REDUCING DISEASE INCIDENCE OF COTTON LEAF CURL VIRUS (CLCUV) IN COTTON (*GOSSYPIUM HIRSUTUM* L.) BY POTASSIUM SUPPLEMENTATION

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Abstract

Severity of various diseases in plants can be reduced by nutrient management. The knowledge of K nutrition on relationship between plants and pests may help in devising strategies to set up high yielding production system by reducing disease incidence. Therefore, two cotton (*Gossypium hirsutum* L.) cultivars, S-12 (CLCuV-susceptible) and CIM-448 (CLCuV-resistant) were supplied with varying concentration of potassium [40, 120, and 236 mg K L^{-1}]. During the experiment, only virus susceptible cultivar S-12 exhibited typical disease symptoms on leaves, while that of CIM-448 did not show any degree of disease incidence. Moreover, at lower K supply, severity of disease incidence significantly enhanced. Although growth of both cotton cultivars decreased at low K concentration, cv. CIM-448 was superior in growth than that of virus susceptible cv. S-12 at all K concentrations. Virus resistant cultivar, cv. CIM-448 had higher leaf epicuticular wax than that of cv. S-12. However, it is interesting to note that diseased leaves of cv. S-12 had greater epicuticular wax content than that of healthy leaves of cv. S-12. Leaf K$^+$ decreased with decrease in K regimes in both cultivars. Diseased leaves of virus susceptible cv. S-12 had higher leaf Ca^{2+} and N than those in healthy leaves of cvs. S-12 and CIM-448, whereas CIM-448 had higher leaf Mg^{2+} as compared to diseased or healthy leaves of S-12. Total soluble proteins, total free amino acids, total soluble sugars did not show any relationship with disease incidence. Photosynthetic rate and stomatal conductance were higher in CIM-448 than that in cv. S-12. Moreover, photosynthetic rate was higher in healthy leaves of cv. S-12 than that of diseased leaves. Chlorophyll '*a*' was higher in cv. CIM-448 than that in the leaves of cv.S12. In conclusion, low supply of K decreased the growth of both cultivars, but it also increased the severity of disease incidence in cv. S-12. Increase in disease incidence in cv. S-12 is possibly associated with lower photosynthetic pigments, lower rates of photosynthesis, and lower K and N use efficiency and low accumulation of Mg^{2+}in the leaves.

Introduction

Growth and productivity of cotton is severely reduced by both abiotic and biotic stresses. Biotic stresses or diseases reduce uptake and utilization of nutrients by plants. It is therefore, plant mineral nutrition management not only used for producing higher crop productivity; it also used to change plant responses to biotic stresses-disease incidences (Walters & Bingham, 2007). Generally, nutrition may cause changes in plant growth characteristics (anatomical, chemical composition) resulting in increase or decrease in tolerance to diseases (Zafar *et al*., 2010; Athar *et al*., 2011; Huber *et al*., 2002). Application of mineral nutrients to mitigate the adverse effects of diseases on plants is suggested by a number of scientists (Graham, 1983; Huber & Graham, 1999, Athar *et al*., 2011; Huber *et al*., 2012). Of all the nutrients that become altered during plant disease or pest attack, potassium is the most effective nutrient. Potassium is involved in morpho-physiological and biochemical processes that influence disease incidence and severity such as protein synthesis, N-metabolism, enzyme activation, hormonal signaling, stomatal movement, osmoregulation, photosynthesis, phloem loading and assimilate translocation (Marschner, 1995; Epstein & Bloom, 2005; Taiz & Zeiger, 2010). A sufficient potassium supply tends to harden plant structures, including stronger cuticle, stronger outer wall of epidermis, stronger cell walls, improved formation of sclerenchymatous tissues, lignification stimulated, silicification which in turn could protect plants from insects and pathogens (Huber & Graham, 1999, Datnoff *et al*., 2006). Moreover, K nutrition can influence disease severity by interacting with hormonal defence pathways such as jasmonic acid signaling (Schachtman & Shin, 2006). It has been observed that K deficiency in Arabidopsis up-regulated jasmonic acid signaling and was reversed at resupply of K (Armengaud *et al*., 2004). In view of these reports, it is hypothesized that application of varying levels of K may alter various physiological and biochemical processes which in turn modulate the disease incidence. For example, inverse relationship between disease incidence and K nutrition was found in soybean and sesame (Mondal *et al*., 2001). Likewise in other studies with cotton, potassium supplementation reduced the fungal disease incidence (Prabbu *et al*., 2007). Experimental evidences about effects of mineral nutrient on disease severity, comparison of mineral concentrations in tissue of resistant and susceptible cultivars or diseased and non-diseased tissues support the usage nutrients to reduce the severity of diseases (Marschner, 1995; Huber & Graham, 2002; Datnoff *et al*., 2006; Athar *et al*., 2011; Huber *et al*., 2012). However, pattern of K-induced biochemical changes and disease incidence still remains to be established.

Cotton is more sensitive to low potassium than most of the other crops (Oosterhuis *et al*., 1997). Thus, the present study was conducted to assess up to what extent low and high K supplies affect mineral nutrient status, biochemical and physiological process of cotton thereby resulting in change in plant vigor and further decrease in incidence/symptoms of cotton leaf curl virus (CLCuV) in two cultivars of cotton differing in disease resistance. Moreover, the present study was also conducted to understand the biochemical basis of differential disease reaction and physiological reasons for variation in growth due to variable K doses.

Materials and Methods

Seeds of two cultivars of cotton (*Gossypium hirsutum* L.), cv CIM-448 (CLCuV-resistant) and cv S-12 (CLCuV-susceptible) were obtained from the Central Cotton Research Institute Multan, Pakistan. The experiment was conducted in a glasshouse with natural sunlight during the summer with mean day temperature of $38.6 \pm 9.4^{\circ}C$ and night temperature of $22.3 \pm 7.6^{\circ}C$, and the day length from 12 to 14 hours.

River sand was thoroughly washed with tap water and then with distilled water. Thirty-six mosaic-cement pots of 28 cm diameter were filled with each of 16.5 kg of dry sand. The experiment was arranged in a randomized complete block design with six blocks. Each block contained two lines and three K concentrations. Results based on $n = 6$. Varying concentrations of K in the Hoagland's nutrient solution (Epstein, 1972) were prepared as follows:

T_1 = K 236 mg L^{-1} = Full strength Hoagland's nutrient solution

T_2 (control) = K 120 mg L^{-1} = Full strength Hoagland's nutrient solution except the amendment in KNO_3 as follows: + 3.076 mL/L 1*M* KNO_3 + 2.92 mL/L 1*M* $NaNO_3$

T_3 = K 40 mg L^{-1} = Full strength Hoagland's nutrient solution except the amendment in KNO_3 as follows: + 1.0256 mL/L 1*M* KNO_3 + 4.97 mL/L 1*M* $NaNO_3$

Seeds of both cultivars were surface sterilized in 5% sodium hypochlorite solution for 10 min and washed with distilled deionized water prior to experimentation. 300 seed of each cultivar were sown in wet sand in plastic trays. After two weeks, 4 seedlings of comparable size were randomly chosen and transplanted equidistant from each other into each mosaic-cemented pot. Appropriate treatment solutions (T_1, T_2, T_3) of K were applied to all the pots before the transplantation of seedlings and thereafter every 7 days, 2 L of the appropriate treatment solution was applied to each pot to regularly maintain the N concentration in sand. During the 7 days, 200 mL of distilled deionized water was applied daily to each pot to compensate evapotranspiration loss. Two plants from each pot were harvested at the onset of the flowering stage. Plant roots were removed from the sand and washed thoroughly with distilled deionized water. After recording the fresh weights of shoots and roots they were dried at $65^{\circ}C$ for one week and dry weights measured.

Epicuticular wax content: Epicuticular wax content was determined following Silva Fernandez *et al*., (1964). Fresh leaves (2.0 g) of the same age and size were excised from healthy and diseased plants. The area of each leaf sample was first measured with a leaf area meter (Delta T Devices, Burwell, Cambridge, England). The leaf samples were then placed in weighed glass vials, and were washed with 40, 30, and 30 mL of carbon tetrachloride for 30 s per wash. The extract thus obtained was filtered, evaporated to dryness, and the remaining wax was weighed. Wax content was expressed on the basis of unit leaf area i.e., wax content ($\mu g/cm^2$).

Water potential: One day prior to measurement of leaf water potential, all plants were watered to field capacity. Next morning at 09:00, a fully expanded young leaf was excised from the healthy and diseased plants and its water potential was measured with a Scholander type pressure chamber (Chas, W. Cook and Sons, Birmingham, U.K.).

Chlorophyll: The determination of chlorophyll content was carried out following the method described by Witham *et al*., (1971). One gram of fresh leaves was taken from the healthy and diseased plants and triturated in 80% acetone (v/v). The optical densities of the extracts were measured at 645, 652 and 663 nm using a spectrophotometer (Hitachi U-2000).

Macronutrients (K^+, Ca^{2+}, Mg^{2+}, N and P): For the analysis of macronutrients a fully expanded youngest leaf from each plant was sampled. A diseased leaf of the comparable age was also excised from each infected plant for analysis of macronutrients. The macronutrients were determined by the methods described by Allen *et al*., (1986). One hundred mg of ground dry leaf samples were digested in 2 mL of sulphuric-peroxide digestion mixture until a clear and almost colorless solution was obtained. After digestion, the volume of the sample was made 100 mL with distilled deionized water. K^+ was determined with a flame photometer (Jenway PFP7), and Ca^{2+} and Mg^{2+} with an atomic absorption spectrometer (Perkin Elmer Analyst 100). P was estimated by the method described by Jackson (1958) using a spectrophotometer (Hitachi U-2000) and N by titration method following Allen *et al*., (1986).

Total soluble proteins: Total soluble proteins were determined as described by Lowry *et al*., (1951). Fresh leaf material from healthy and diseased plants (0.2 g) was homogenized in 4 mL of sodium phosphate buffer solution (pH = 7.0) and centrifuged. The extract was used for the estimation of soluble proteins and free amino acids. The sample extracts were reacted with a Folin phenol reagent and the optical densities read at 620 nm using a spectrophotometer (Hitachi U-2000).

Total free amino acids: Total free amino acids were determined following procedures of Hamilton & Van Slyke, (1943). For estimation of total free amino acids, 1 mL of each sample as extracted for soluble protein determinations was treated with 1 mL of 10% pyridine and 1 mL of 2% ninhydrin solution. The optical densities of the solutions were read at 570 nm using a spectrophotometer (Hitachi U-2000).

Total soluble sugars: Total soluble sugars were estimated following procedures of Malik & Srivastava, (1985). Well ground dry leaf material (0.1 g) of each sample was homogenized in 80% ethanol and centrifuged at $2900 \times g$. The residue was retained and repeatedly washed with 80% ethanol to remove all traces of soluble sugars. The resulted filtrate was diluted up to 100 mL with distilled deionized water and reacted with anthrone reagent.

Starch: The residue from the total soluble sugars was treated with 6.5 mL 65% hydrogen perchloric acid and 3mL distilled deionized water and samples were kept in refrigerator at below 0^oC for 15 min., and then centrifuged at $2900 \times g$. The above procedure repeated three times and filtrate was diluted up to 100 mL with distilled deionized water. The filtrate was reacted with anthrone reagent and the absorbance of the colored solutions read at 625 nm.

Gas exchange parameters: Measurements of gas exchange parameters were made on the intact fully young leaf of each plant using an ADC LCA-4 portable infrared gas analyzer (Analytical Development, Hoddesdon, UK). These measurements were made from 9.30 to 11.30 a.m. with the following specifications/adjustments: leaf surface area, 6.25 cm^2; ambient temperature, 45 ± 3^oC; ambient CO_2 concentration, 352 $\mu mol \; mol^{-1}$; temperature of leaf chamber varied from 41.0 to 49.0^oC; leaf chamber gas flow rate, 410 ml min^{-1}; RH of the chamber ranged from 25.4 to 41.2 %; PAR (Q_{leaf}) at leaf surface during noon was maximum up to 1032 $\mu mol \; m^{-2} \; s^{-1}$; ambient pressure 98.8 kPa.

Leaf stomatal conductance: Stomatal conductance of the intact fully young leaf was measured with an automatic porometer (MK_3; Delta-T Devices, Cambridge) from 9.30 to 11.30 a.m.

Statistical analysis of data: The results for each variable (based on $n = 6$) were subjected to a two-way analysis of variance using a COSTAT package (Cohort Software, Berkeley, USA) and the least significant differences (LSD) were calculated following Snedecor & Cochran, (1980).

Results

The disease incidence was recorded following Ali *et al*., (1995) considering vein thickening and leaf curling as the selection criteria. The disease occurred only on S-12, whereas all the plants of CIM-448 remained free of disease. The disease incidence on S-12 was more severe at lower levels of K while the plants of this cultivar at the highest level of K (236 mg L^{-1}) were almost free of disease and disease incidence was only 8.33% (Fig. 1).
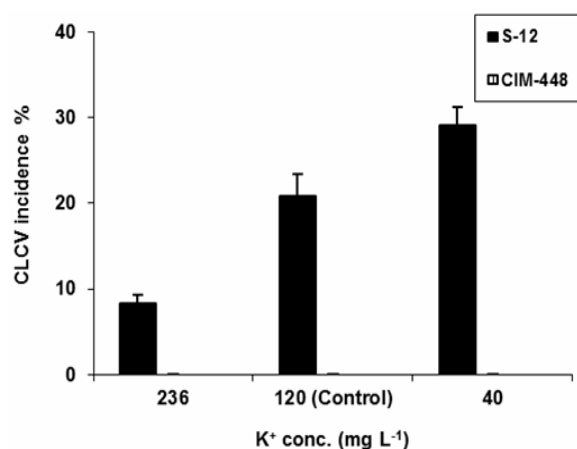

Fig. 1. Cotton leaf curl virus incidence (%) in S-12 and CIM-448 at the flowering stage grown in sand culture under different K regimes in Hoagland's nutrient solution.

Varying K levels of the growth medium had a significant effect on fresh or dry matters of shoots of the two cultivars differing in resistance to CLCuV (Table 1, Fig. 2). There was an overall difference between the two cultivars in shoot dry biomass since the interaction term (T × Cv) was non-significant for this variable (Table 1). Overall, CIM-448 was better in growth than S-12 at all external K regimes (Fig. 2).

Chlorophyll a decreased consistently in all the cultivars with decrease in the external K levels. CIM-448 had higher chlorophyll a accumulation than that in the healthy and diseased leaves of S-12. Chlorophyll b in the healthy and diseased leaves of S-12 was minimum at intermediate K regime (120 mg L^{-1}) and it was high ($p \leq 0.001$) at the higher and lower K regimes (Table 2). In contrast in CIM-448, it was high at 120 mg L^{-1} of K and low at the remaining, two K regimes (Fig. 3). Chlorophyll a/b ratio in the healthy and diseased leaves of S-12 remained high at 120 mg L^{-1} of K and low at the lower extreme external K regimes. CIM-448 had maximum chlorophyll a/b at the highest K regime but its chl. a/b ratio at the other two levels of K remained unchanged (Fig. 3).

Different K regimes of the growth medium had a significant effect on the epicuticular wax content of the two cultivars. Wax content in the healthy leaves of S-12 was high at the highest K regime and low at the two lower levels of K, whereas in the diseased leaves of S-12 and healthy leaves of CIM-448, it remained unaffected with the decrease in external K levels (Fig. 3). The cultivar difference was inconsistent for this variable. The significant increase in wax content on the diseased leaves of S-12 may have been due to the curled and shriveled surface of these leaves with many slight grooves. Although it was tried to make the leaf surface plan and smooth by slightly pressing the leaf, it was not possible to make it fully smooth. Thus in views of this technical reason inflated values of epicuticular wax content of diseased leaves are expected.

K^+ concentration in the healthy leaves of S-12 was maximum at 236 mg L^{-1} of K and remained unaffected at the lower two K regimes, whereas in the diseased leaves of S-12, it was high at 120 mg L^{-1} of K and low ($p \leq 0.001$) at the lowest K regime (40 mg L^{-1}) (Table 3). In CIM-448, K^+ concentration remained unaffected at the higher K regimes and decreased only at the lowest K regime (Fig. 4). S-12 was better in accumulation of K as compared to CIM-448.

Ca^{2+} concentration in the healthy leaves of S-12 remained unaffected at varying K regimes whereas in diseased leaves of S-12 and healthy leaves of CIM-448, it increased significantly ($p \leq 0.001$) with decease in K regimes (Table 3; Fig. 4). Cultivar difference was only evident at the lowest K regime at which diseased leaves of S-12 were the highest in Ca^{2+} accumulation of all types of leaves or cultivars (Table 3).

Mg^{2+} concentration in the healthy leaves of S-12 and CIM-448 increased significantly ($p \leq 0.001$) with decrease in the external K, whereas in the diseased leaves of S-12 it remained unaffected at the highest K regime and increased only at 40 mg L^{-1} of K (Tables 3, Fig. 4). Cultivars were also significantly different at all the external K regimes and CIM-448 was better in accumulation of Mg^{2+} as compared to S-12 (Table 3, Fig. 4).

Table 1. Mean squares from analyses of variance of data for shoot fresh and dry weights of two cultivars of cotton at the flowering stage grown in sand culture under different K regimes in Hoagland's nutrient solution.

Source of variation	df	Shoot F. wt.	Shoot D. wt.
Blocks	5	34.454 NS	1.963 NS
Treatments (T)	2	190.542 *	10.303 *
Cultivars (Cv)	1	57.760 NS	21.499 **
T × Cv	2	30.887 NS	2.814 NS
Error	25	39.049	2.446

*, ** = Significant at 0.05 and 0.01 levels, respectively

NS = Non-significant

Table 2. Mean squares from analyses of variance of data for chlorophyll a, b, a/b ratio and wax content of healthy (H) and diseased (D) leaves of CLCuV-resistant or CLCuV-susceptible cotton cultivars/lines at the flowering stage grown in sand culture under different K regimes in Hoagland's nutrient solution.

Source of variation	Df	Chl a	Chl b
Blocks	5	0.0270 *	0.0018 NS
Treatments (T)	2	0.5251 ***	0.0055 NS
Cultivars (Cv)	2	0.3341 ***	0.0752 ***
T × Cv	4	0.0583 ***	0.0268 ***
Error	40	0.0092	0.0023

*, *** = Significant at 0.05 and 0.001 levels, respectively

NS = Non-significant

Table 3. Mean squares from analyses of variance of data for water potential, potassium, calcium, magnesium, nitrogen and phosphorus of healthy (H) and diseased (D) leaves of CLCuV-resistant or CLCuV-susceptible cotton cultivars/lines at the flowering stage grown in sand culture under different K regimes in Hoagland's nutrient solution.

Source of variation	df	Water potential	Potassium
Blocks	5	0.004 NS	311.4 NS
Tmts. (T)	2	0.976 ***	14242.1 ***
Cults. (Cv)	2	6.469 ***	25739.0 ***
T × Cv	4	0.351***	4923.5 **
Error	40	0.003	1269.3

, * = Significant at 0.01 and 0.001 levels, respectively
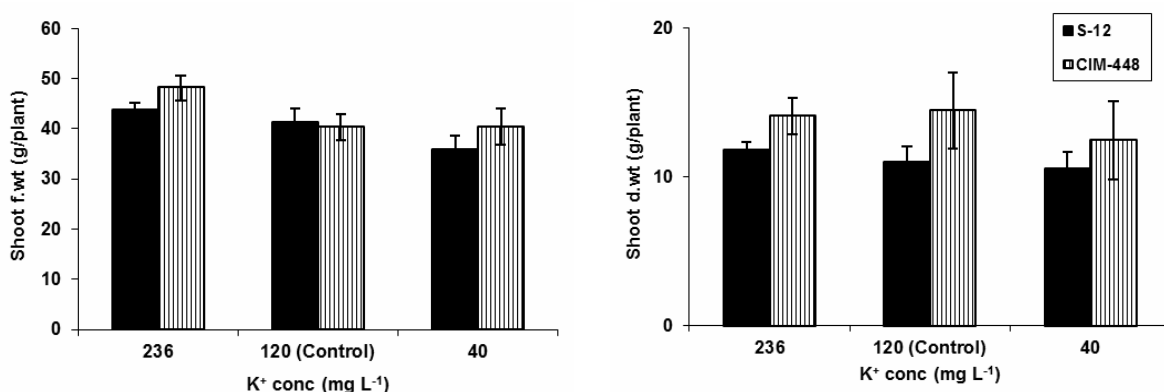
NS = Non-significant

Fig. 2. Shoot fresh and dry weights (g/plant) of two cultivars of cotton at the flowering stage grown in sand culture under different K regimes in Hoagland's nutrient solution.
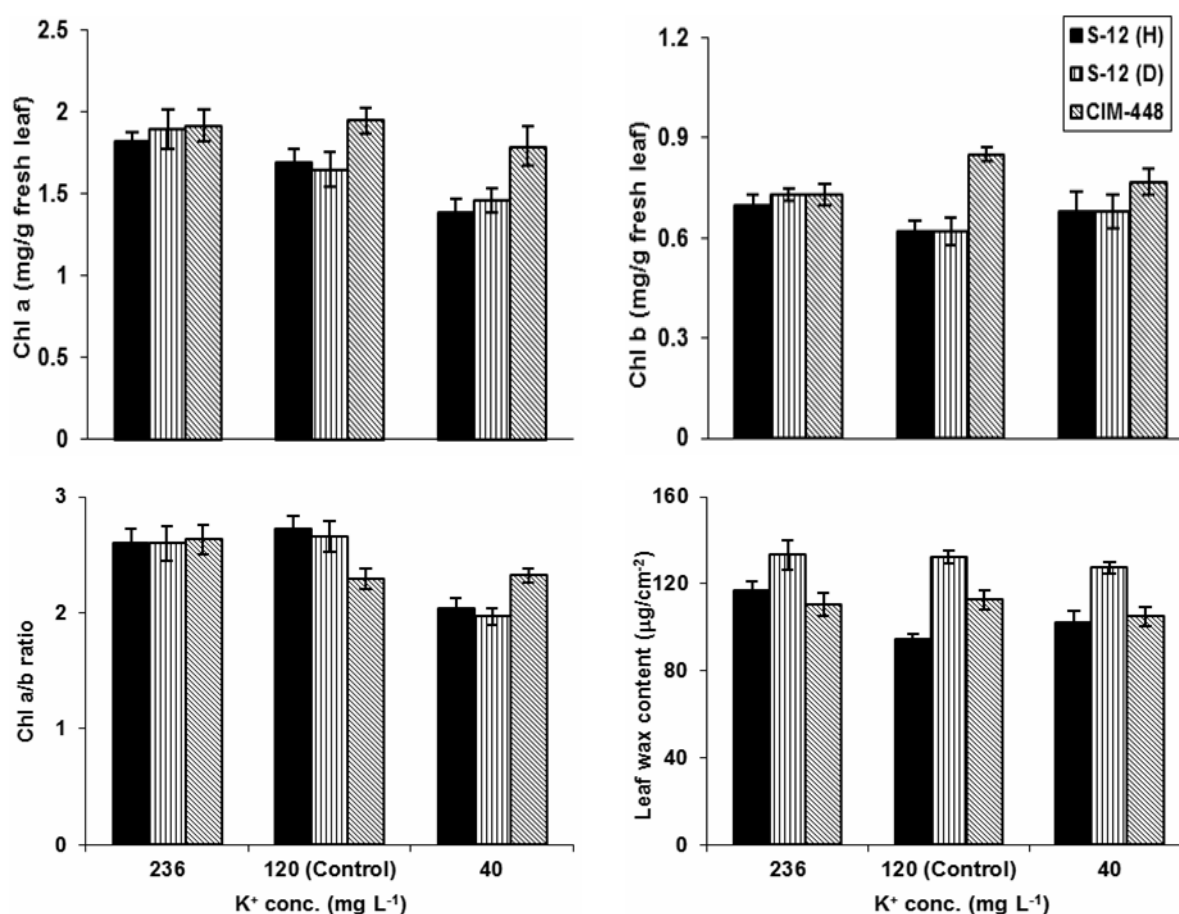
Fig. 3. Chlorophyll a, b, a/b ratio (mg g^{-1} fresh tissue) and leaf wax content (µg cm^{-2}) in healthy (H) and diseased (D) leaves of CLCuV-resistant or CLCuV-susceptible cotton cultivars/lines at the flowering stage grown in sand culture under different K regimes in Hoagland's nutrient solution.

A consistent increase in N concentration was observed in the healthy leaves of S-12 with decrease in K regimes, whereas in the diseased leaves of S-12, it was high at 40 mg L^{-1} of K but low at the two higher external K regimes. In CIM-448, it remained unaffected at the two higher K regimes, but it increased only at the lowest K regime (40 mg L^{-1}) (Fig. 4). Comparison of the varieties for this variable shows that diseased leaves of S-12 had significantly greater concentration of N as compared to healthy leaves of both S-12 and CIM-448 (Table 3, Fig. 4).

Phosphorus concentration in the healthy leaves of S-12 was maximum at the highest K regime (236 mg L^{-1}) and it remained unaffected at the lower two K regimes, whereas in the diseased leaves of the same cultivar it remained unaffected at the two higher K regimes and decreased only at the lowest K regime (40 mg L^{-1}) (Fig. 4). In CIM-448, P concentration was minimum at 120 mg L^{-1} of K but high at the other two K regimes. The cultivar difference was not consistent for this variable (Table 3).

Water potential of the healthy leaves of S-12 and CIM-448 decreased significantly with decrease in the external K regimes, whereas that of the diseased leaves of S-12, it was low at 120 mg L^{-1} of K and high at the two extreme K levels (Table 3, Fig. 4). Cultivar difference was clear at all the external K regimes. CIM-448 had

higher leaf water potential as compared to S-12 at all K regimes (Fig. 4).

Different K regimes had significant (p≤0.001) effect on the soluble proteins (Table 4, Fig. 5). In the healthy leaves of S-12, soluble proteins decreased with decrease in the external K, whereas in the diseased leaves of the same cultivar and healthy leaves of CIM-448 these remained unaffected at different K regimes (Fig. 5). Cultivar difference was non-consistent for this variable at varying external K levels (Fig. 5).

Varying K levels of the growth medium had no significant effect on free amino acids of all the cultivars (Table 4, Fig. 5). There was an overall difference between the cultivars in free amino acids since the interaction term (T x Cv) was non-significant for this variable. Thus, it was not possible to compare the cultivars at any of the external K regimes. However, free amino acids were higher in S-12, particularly in its diseased leaves as compared to those in CIM-448 (Fig. 5).

Total soluble sugars decreased consistently with decrease in the external levels of K in all the cultivars. Cultivar difference was prominent at the higher K regimes as compared to that at the lowest K regime (Fig. 5). CIM-448 accumulated higher concentration of soluble sugars in its leaves as compared to S-12.
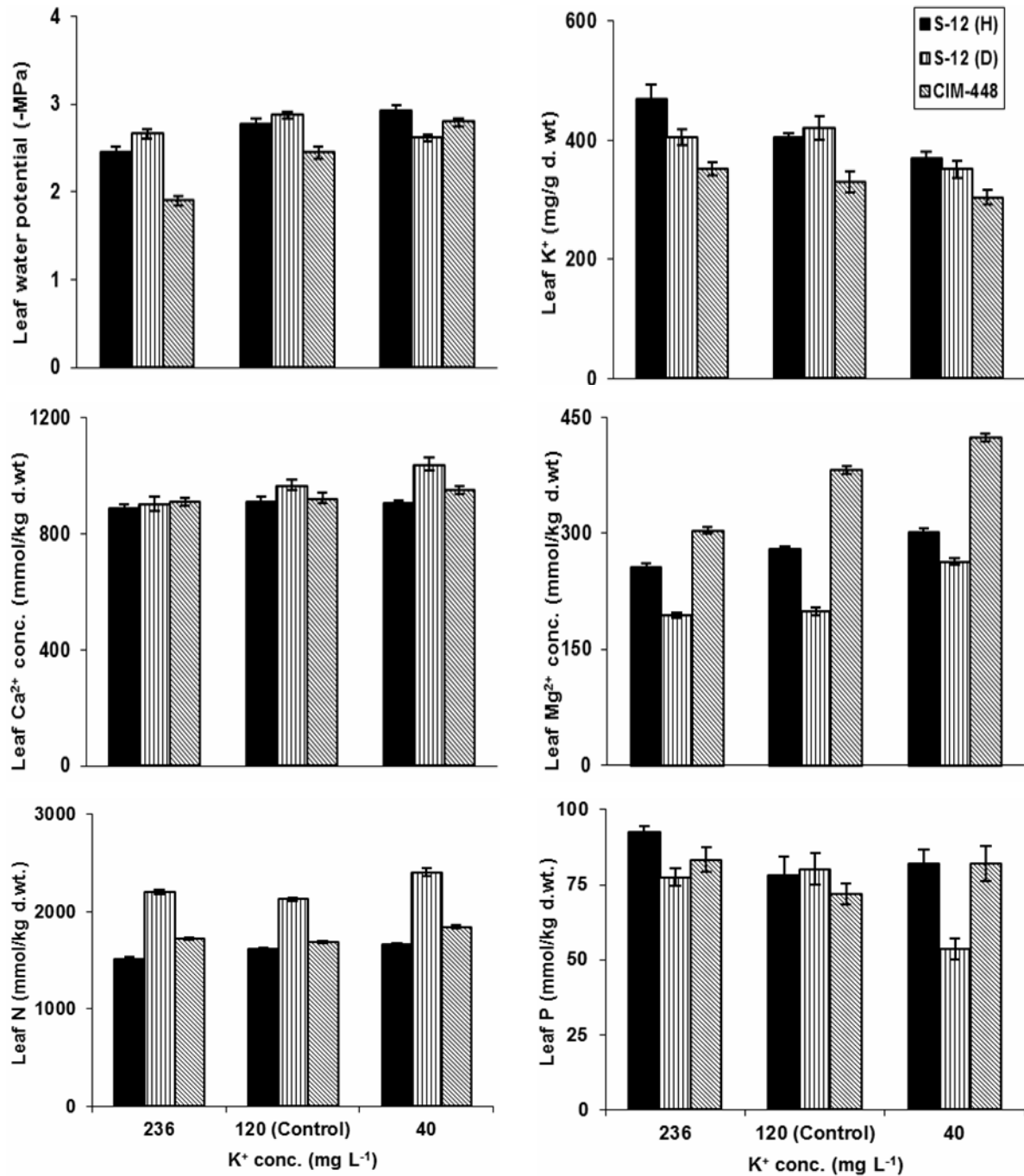
Fig. 4. Leaf water potential (-MPa), potassium, calcium, magnesium, nitrogen and phosphorus concentration (mmol kg^{-1} d. wt.) in healthy (H) and diseased (D) leaves of CLCuV-resistant or CLCuV-susceptible cotton cultivars/lines at the flowering stage grown in sand culture under different K regimes in Hoagland's nutrient solution.

Starch content in the healthy leaves of S-12 was maximum at the 120 mg L^{-1} of K and low at the other two external K levels. In CIM-448, starch content remained unaffected at higher K regimes and decreased significantly only at the lowest K regime (40 mg L^{-1}) (Table 4, Fig. 5). Cultivars differed significantly at the highest K regime but not at two lower K regimes.

Net CO$_2$ assimilation rate (A) in the healthy leaves of S-12 decreased significantly at the lowest external K regime, whereas in the diseased leaves of S-12, it remained unaffected at higher K regimes and decreased significantly (p≤0.01) only at the lowest K regime (40 mg L^{-1}) (Table 5, Fig. 6). In CIM-448, it changed only at the highest and the lowest K regimes, while it remained unaffected at the intermediate K regime (120 mg L^{-1}) (Table 5). Comparison of the cultivars for this variable shows that CIM-448 had significantly greater net CO$_2$ assimilation rate than that of healthy and diseased leaves of S-12 at all external K regimes (Table 5, Fig. 6).

Table 4. Mean squares from analyses of variance of data for total soluble proteins, free amino acids, total soluble sugars and starch of healthy (H) and diseased (D) leaves of CLCuV-resistant or CLCuV-susceptible cotton cultivars/lines at the flowering stage grown in sand culture under different K regimes in Hoagland's nutrient solution.

Source of variation	df	Soluble proteins	Free amino acids
Blocks	5	1.03 NS	9784.11 NS
Treatments (T)	2	10.59 **	4681.19 NS
Cultivars (Cv)	2	4.75 ***	184707.19 **
T × Cv	4	7.01 ***	52504.74 NS
Error	40	1.31	25706.31

, * = Significant at 0.01 and 0.001 levels, respectively
NS = Non-significant

Table 5. Mean squares from analyses of variance of data for net CO_2 assimilation rate and transpiration of healthy (H) and diseased (D) leaves of CLCuV-resistant or CLCuV-susceptible cotton cultivars/ lines at the flowering stage grown in sand culture under different K regimes in Hoagland's nutrient solution.

Source of variation	df	Net CO_2 assimilation rate	Transpiration
Blocks	5	0.09 NS	0.95 NS
Tmts. (T)	2	17.92 ***	3.01 ***
Cults. (Cv)	2	56.78 ***	20.03***
T × Cv	4	2.30 **	0.80 NS
Error	40	0.50	0.84

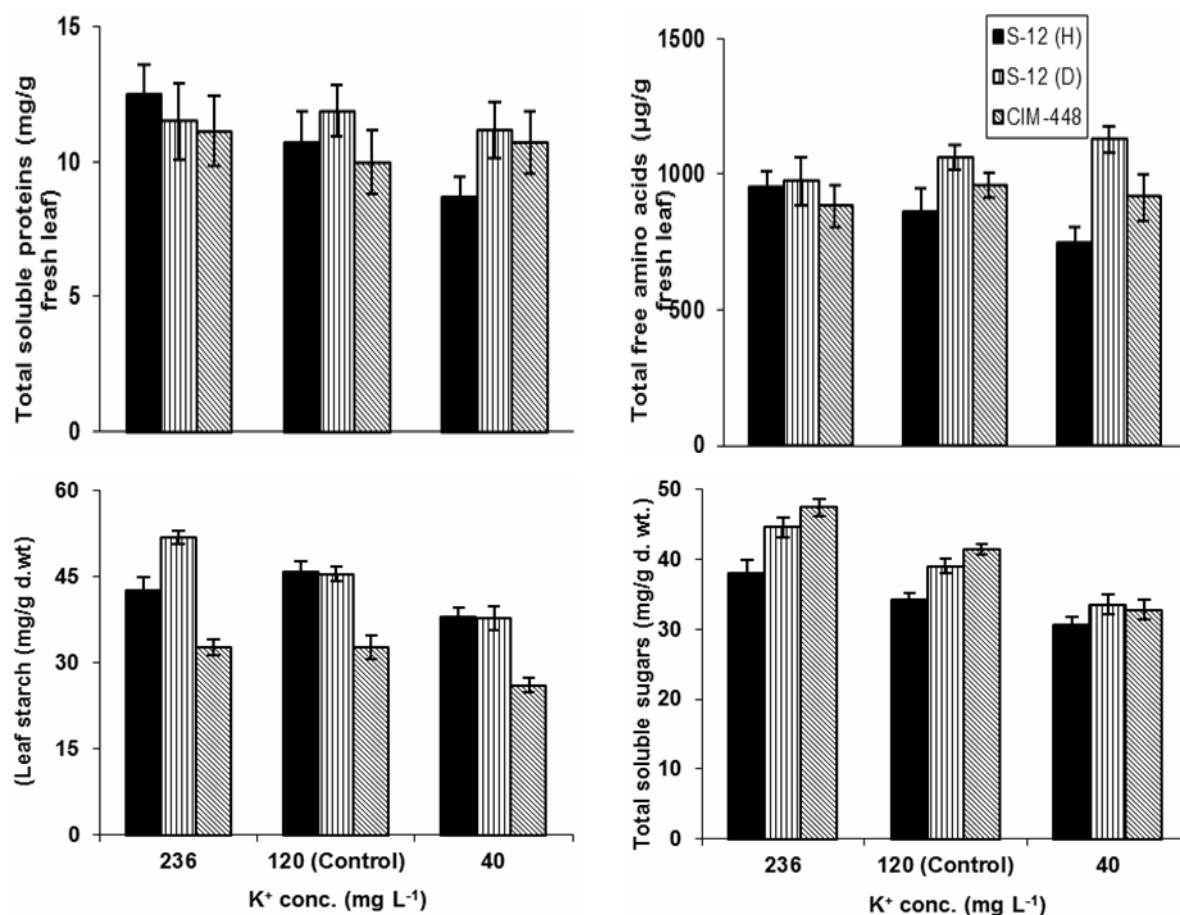*,**, *** = Significant at 0.05, 0.01 and 0.001 levels, respectively
NS = Non-significant

Fig. 5. Total soluble proteins (mg g^{-1} fresh leaf tissue), total free amino acids (µg g^{-1} fresh leaf tissue), total soluble sugars (mg g^{-1} dry leaf tissue) and starch (mg g^{-1} dry leaf tissue) in healthy (H) and diseased (D) leaves of CLCuV-resistant or CLCuV-susceptible cotton cultivars/lines at the flowering stage grown in sand culture under different K regimes in Hoagland's nutrient solution.

Fig. 6. Net CO_2 assimilation rate (mmol CO_2 m^{-2} s^{-1}), transpiration (mmol H_2O m^{-2} s^{-1}), water use efficiency (mmol CO_2/mmol H_2O), stomatal conductance (cm s^{-1}), substomatal CO_2 concentration (mmol mol^{-1}) and relative substomatal CO_2 in healthy (H) and diseased (D) leaves of CLCuV-resistant or CLCuV-susceptible cotton cultivars/lines at the flowering stage grown in sand culture under different K regimes in Hoagland's nutrient solution.
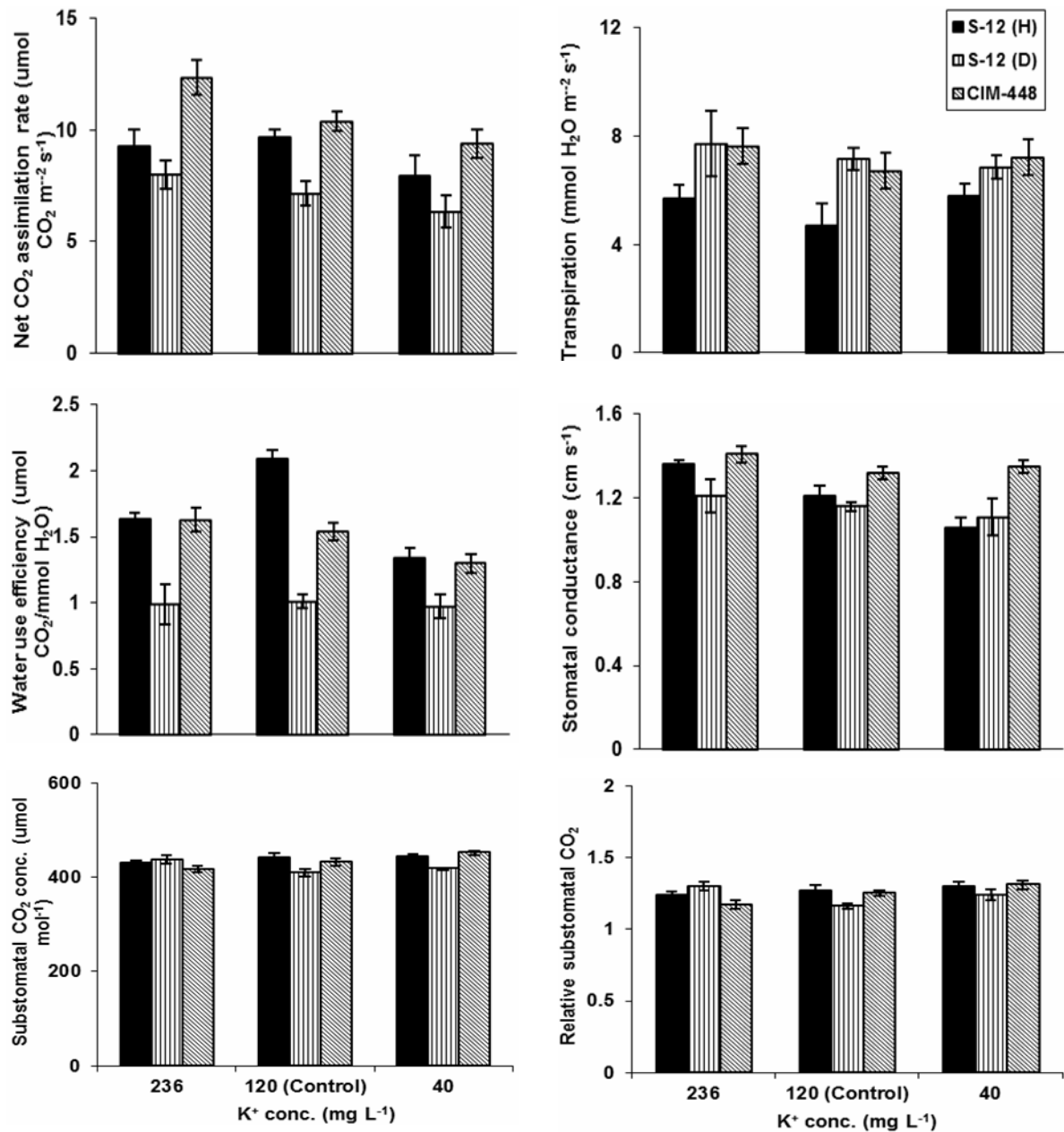
Varying concentrations of K had a significant effect on the transpiration rate of both cultivars. The interaction term (T x Cv) was non-significant in this variable. Thus, it was not possible to compare the cultivars at any of the external K regimes (Table 5, Fig. 6).

Water use efficiency (*WUE*), calculated as A/E, was maximum at 120 mg L^{-1} of K and decreased significantly at both the high and low external K regimes, whereas in the diseased leaves of the same cultivar, it remained unchanged at all the external K regimes (Table 5, Fig. 6). In CIM-448, it remained unaffected at the higher two K regimes and decreased only at the lowest K regime (40

mg L^{-1}) (Fig. 6). The cultivar difference was not consistent for this variable.

Stomatal conductance in the healthy and diseased leaves of S-12 decreased significantly (p≤0.001) with decrease in the external N regimes, whereas in CIM-448, it was maximum at 236 mg L^{-1} of K and remained unchanged at the two lower levels of K. CIM-448, generally had higher stomatal conductance than S-12 (Fig. 6).

Substomatal CO_2 concentration (*Ci*) in the healthy leaves of S-12 remained unaffected at all external K regimes, whereas in the diseased leaves of S-12, it was maximum at the highest K regime and remained

unaffected at the two lower K regimes. In CIM-448, it remained unaffected at the higher K regimes but at the lowest K regime it was maximum (Fig. 6).

The ratios of substomatal CO_2 to ambient CO_2 (C_i/C_a) in the healthy leaves of S-12 decreased with increase in the external K levels. In contrast, in the diseased leaves of S-12 the pattern of increase or decrease in Ci/Ca was not consistent at varying external K regimes. In CIM-448, it consistently increased with decrease in K regimes (Fig. 6).

Discussion

In the present study it was found that disease resistance, particularly in CLCuV susceptible S-12, was decreased with decreasing levels of K of the growth medium. This might have been due to lower K supply reduced the strength of epidermal wall structures and epicuticular wax deposition (Huber & Graham, 1999, Datnoff *et al*., 2006). However, leaf epicuticular wax content was higher in the diseased leaves of S-12 as compared to the healthy leaves of S-12 and CIM-448 and cannot be explained in line with the earlier findings of Ashraf & Zafar, (1999; 2000) who observed high leaf epicuticular wax content in disease resistant cotton cultivars.

From the results, it is evident that although decreasing K levels of the growth medium caused an inhibitory effect on the growth of both cultivars (S-12 and CIM-448), the adverse effects on growth of S-12 was more pronounced. This differential response of the two cultivars to external K supply can be explained in view of the argument that it is due to their physiological behavior for K use efficiency that resulted in differences in growth. For example, working with sweet potato, George *et al*., (2002) observed genotypic variation for K^+ uptake, root dry matter, and protein content under varying concentrations of K^+. In another study with cotton, Zhang *et al*., (2007) also found differential responses of cotton cultivars to K^+ application. These results can be explained in view of the arguments of Rengel & Damon, (2008) that some genotypes can have higher acquisition of K^+ from soil (uptake efficiency) and/or higher dry matter production per unit of K^+ taken up (utilization efficiency). Thus, CIM-448 is efficient in K^+-utilization efficiency.

Analysis of K^+ of the healthy and diseased leaves showed inconsistent pattern of K^+ accumulation. However, high concentration of K^+ in the healthy leaves of S-12 at both higher and lower external K^+ levels as compared with diseased leaves of S-12 is parallel to what has earlier been observed by Ashraf & Zafar, (1999; 2000) who found low accumulation of K^+ in diseased S-12 plants grown under normal conditions. However, since diseased leaves of S-12 severely curled and had substantial/partial blockage of vascular system of these leaves, it is expected that K^+ might not have transported from old leaves to younger leaves. In contrast, the lower accumulation of K^+ in disease resistant CIM-448 cannot be explained in view of earlier argument because the vascular tissue in this cultivar remained unblocked. However, higher accumulation of K^+ in healthy and diseased leaves of S-12 than that in CIM-448 can be explained as S-12 is K^+-uptake efficient while CIM-448 is K^+-use efficient. In the present study, a positive correlation was found between K^+ utilization efficiency at low available K^+ and relative shoot dry weight in two cotton cultivars as has earlier been observed in rice at the tillering stage (Yang *et al*., 2003). Similarly, in sweet potato, K^+ utilization

efficiency was positively correlated with total plant biomass and root yield (George *et al*., 2002). However, efficient utilization of K^+ in CIM-448 during the vegetative stage may not translate into K efficiency for economic yield as has been suggested by Rengel & Damon, (2008).

In the present study, accumulation of Ca^{2+} in the leaves of cotton cultivars with decrease of the external K^+ levels of the growth medium is not associated with disease resistance. Increase in accumulation of Ca^{2+} in the diseased leaves of S-12 might have been due to whitefly induced activation of Ca^{2+}-channels at vacuoles, chloroplast, endoplasmic reticulum thereby increase Ca^{2+} influx and cytosolic free Ca^{2+} concentrations (Atkinson *et al.,* 1990). Magnesium concentrations increased in both cultivars with decrease in K concentrations of the external growth medium. These results are quite parallel to the early findings of Fecenko, (1982), Sonneveld & Voogt, (1990), and Garcia *et al*., (1999) who report that high Mg^{2+} contents may occur in plants supplied with low levels of K. High Mg^{2+} contents in disease resistant-CIM-448 can be related to its high Chl a content as found in the present study. Increase in accumulation of N with concurrent reduction in K accumulation in both cultivars at low potassium supply indicating reduction in N use efficiency. This finding is further supported by the fact that K-deficiency caused a significant reduction in soluble proteins only in the diseased leaves of S-12, whereas it remained unchanged in healthy leaves of S-12 and CIM-448. The results for soluble proteins in the healthy leaves of S-12 are similar to those of Wyn Jones *et al*., (1979) who found that K is required in higher concentrations for protein synthesis.

Reduction in chlorophyll a in both cotton cultivars due to K deficiency may have been due to the enhanced activation of chlorophyllase. Ashraf & Naz, (1994) found similar results in some arid zone grasses under K deficiency. The higher chlorophyll a and chlorophyll a/b ratios in disease resistant CIM-448 can be related to its higher accumulation of Mg^{2+}, as chlorophyll biosynthesis is catalyzed by Magnesium-chelatase (Walker & Weinstein, 1991).

Higher stomatal conductance in plants is known to increase CO_2 diffusion into leaves thereby favoring higher photosynthetic rates. Higher net assimilation rate could in turn favor a higher biomass production (Taiz & Zeiger, 2010). The results for stomatal conductance and net assimilation rate presented here revealed that K deficiency caused a marked reduction in both net CO_2 assimilation rate and stomatal conductance. However, disease resistant CIM-448 had higher stomatal conductance and net CO_2 assimilation rate compared with S-12 diseased and healthy plants at all external K levels of the growth medium. These results are in agreement with those of Raza *et al*., (2006; 2007) who found that in wheat, higher stomatal conductance favored higher yield. This argument is further supported by the fact that disease resistant CIM-448 was higher in sugar accumulation as compared to S-12 (both healthy and diseased leaves), and positively correlated with higher rate of photosynthesis.

In conclusion, this study clearly depicts that low K levels had a significant effect on disease severity in S-12. Low K had a significant inhibitory effect on growth of the two cotton cultivars differing in resistance to CLCuV. Higher disease resistance of CIM-448 to CLCuV was found to be associated with its high Chl. a content, high rates of photosynthesis, and high accumulation of Mg^{2+} in the leaves.

References

Ali, M., Z. Ahmad, M. Tanveer and T. Mahmood. 1995. Cotton leaf curl virus in the Punjab-Current Situation and Review of Work. Central Cotton Research Institute, Multan/CLCV Project, Ministry of Food, Agriculture and Livestock, Government of Pakistan/Asian Development Bank.

Allen, S.E., H.M. Grimshaw and A.P. Rowland. 1986. Chemical analysis. In: *Methods in Plant Ecology,* (Eds.): P.D. Moore and S.B. Chapman. (2nd Ed) Blackwell Scientific Publications, Oxford, pp. 285-344.

Armengaud, P., R. Breitling and A. Amtmann. 2004. The potassium-dependent transcriptome of *Arabidopsis* reveals a prominent role of jasmonic acid in nutrient signaling. *Plant Physiol.*, 136: 2556-2576.

Ashraf, M. and F. Naz. 1994. Responses of some arid zone grasses to K deficiency. *Acta Physiol. Plant.*, 16(1): 69-80.

Ashraf, M. and Z.U. Zafar. 1999. Some physiological characteristics in resistant and susceptible cotton cultivars infected with cotton leaf curl virus. *Biol. Plant.*, 42(4): 615-620.

Ashraf, M. and Z.U. Zafar. 2000. Effect of low and high regimes of calcium on two cultivars of cotton (*Gossypium hirsitum* L.) differing in resistance to cotton leaf curl virus (CLCuV)-Growth and macronutrients. *Agrochimica*, 44: 89-100.

Athar, H.R., A.R. Bhatti, N. Bashir, Z.U. Zafar, A. Abida and A. Farooq. 2011. Modulating infestation rate of white fly (*Bemicia tabaci*) on okra (*Hibiscus esculentus* L.) by nitrogen application. *Acta Physiol. Plant.*, 33: 843-850.

Atkinson, M.M., L.D. Keppler, E.W. Orlandi, C.J. Baker and C.F. Mischke. 1990. Involvement of plasma membrane calcium influx in bacterial induction of the K^+/H^+ and hypersensitive responses in tobacco plant. *Plant Physiol.*, 92: 215-221.

Datnoff, L.E., W. Elmer and D.M. Huber (Eds.). 2006. *Mineral Nutrition and Plant Disease*. APS Press, St. Paul, MN

Epstein, E. 1972. Mineral Nutrition of Plants: Principles and Perspectives. Wiley, New York.

Epstein, E. and A.J. Bloom. 2005. *Mineral Metabolism- Mineral Nutrition of Plants: Principles and perspectives* 2nd edn. Sinuar Associates, Inc. Publishers, Saunderland, Massachusetts.

Fecenko, J. 1982. Uptake of magnesium and ion interaction. *Agrochemia* 22, 253-255.

Garcia, M., C. Daverede, P. Gallego and M. Toumi. 1999. Effect of various K-Ca ratios on cation nutrition of grapes grown hydroponically. *J. Plant Nut.* 22, 417-425.

George, M.S., G. Lu and W. Zhou. 2002. Genotypic variation for potassium uptake and utilization efficiency in sweet potato (*Ipomoea batatus* L.). *Field Crops Res.*, 77:7-15.

Graham, R.D. 1983. Effect of nutrient stress on susceptibility of plants to disease with particular reference to the trace elements. *Adv. Bot. Res.*, 10: 221-276.

Hamilton, P.B. and D.D. Van Slyke. 1943. Amino acid determination with ninhydrin. *J. Biol. Chem.*, 150: 231-233.

Huber, D., V. Romheld and M. Weinmann. 2012. Relationship between Nutrition, Plant Diseases and Pests. In: *Marschner's Mineral Nutrition of Higher Plants*, (Ed.): P. Marschner, 3rd Edition. Academic Press, U.K. pp. 283-298.

Huber, D.M. and R.D. Graham. 1999. The role of nutrition in crop resistance and tolerance to diseases: Fundamental Mechanisms and Implications. In: *Mineral Nutrition of Crops Food Products Press*, (Ed.): Z Rengel. the Haworth Press, Inc., New York, pp. 169-206.

Huber, D.M. and R.D. Graham. 2002. The role of nutrition in crop resistance and tolerance to diseases. In: Mineral Nutrition of Crops. pp 169-204

Jackson, M.L. 1958. Soil chemical analysis. Prentice Hall, Inc., Englewood Cliffs, New Jersey.

Lowry O.H., N.J. Rosebrough, A.L. Farr and R.J. Randal. 1951. Protein measurement with Folin phenol reagent. *J. Biol. Chem.,* 193: 265-275.

Malik, C.P. And A.K. Srivastava. 1985. Text Book of Plant Physiology. Kalyani Publisher, New Delhi, India.

Marschner, H. 1995. *Mineral Nurrition of Higher Plants*. (2nd Ed) Academic Press, London.

Mondal S. S., C.K. Pramanik and J. Das. 2001. Effect of nitrogen and potassium on oil yield, nutrient uptake and soil fertility in soybean (*Glycine max*) – sesame (*Sesamum indicum*) intercropping system. *Indian J. Agric. Sci.,* 71: 44-46.

Oosterhuis D.M., C.W. Bednarz and A. Stegar. 1997. Potassium deficiency in cotton: Physiological aspects and tissue sampling, Beltwide Cotton Conference, Orleans, USA, pp 610

Prabhu A.S., N.K. Fageria and D.M. Huber. 2007. Potassium nutrition and plant diseases. In: *Mineral Nutrition and Plant Disease.* (Eds.) L.E. Datnoff., W.H. Elmer and D.M. Huber. The American Phytopathological Society Press, Saint Paul.

Raza, S.H., H.R. Athar and M. Ashraf. 2006. Influence of exogenously applied glycine betaine on the photosynthetic capacity of two differently adapted wheat cultivars under salt stress. *Pak. J. Bot.*, 38(2): 341-351.

Raza, S.H., H.R. Athar, M. Ashraf and A. Hameed. 2007. Glycine betaine-induced modulation of antioxidant enzymes activities and ion accumulation in two wheat cultivars differing in salt tolerance. *Environ. Expt. Bot.*, 60(3): 368-376.

Rengel, Z. and M.P. Damon. 2008. Crops and genotypes differ in efficiency of potassium uptake and use. *Physiol. Plant.,* 133: 624-636.

Schachtman, D.P. and R. Shin. 2006. Nutrient sensing and signaling: NPKS. *Annu. Rev. Plant Biol.*, 58: 47-69.

Silva Fernandes, A.M., E.A. Baker and J. T. Martin. 1964. Studies on plant cuticle. VI. The isolation and fractionation of cuticular waxes. *Ann. Appl. Biol.* 53: 43-58.

Snedecor, G.W. and W.G. Cochran. 1980. *Statistical Methods*. 7th edition. The Iowa State. University Press, Ames.

Sonneveld, C. and W. Voogt. 1990. Responses of tomatoes (*Lycopersicon esculantum*) to an unequal distribution of nutrients in the root environment. *Plant and Soil.,* 124: 251-256.

Taiz L. and E. Zeiger. 2010. Plant Physiology, 5th edn. Sinauer Associates, Inc., Publishers, Sunderland, Massachusetts.

Walker, C.J. and J.D. Weinstein. 1991. *In vitro* assay of the chlorophyll biosynthetic enzyme Mg-chelatase: Resolution of the activity into soluble and membrane-bound fractions. *Proc. Natl.Acad. Sci*., 88: 5789-5793.

Walters, D.R. and I.J. Bingham. 2007. Influence of nutrition on disease development caused by fungal pathogens: implications for plant disease control. *Ann. Appl. Biol.*, 151: 307-324.

Witham, F.H., D.F. Blaydes and R.M. Devlin. 1971. Experiment in Plant Physiology. Van Norstand Reinhold Co., New York.

Wyn Jones, R.G., C.J. Brady and J. Spiece. 1979. Ionic osmotic relations in plant cells. In: Recent Advances in the Biochemistry of Cereals. Academic Press, New York, pp 63-103.

Yang, X.E., J.X. Liu, W.M. Wang, H. Li, A.C. Luo, Z.Q. Ye and Y. Yang. 2003. Genotypic differences and some associated plant traits in potassium internal use efficiency of lowland rice (*Oryza sativa* L.). *Nutr. Cycl. Agroecosyst.*, 67: 273-282.

Zafar, Z.U., H.R. Athar and M. Ashraf. 2010. Responses of two cotton (*Gossypium hirsutum* L.) cultivars differing in resistance to leaf curl virus disease to nitrogen nutrition. *Pak. J. Bot.,* 42(3): 2085-2094.

Zhang, Z., X. Tian, L. Duan, B. Wang, Z. He and Z. Li. 2007. Differential responses of conventional and Bt-transgenic cotton to potassium deficiency. *J. Plant Nutr.*, 30: 659-670.