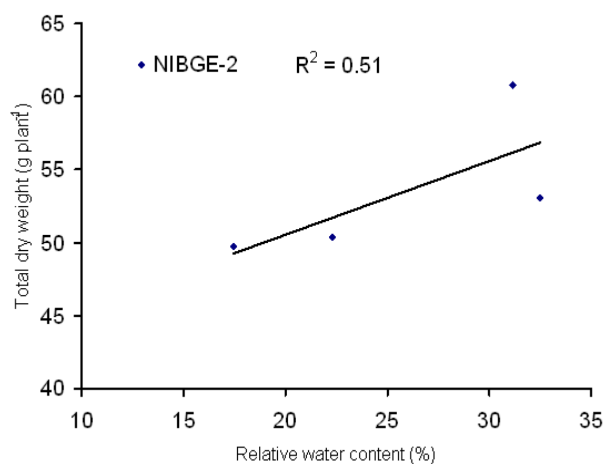

Figure1. Relationship between total dry weight and relative water content at various rates of K^+ and Na^+ in soil

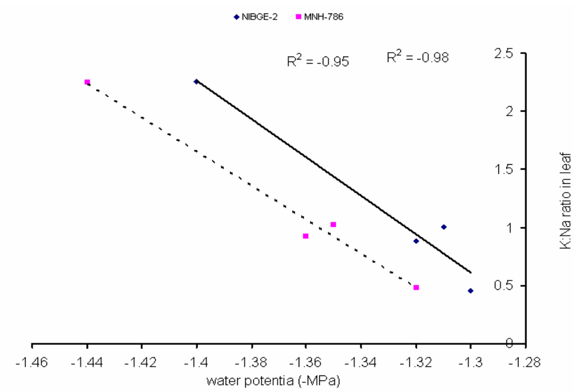

Figure2. Relationship between water potential and $K^+ : Na^+$ ratio in leaf of both genotypes under various rates of K^+ and Na^+ in soil

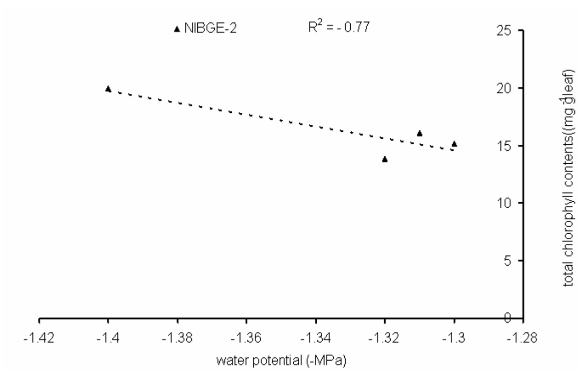

Figure 3. Relationship between water potential and total chlorophyll contents of NIBGE-2 under various rates of K^+ and Na^+ in soil

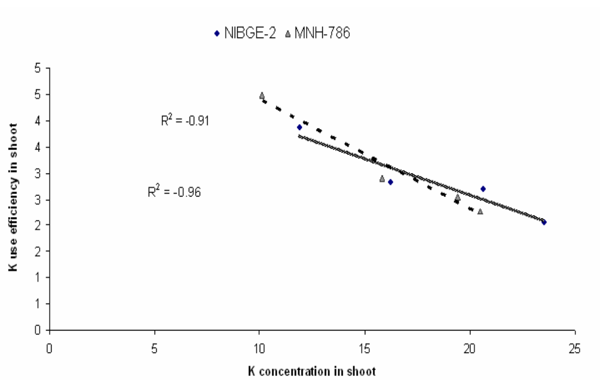

Figure 4. Relationship between K^+ concentration and K use efficiency in shoot of two cotton varieties

in soil. The variety NIBGE-2 proved better in relative water contents which resulted in higher shoot dry matter and it may be tested under field conditions for their response to different K, Na rates. In case of deficient soil K, plant growth can be promoted in cotton varieties by adding modest amount of Na in normal soil because Na substitutes some non specific functions of K.

Acknowledgement

Authors are grateful to Higher Education Commission of Pakistan for providing financial support under the project "Development of S&T Manpower through Indigenous Ph. D. Scholarship Scheme."

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