

INFLUENCE OF DIFFERENT PHOSPHORUS REGIMES ON DISEASE RESISTANCE IN TWO COTTON (*GOSSYPIUM HIRSUTUM* L.) CULTIVARS DIFFERING IN RESISTANCE TO COTTON LEAF CURL VIRUS (CLCUV)

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Abstract

In recent years, plant diseases are major limiting factor in agricultural production. However, severity of disease incidence can be reduced by nutrient management. Therefore, understanding physiological and biochemical changes in relation to disease incidence will help in devising economic approaches to control crop damages due to different diseases. Therefore, two cotton (*Gossypium hirsutum* L.) cultivars, S-12 (CLCuV-susceptible) and CIM-448 (CLCuV-resistant) were grown at varying concentration [62, 31(control) 15.5 and 3.88 mg P L⁻¹] of phosphorus supplied with Hoagland's nutrient solution. Throughout the experiment, cv. CIM-448 remained free from CLCuV disease, whereas plants of cv. S-12 showed a degree of disease symptoms. Increasing P supply caused a consistent increase in growth of both cotton cultivars with concomitant increase in disease symptoms in only cv. S-12. The CLCuV-resistant cultivar, CIM-448 was higher in growth than that of cv. S-12 at all external phosphorus regimes. Leaf epicuticular wax content was greater in diseased leaves of CLCuV-susceptible S-12 as compared to its healthy leaves and CLCuV-resistant cultivar CIM-448. Lowering in P supply caused a decline in leaf K⁺ in both healthy leaves of S-12 and CIM-448 but it remained unchanged in diseased leaves of S-12. However, diseased leaves of S-12 had significantly higher leaf K⁺ and Ca²⁺ as compared to those of healthy S-12 and CIM-448. Leaf Mg²⁺ concentration was higher in CIM-448 as compared to that to diseased or healthy leaves of S-12 at all P levels. Accumulation of N remained unchanged with decrease in P levels. Leaf P content was decreased with decrease in external P levels in all the cultivars. Total soluble proteins and amino acids increased with the decrease of P levels. Total soluble sugars were lower in healthy leaves of S-12 as compared to diseased leaves of S-12 and CIM-448 at all external P levels whereas starch content decreased in diseased leaves of S-12 with decrease in P levels but it remained unchanged in diseased leaves of S-12 and CIM-448. Net CO₂ assimilation rate decreased in both cultivars with decrease in P levels. In conclusion, low P supplies decreased the severity of disease in S-12. Disease resistance in S-12 and CIM-448 was positively associated with P and Mg²⁺ accumulation, photosynthetic rate and low accumulation of soluble sugars, soluble proteins and total free amino acids.

Introduction

In Pakistan, growth and crop productivity of cotton is severely affected by cotton leaf curl virus (CLCuV) during the last three decades. Cotton leaf curl virus is transmitted by the whitefly *Bemisia tabaci* (Rybicki & Fauquet, 1998). Under the severe attack of the virus particularly at initial growth stages, CLCuV disease caused considerable yield losses (Akhtar *et al.*, 2003). However, plant scientists have recommended some effective management practices which include cultivation of virus resistant varieties and effective management of causal agents through genetic engineering (Mansoor *et al.*, 2003) or cultural practices (Hilji *et al.*, 2001; Athar *et al.*, 2011). Various cultural practices to manage whitefly include avoidance of causal agents by modulating host plant metabolism or removal of insects using insecticides or by manipulating their behavior. Mineral nutrients are essential for crop growth and productivity, use of different mineral nutrients may alter plant metabolism and thus played a role in plant-disease interactions (Hilje *et al.*, 2001; Zafar *et al.*, 2010). However each mineral nutrient changes resistance of plants to bacterial or viral disease varied in different plant species (Huber *et al.*, 2012).

Phosphorus is second to nitrogen as the most limiting element for plant growth (Epstein & Bloom, 2005; Taiz & Zeiger, 2010). It is also involved in the regulation of metabolic pathways particularly photosynthetic metabolism in chloroplasts (Epstein & Bloom, 2005; Taiz & Zeiger, 2010). For example, P deficiency decreases photosynthetic CO₂ assimilation rate in *Glycine max* (Fredeen *et al.*, 1989), *Helianthus annuus* and *Zea mays*

(Jacob & Lawlor, 1992), reduces translocation of carbohydrates from source tissue to sink (Epstein & Bloom, 2005; Taiz & Zeiger, 2010), decreases number of leaves and total leaf area, and reduced transport of hormones (Epstein & Bloom, 2005). It is generally accepted that alternation in plant nutrient status may alter the susceptibility to viruses (Marschner, 1995; Hilji *et al.*, 2001; Zafar *et al.*, 2010; Athar *et al.*, 2011). For instance, Bawden (1995) reported that N and P increased susceptibility of tobacco plant to tobacco mosaic virus and P was more effective than N in increasing the susceptibility in tobacco. Working with the nutrition status of the CLCuV-diseased and healthy leaves of cotton, Rashid *et al.*, (1995) found that the content of N, P, K and micronutrients, Zn, Cu, Fe, and Mn was the same in healthy and diseased leaves. In contrast, Ahmad (1995) reported that the leaves of diseased plants of S-12 and CIM-240 had higher concentration of N than the healthy leaves of the same cultivars. Likewise in other studies with cotton, higher N application has been shown to increase N, amino acids and the incidence of CLCuV-disease (Zafar *et al.*, 2010). Similarly, Athar *et al.*, (2011) reported that higher N application to okra increased the susceptibility of plants as measured in terms of number of whitefly eggs and nymphs.

In view of all these studies, the present study was aimed to assess the pattern of accumulation of soluble proteins, amino acids, soluble sugars and photosynthetic capacity in two high yielding cotton cultivars at high and low levels of P. From a number of earlier studies it is now well evident that cv S-12 is highly susceptibility to CLCuV (Mansoor *et al.*, 1993; Zafar *et al.*, 2010) and

CIM-448 resistant to this disease (Ali *et al.*, 1995; Zafar *et al.*, 2010). It is therefore, the present study was also aimed to assess how low and high P supplies affects the incidence of cotton leaf curl virus (CLCuV) in two cultivars of cotton differing in disease resistance.

Materials and Methods

Two cotton cultivars cv CIM-448 (CLCuV-resistant) and cv S-12 (CLCuV-susceptible) were used in the present study. Seeds of cotton were obtained from the Central Cotton Research Institute Multan, Pakistan. Three hundred seeds of each cotton cultivar were disinfected with 5% sodium hypochlorite solution and sown in plastic trays for two weeks, after which seedlings of uniform size were transplanted in pots filled with 16.5 kg river sand washed with tap water. Varying concentrations of P (3.88, 15.5, 31 and 62 mg L⁻¹) were applied in full strength Hoagland's nutrient solution. In order to maintain the nutrients status of other mineral nutrients, amendments were made in Hoagland's nutrient solution using 1M (NH₄)₂SO₄ and 1M NH₄H₂PO₄ following Taiz & Zeiger (2010). Nutrient solutions were replaced on weekly basis. However, 200 mL water was applied daily to each pot to compensate evapo-transpirational water loss. The experiment was arranged in a randomized complete block design with six blocks. Each block contained two lines and four Phosphorus concentrations. At the onset of the flowering stage, 2 plants from each pot were harvested, separated into shoots and roots and their fresh weights measured. Both shoots and roots were oven-dried at 70°C for one week and their dry weights measured. At the time of harvest following physiological parameters were measured.

Epicuticular wax content: Fully developed leaves of same age were taken from healthy and diseased plants of both cotton cultivars and their leaf area measured using leaf area meter (Delta T Devices, Burwell, Cambridge, England). The leaf samples were washed with 40, 30, and 30mL of carbon tetrachloride for 30 s per wash. The extract thus obtained was evaporated to dryness, and the remaining wax was weighed. Wax content was expressed on the basis of unit leaf area (µg/cm²).

Water potential: A fully expanded young leaf was excised and its water potential was measured using 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). Hundred milligram fresh leaves was ground in 80% acetone (v/v) and centrifuged at 10000 x g for 5 minutes. The absorbance of the supernatants was measured at 645, 652 and 663 nm using a spectrophotometer (Hitachi U-2000, Tokyo Japan).

Macronutrients (K, Ca, Mg, N, and P): Macronutrients in a fully expanded youngest leaf from each plant were determined by the methods described by Allen *et al.*, (1986). Oven dried grounded leaf material (100 mg) were

digested in 2mL of sulphuric-peroxide digestion mixture at 250°C. The volume of digested sample was made 100mL with distilled water. Potassium contents were measured using a flame photometer (PFP7 Jenway), while those of Ca and Mg were measured with an atomic absorption spectrometer (Analyst 100, Perkin Elmer). 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 in leaves of cotton cultivars were determined following Lowry *et al.*, (1951). Fresh leaf material from healthy and diseased plants (0.2g) was ground in 5mL of phosphate buffer solution (pH = 7.0). One mL of sample extracts were mixed with 0.5mL of Folin phenol reagent in a test tube and allowed to stand for 10 minutes, after which the absorbance was measured at 620nm using a spectrophotometer (Hitachi U-2000).

Total free amino acids: Total free amino acids in leaves of cotton cultivars were estimated according to the method of Hamilton and Van Slyke (1943). One mL of each sample extract was treated with 1mL of 2% ninhydrin solution and 1mL of 10% pyridine. The mixture was heated in boiling water for 20 min and then cooled at room temperature. The mixture was diluted up to 20mL with distilled water and absorbance of the mixture was measured at 570nm using a spectrophotometer (Hitachi U-2000).

Total soluble sugars: Total soluble sugars in oven dried leaves of cotton cultivars were determined according to the method of Malik & Srivastava (1985). Hundred milligram oven-dried and ground leaf materials was mixed with 80% ethanol and filtered. The volume of filtrate was made 100mL with distilled water. The diluted filtrate was treated with anthrone reagent and optical density was read at 625nm.

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°C for 15 min., and then centrifuged at 2900×g. The above procedure repeated three times and volume of the extract was made 100mL with distilled water. The diluted filtrate was treated with anthrone reagent and optical density was read at 625nm.

Gas exchange parameters: Gas exchange parameters were measured with ADC LCA-4 portable photosynthesis system (Analytical Development, Hoddesdon, UK). The analyzer was calibrated prior to measurements and checked for leaks. Measurements were taken between 10:30 am to 13:30 pm on fully developed and young leaves using a reference CO₂ concentration 380µmol mol⁻¹. Leaf area was adjusted to 11.25cm², while temperature of leaf chamber was 32°C. Gas flow rate to leaf chamber was adjusted to 200ml min⁻¹. PAR (Q_{leaf}) at leaf surface during noon varied from 800 to 1600µmol m⁻² s⁻¹.

Leaf stomatal conductance: Leaf stomatal conductance of plants of cotton cultivars was measured with an automatic porometer (MK₃; Delta-T Devices, Cambridge).

Statistical analysis of data: The data for each variable obtained from the experiment were subjected to a 2-way ANOVA using a COSTAT package (Cohort Software, Berkeley, USA). The mean values were compared with least significant difference (LSD) test 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 higher levels of P while the plants of this cultivar at the lower levels of P (15.5 and 3.88 mg L⁻¹) were less affected to CLCuV and almost free of disease at 3.88mg L⁻¹ of P, where disease incidence was only 7.50% (Fig. 1).

Different P levels of the growth medium had significant ($p \leq 0.01$) effect on fresh biomass of shoots of the two cultivars differing in resistance to CLCuV (Table 1 & Fig. 2), whereas they had non-significant effect on dry biomass of shoots. The interaction term (T × Cv) was non-significant in both variables (Table 1). However, CIM-448 was generally better in growth than S-12 at all external P regimes (Fig. 2).

Chlorophyll a in the healthy and diseased leaves of S-12 was high at 31 mg L⁻¹ of P and low at the other external P regimes. In CIM-448, chlorophyll a decreased significantly ($p \leq 0.001$) with decrease in P regimes except at the lowest P regime (3.88mg L⁻¹) where it was much as at 15.5mg L⁻¹ (Table 2 & Fig. 3). Cultivar difference was non-consistent for this variable. Chlorophyll b in the healthy leaves of S-12 was high at 31mg L⁻¹ of P and low at the P regimes except at the lowest P level where it was as much as in the diseased leaves of the same cultivar at 62 mg L⁻¹ of P. In contrast, chlorophyll b was low at the highest and lowest P regimes of the growth medium. In CIM-448, chlorophyll b content decreased significantly with decrease in P regimes except at the lowest P level where it was almost equal to that at 15.5 mg L⁻¹ (Table 2 & Fig. 3).

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 P regimes in Hoagland's nutrient solution.

Source of variation	df	Shoot F. wt.	Shoot D. wt.
Blocks	5	55.215 NS	11.729 NS
Treatments (T)	3	614.563 **	23.623 NS
Cultivars (Cv)	1	164.854 NS	68.856 NS
T × Cv	3	3.262 NS	11.775 NS
Error	35	120.080	20.375

** = Significant at 0.01 level, NS = Non-significant

Table 2. Mean squares from analyses of variance of data for chlorophyll a, b, a/b ratio and leaf water potential 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 P regimes in Hoagland's nutrient solution.

Source of variation	df	Chl a	Chl b	Chl a/b ratio	Water potential
Blocks	5	0.0608 **	0.00211 NS	0.0438 NS	0.003 NS
Treatments (T)	3	0.2449 ***	0.04210 ***	0.1074 **	0.061 NS
Cultivars (Cv)	2	0.0256 NS	0.01885 ***	0.0934 *	0.212 ***
T × Cv	6	0.1697 ***	0.04410 ***	0.0436 NS	0.141 ***
Error	55	0.0171	0.00033	0.0213	0.005

*, **, *** = Significant at 0.05, 0.01 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 P regimes in Hoagland's nutrient solution.

Source of variation	df	Wax content	Potassium	Calcium	Magnesium	Nitrogen	Phosphorus
Blocks	5	0.0029 NS	506.2 NS	4271.6 NS	2551.0 NS	122.7 NS	252.8 NS
Tmts. (T)	3	0.0609 NS	7704.6 ***	65789.1 ***	11917.7 ***	44695.3 ***	1745.1 ***
Cults. (Cv)	2	0.2118 ***	37660.8 ***	141509.7 ***	79074.1 ***	150984.3 ***	308.7 NS
T × Cv	6	0.1410 ***	2118.6 **	36786.1 ***	2980.0 ***	29086.8 ***	283.7 ***
Error	55	0.0054	639.4	3798.3	249.2	385.9	162.5

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

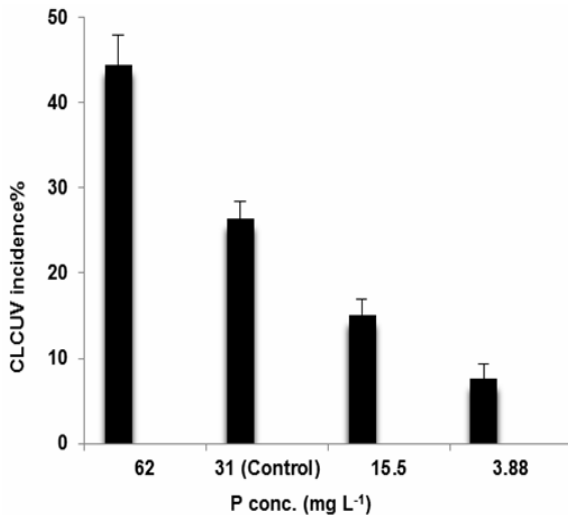


Fig. 1. Cotton leaf curl virus incidence (%) in S-12 at the flowering stage grown in sand culture under different P regimes in Hoagland's nutrient solution.

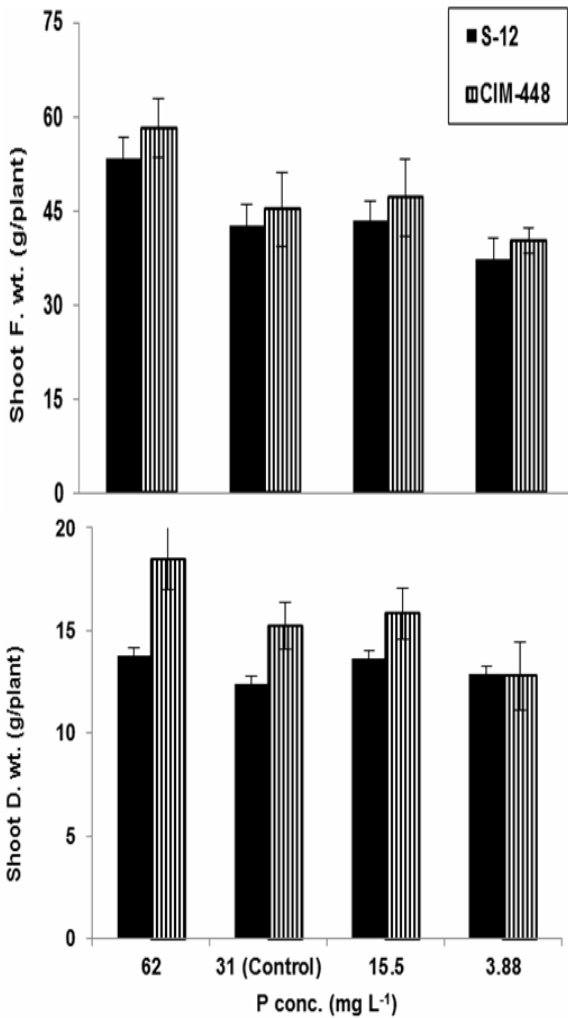


Fig. 2. Shoot fresh and dry weights (g/plant) of two cultivars of cotton at the flowering stage grown in sand culture under different P regimes in Hoagland's nutrient solution.

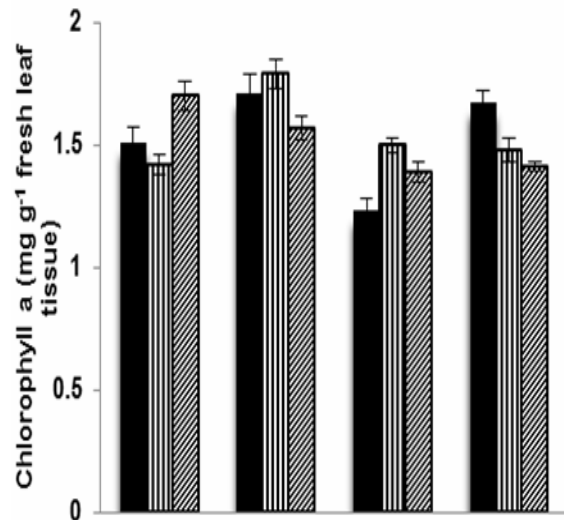
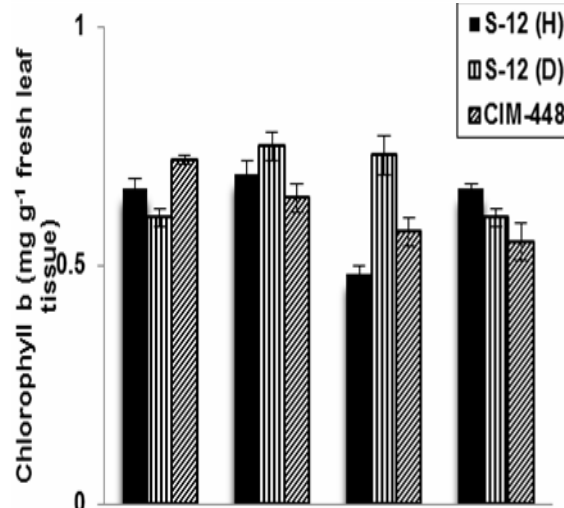
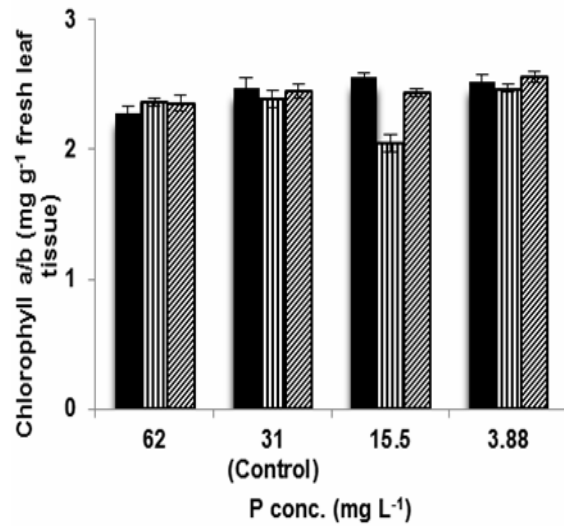


Fig. 3. Chlorophyll a, b, a/b (mg g⁻¹ fresh tissue) and leaf water potential (-MPa) in healthy (H) and diseased (D) leaves of CLCuV-resistant or CLCuV-susceptible cotton cultivars/lines at the flowering stage grown in sand culture under different P regimes in Hoagland's nutrient solution.

Varying P levels of the growth medium had significant ($p \leq 0.01$) effect on chlorophyll a/b in two cultivars differing in resistance to CLCuV. Since the interaction term (T p Cv) was non-significant for this variable, there was an overall difference between the two cultivars at varying P levels (Table 2 & Fig. 3). Different levels of P had no significant effect on water potential of the cultivars (Table 2) but the cultivar difference was significant. At the higher three P regimes diseased leaves of S-12 had lower water potential as compared to healthy leaves of S-12 and CIM-448 (Fig. 3).

Epicuticular wax content in the healthy leaves of S-12 was high only at 15.5 mg L⁻¹ of P, but remained unchanged at all other external P regimes of the growth medium, whereas in the diseased leaves of S-12 it was maximum at intermediate P regimes (31 and 15.5 mg L⁻¹) and low at the other external P regimes. In CIM-448, wax content decreased with decrease in P regimes (Table 3 & Fig. 4). Cultivar difference was apparent at the lower P regimes where S-12 was better in wax accumulation, particularly in the diseased leaves of S-12, than CIM-448 (Fig. 4). 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 view of this technical reason inflated values of epicuticular wax content of diseased leaves are expected.

K⁺ concentration in the healthy leaves of S-12 and CIM-448 remained unaffected at different P regimes except at the lowest P regime (3.88 mg L⁻¹) where it decreased significantly. In contrast, in the diseased leaves of S-12 it remained unaffected at all external P regimes (Fig. 4). Comparison of the cultivars for this variable shows that S-12 had significantly greater concentration of K⁺ in its leaves, particularly in the diseased leaves, than that in CIM-448 (Table 3). In the healthy leaves of S-12, Ca²⁺ concentration was high only at 15.5 mg L⁻¹ of P, but it remained unaffected at all the other external P regimes. In contrast, in the diseased leaves of S-12 Ca²⁺ concentration was low at the lowest P regime (3.88 mg L⁻¹) but it remained unaffected at all higher levels of P (Fig. 4). In CIM-448 the accumulation of Ca²⁺ was non-consistent at different P regimes. Diseased leaves of S-12 had greater amount of K⁺ as compared to the healthy leaves of S-12 and CIM-448 (Fig. 4).

Mg²⁺ concentration in the healthy leaves of S-12 remained unaffected at the external levels of P except at

the lowest P regime where it was considerably low. In the diseased leaves of S-12, Mg²⁺ concentration increased with decrease in P regimes except at the lowest P regime (3.88 mg L⁻¹) where it was low. In CIM-448, Mg²⁺ concentration was maximum at 15.5 mg L⁻¹ of P and low at the other P regimes (Fig. 4). CIM-448 was better in Mg²⁺ concentration as compared to S-12.

Nitrogen concentration was inconsistent at different levels of P in the healthy leaves of S-12 and CIM-448, whereas in the diseased leaves of S-12 it decreased significantly with decrease in P regimes (Fig. 4). Comparison of the cultivars shows that the diseased leaves of S-12 had significantly higher N as compared to other leaf types or cultivars at the first three higher P levels (Table 3).

Varying P levels of the growth medium had a significant ($p \leq 0.001$) effect on the leaf P concentration. In the healthy and diseased leaves of S-12, leaf P concentration decreased significantly only at the lowest P regime, but it remained unaffected at all higher P regimes (Table 3). In contrast, in CIM-448 it was maximum at 31 mg L⁻¹ of P and low at the other external P regimes (Fig. 4). Soluble protein content in the healthy leaves of S-12 remained unaffected at different P regimes except at the lowest P regime where it increased significantly ($p \leq 0.001$), whereas in the diseased leaves of S-12 it increased with decrease in P regimes except at the lowest P regime where it was almost equal to that at 15.5 mg L⁻¹ of P. In CIM-448 it was maximum at the lowest P regime (3.88 mg L⁻¹), but pattern of accumulation of soluble proteins was non-significant at other external P regimes (Table 4 & Fig. 5).

Free amino acids in the healthy leaves of S-12 remained unchanged at different P regimes except at the lowest P regime (3.88 mg L⁻¹) where it was significantly high, whereas in the diseased leaves of the same cultivar it was minimum at 15.5 mg L⁻¹ of P and high at the other P levels. In CIM-448, free amino acids were maximum at 31 mg L⁻¹ of P and low at other P regimes of the growth medium (Table 4 & Fig. 5). Cultivar difference remained inconsistent at different P regimes. Total soluble sugars remained unchanged at various levels of P except at the lowest P regime where they were significantly low, whereas in the diseased leaves of S-12 and healthy leaves of CIM-448 they decreased with decrease in the external levels of P (Fig. 5). Cultivar difference was evident at 31 and 3.88 mg L⁻¹ of P where CIM-448 was high in total soluble sugars as compared to S-12 (Fig. 5).

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 P regimes in Hoagland's nutrient solution.

Source of variation	df	Soluble proteins	Free amino acids	Total soluble sugars	Starch
Blocks	5	0.49 NS	13464.45 NS	36.90 NS	8.07 NS
Treatments (T)	3	12.88***	51138.42 *	1353.89 ***	94.19 *
Cultivars (Cv)	2	4.23 ***	131564.05 ***	5411.05 ***	17.35 NS
T × Cv	6	3.34 ***	46281.31 **	143.50 ***	183.27 ***
Error	55	0.29	13246.87	29.00	24.93

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

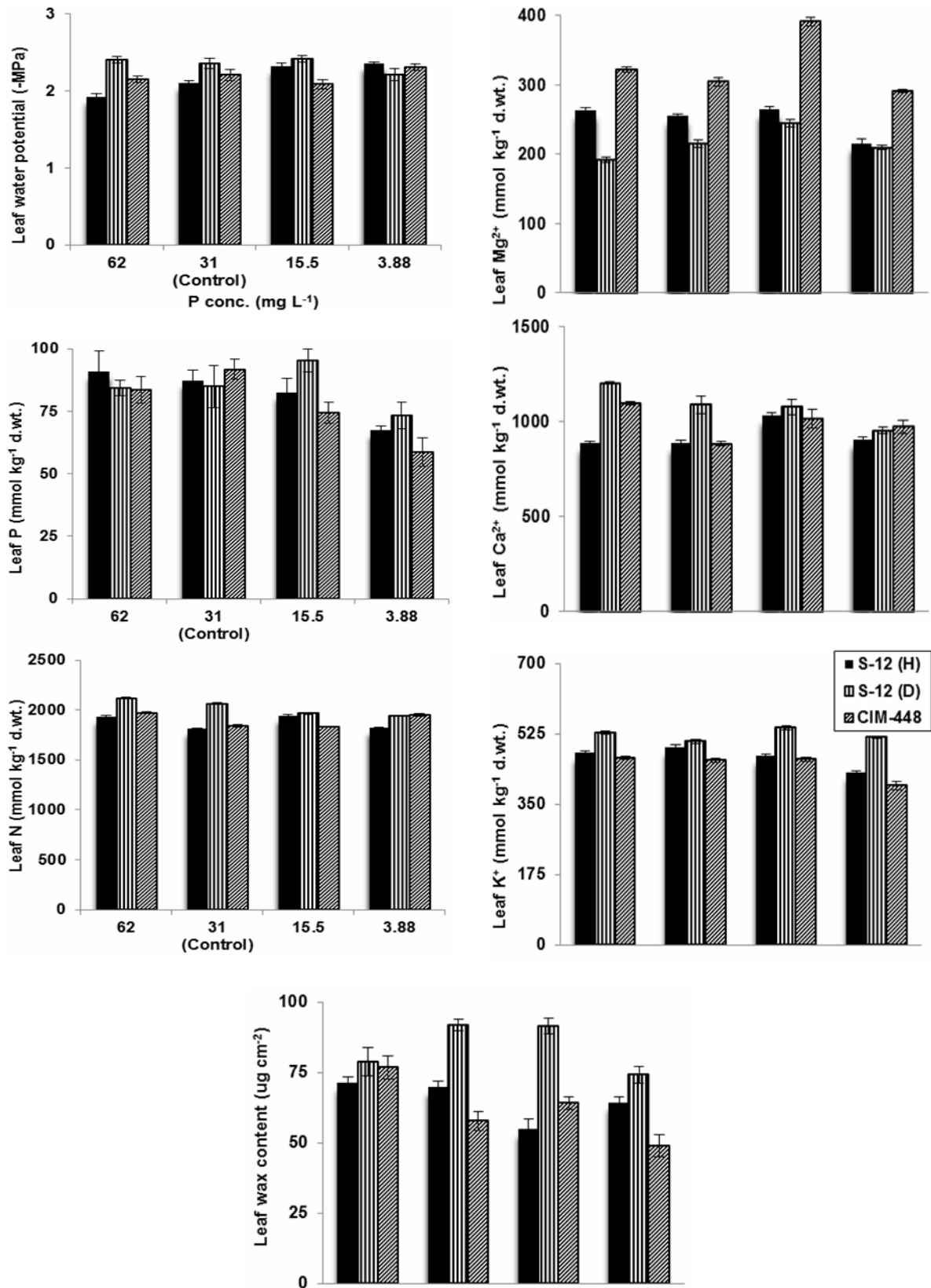


Fig. 4. Leaf wax contents ($\mu\text{g cm}^{-2}$), potassium, calcium, magnesium, nitrogen and phosphorus concentration (mmol kg^{-1} d. wt.) in healthy (H) and diseased (D) leaves of CLCuV-resistant or CLCuV-susceptible cotton cultivars/lines at the flowering stage grown in sand culture under different P regimes in Hoagland's nutrient solution.

Table 5. Mean squares from analyses of variance of data for net CO₂ 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 P regimes in Hoagland's nutrient solution.

Source of variation	Df	Net CO ₂ assimilation rate	Transpiration	Water use efficiency	Stomatal conductance	Substomatal CO ₂ concentration	Relative substomatal CO ₂
Blocks	5	4.94 NS	5.55 NS	0.29 NS	0.003 NS	837.30 NS	0.008 NS
Tmts. (T)	3	58.49 ***	2.94 NS	2.29 ***	0.145 ***	9824.25 ***	0.152 ***
Cults. (Cv)	2	21.07 *	13.01 **	0.35 NS	0.081 ***	1418.19 NS	0.034 NS
T × Cv	6	10.96 *	5.30 NS	0.45 *	0.186 ***	2475.05 *	0.052 **
Error	55	4.22	2.53	0.16	0.003	1005.76	0.013

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

Starch content in the healthy leaves of S-12 decreased significantly with the decrease in the external P regimes. In the diseased leaves of S-12 it remained unaffected at the external P regimes except at the lowest P regime (3.88mg L⁻¹) where it was significantly high (Table 4 & Fig. 5). In contrast, in CIM-448, starch content remained unchanged at different P levels except at the highest level where it was

significantly high (Fig. 5). Net CO₂ assimilation rate (A) in the healthy leaves of S-12 decreased significantly with decrease in the external P regimes, whereas in the diseased leaves of S-12 it was maximum at 31mg L⁻¹ of P and low at the other P levels. In CIM-448, it was low only at the lowest P regime while remained unaffected at all higher P regimes (Fig. 6).

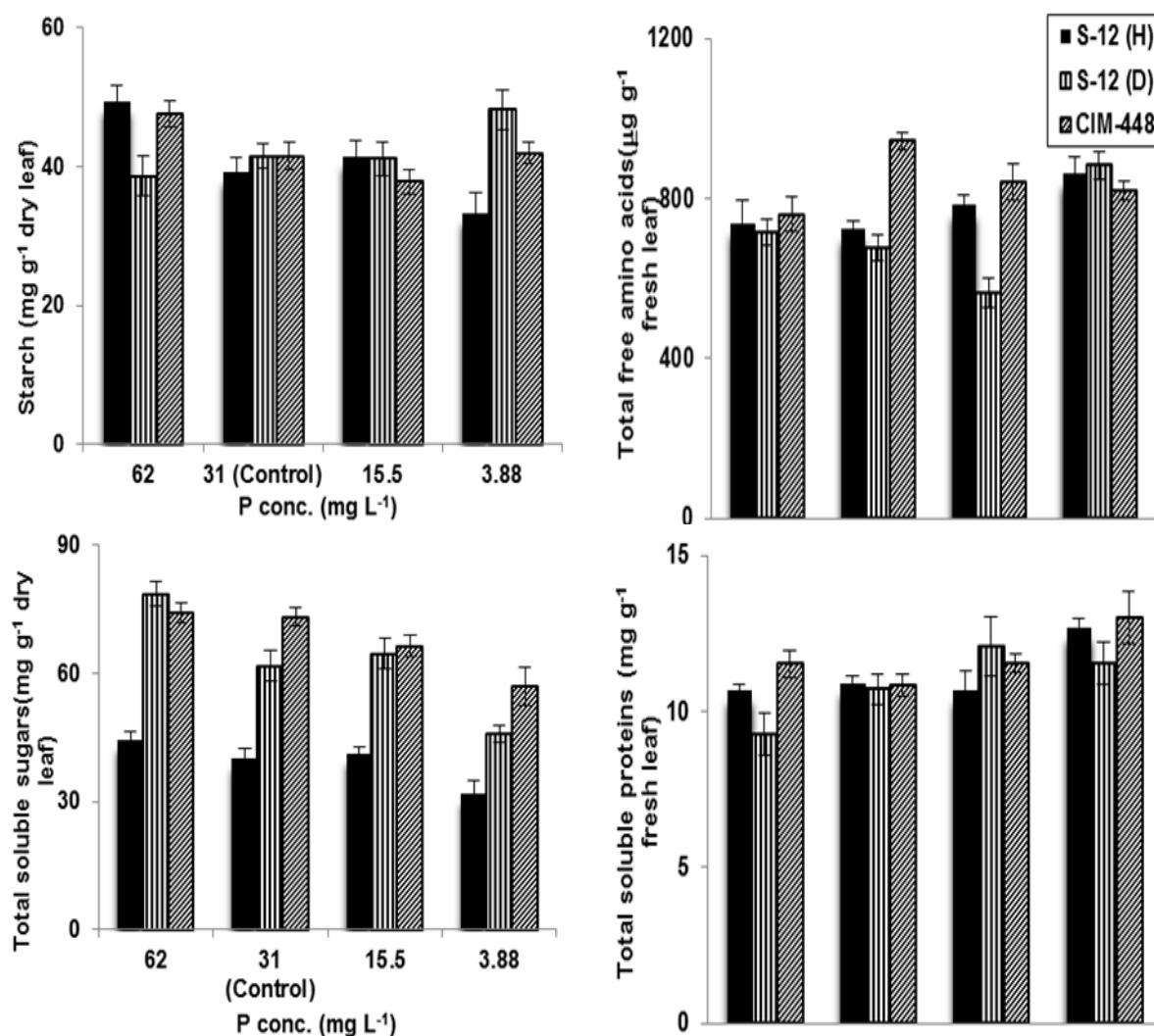


Fig. 5. Total soluble proteins (mg g⁻¹ fresh leaf tissue), total free amino acids (μg g⁻¹ fresh leaf tissue), total soluble sugars (mg/g dry leaf tissue) and starch (mg/g dry leaf tissue) in healthy (H) and diseased (D) leaves of CLCuV-resistant or CLCuV-susceptible cotton cultivars/lines at the flowering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

Water use efficiency (*WUE*) remained unchanged at all the external P regimes in the healthy leaves of S-12, whereas in the diseased leaves of S-12 and healthy leaves of CIM-448, it was high at the highest P regime and remained unchanged at all other lower P regimes. The cultivar difference was non-significant for this variable (Table 5 & Fig. 6).

Varying concentrations of P had no significant effect on the transpiration rate. The interaction term (T x Cv) was also non-significant in this variable, thus it was not possible to compare the cultivars at each external P regime (Table 5 & Fig. 6). However, S-12 had generally higher transpiration rate as compared to CIM-448 (Fig. 6). Stomatal conductance in the healthy leaves of S-12 decreased significantly ($p \leq 0.001$) with decrease in the external P regimes except at the lowest P regime where it again increased. In contrast in the diseased leaves of S-12 and healthy leaves of CIM-448, it was high at 31 mg L⁻¹ of P and low at the other external P regimes (Table 5 & Fig. 6).

Substomatal CO₂ concentration (*C_i*) in the healthy leaves of S-12 was inconsistent at different P regimes, whereas in the diseased leaves of S-12 it decreased with decrease in the external levels of P. In CIM-448, it remained unaffected at all the P regimes (Table 5 & Fig. 6). Cultivar difference was non-significant for this variable (Table 5). The ratios of substomatal CO₂ to ambient CO₂ (*C_i/C_a*) in the healthy leaves of S-12 increased with decrease in the external P regimes except at the lowest P regime, where it was low. In the diseased leaves of S-12 and healthy leaves of CIM-448 it was low at 31 mg L⁻¹ of P and high at the other P regimes (Fig. 6).

Discussion

Diseases are major factors that affect the efficient use of fertilizers by reducing the crop yield, quality, and aesthetic value (Hilji *et al.*, 2001). Although disease resistance is genetically controlled, it is mediated through physiological and biochemical processes interrelated with the nutritional status of the plant (Epstein & Bloom, 2005; Zafar *et al.*, 2010; Athar *et al.*, 2011). Nutrition of plant may determine its resistance or susceptibility to disease and the apparent virulence and ability of pathogen to survive (Marschner, 1995; Ashraf & Zafar, 1999; 2000; Huber & Graham, 2002).

In the present study it was found that disease resistance, particularly in susceptible S-12, was increased with decreasing levels of P of the growth medium and plants of cultivar, S-12 were almost free of disease incidence at the lowest P regime (3.88 mg L⁻¹). The data for fresh and dry biomass of two cultivars differing in disease resistance showed that the virus resistant CIM-448 produced significantly higher fresh and dry biomass as compared to disease susceptible S-12 at all external P regimes. This differential response of the two cotton cultivars to external P supply may have been due to the different genetic make-up of the cultivars that determine variable physiological behavior resulting in difference in

growth under varying concentrations of P (Daigger *et al.*, 1976; Evans & Wardlaw, 1996; George *et al.*, 2002). The disease incidence on S-12 was more severe at higher P levels, whereas low a lower P levels and almost free of disease at the lowest P level. Similar finding were observed long ago by Last (1962). As described earlier, K plays a vital role in the energy status of plants, translocation and storage of assimilates, and maintenance of tissue water relations (Marschner, 1995). Data regarding K concentrations of the healthy and diseased leaves showed differential pattern of accumulation. For instance, in diseased leaves of S-12, K accumulation was higher, whereas the reverse was true in the healthy leaves of S-12 and CIM-448. These results are not in agreement with those of Ashraf & Zafar (1999) who found lower accumulation of K in diseased S-12 plants grown under normal conditions. Similarly, fungal disease resistant variety of flax took up more K than susceptible variety (Dastur & Bhatt, 1964).

Calcium acts as a second messenger for various plant responses to environmental signals such as pathogen infection (Marschner, 1995; Taiz & Zeiger, 2010). However, higher Ca²⁺ content of the healthy leaves of S-12 and CIM-448 at the highest and lowest P levels can be related to its higher resistance to disease examined in the present study. Such a relation between Ca²⁺ content and disease resistance was earlier reported (Ashraf & Zafar, 1999, 2000). However, higher accumulation of calcium in the diseased leaves of S-12 compared to the healthy leaves of S-12 and CIM-448 cannot be related to the above study. However, it may have been due to activation of Ca²⁺ channels of vacuoles, endoplasmic reticulum and chloroplast by pathogenic infection (Atkinson *et al.*, 1990) to increase free cytosolic Ca²⁺. Magnesium contents in the leaves of disease resistant CIM-448 were higher as compared to S-12 healthy and diseased leaves of S-12. However, phosphorous accumulation was decreased with decrease in P levels. Higher accumulation of P by diseased leaves of S-12 at lower external P levels, can be related to its disease resistance at the lowest P level as shown in the present study. Furthermore, N accumulation in S-12 diseased leaves was significantly higher at all external P levels than the healthy leaves of S-12 or CIM-448. These results can be explained with the earlier findings of Spencer (in Bawden, 1995) who found that more local lesions were produced in tobacco (*Nicotiana glutinosa*) and French bean due to Tobacco Mosaic Virus (TMV), a type of Gemini virus (CLCuV is also Gemini virus), by high amount of nitrogen. Similarly, Bawden and Kassanis (in Bawden, 1995) found that susceptibility of tobacco to TMV was increased by additional nitrogen. They found P as more important than N in increasing susceptibility. According to Marschner (1995), nutritional factors, which favor the growth of host plant, also favor the viral multiplication.

Data for leaf epicuticular wax contents showed that diseased leaves of S-12 had considerably higher epicuticular wax content. It may have been partly due to the reason that the diseased leaves might have secreted high amount of wax on their surface to avoid to further attack of whitefly (Ashraf & Zafar, 1999, 2000).

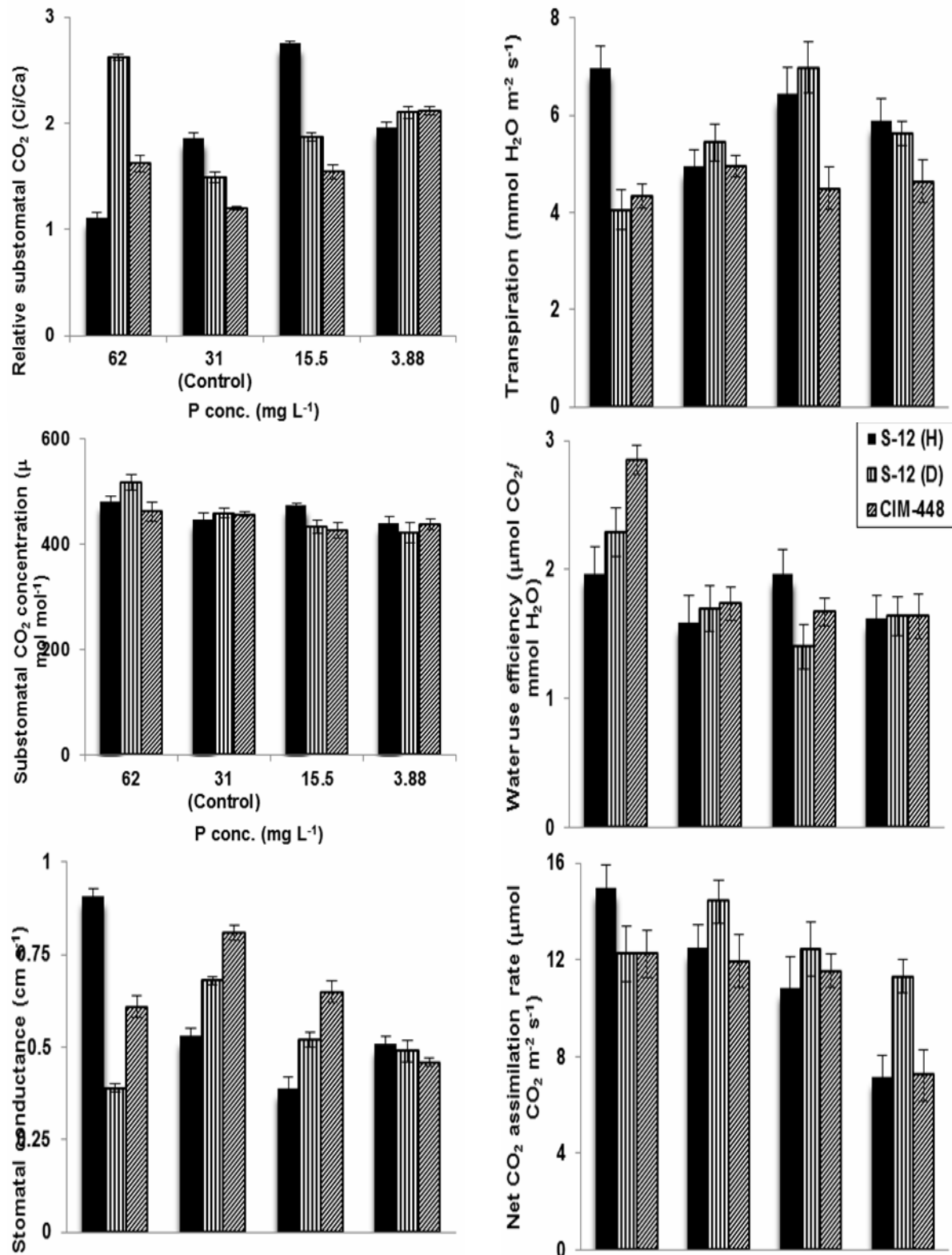


Fig. 6. Net CO₂ assimilation rate (μmol CO₂ m⁻² s⁻¹), transpiration (mmol H₂O m⁻² s⁻¹), water use efficiency (μmol CO₂/mmol H₂O), stomatal conductance (cm s⁻¹), substomatal CO₂ concentration (μmol mol⁻¹) and relative substomatal CO₂ in healthy (H) and diseased (D) leaves of CLCuV-resistant or CLCuV-susceptible cotton cultivars/lines at the flowering stage grown in sand culture under different K regimes in Hoagland's nutrient solution.

The accumulation of chlorophyll a and b, and a/b ratio showed inconsistent pattern in both cotton cultivars under varying external P levels. Similarly, data for water potential and soluble proteins showed inconsistent relationship between the two cultivars grown at different levels of P. However, a remarkable difference in both cultivars was observed in accumulation of free amino acids. The disease resistant cultivar CIM-448 accumulated higher amounts of free amino acids in the leaves. These results are quite parallel to those of Khalifa & Gameel (1983) who observed high accumulation of free amino acids in CLCuV resistant cotton cultivars as compared to CLCuV susceptible cultivars. However, these results are in contrast to what earlier observed in okra plants by Athar *et al.*, (2011) who reported that high leaf amino acids increased the susceptibility of plants.

Data for soluble sugars of two cotton cultivars differing in disease resistance showed that disease resistant cultivar CIM-448 accumulated higher amounts of soluble sugars in the leaves under P deficiency. These results are in agreement with those of Rehmat (1995) who found that CLCuV tolerant cotton cultivar had higher reducing sugars whereas lower in CLCuV susceptible. However, higher accumulation of soluble sugars in S-12 diseased leaves as compared to S-12 healthy leaves cannot be explained in view of above argument. In addition, decrease in soluble sugar concentration with P deficiency in both cultivars. These results are similar to the earlier findings of De Groot *et al.*, (2003a) who found reduction in soluble sugars due to P deficiency.

Data for net CO₂ assimilation rate (Pn) and stomatal conductance of both cotton cultivars showed that P deficiency reduced CO₂ fixation in both cultivars and this reduction was not due to stomatal limitations. These results are similar to Pieters *et al.*, (2001) who found that P deficiency caused Pn by 75% under the growing conditions. It may have been due to decrease in RuBP pool size in some plant species. For example, in *Glycine max* (Fredeen *et al.*, 1989) and *Helianthus annuus* (Jacob & Lawlor, 1992) decrease in RuBP pool size was caused by insufficient ATP in *Helianthus annuus* and *Zea mays* (Jacob & Lawlor, 1992). In addition, P deficiency affected the activity of Calvin cycle enzymes, RuBP regeneration, and/or Rubisco activity (Brooks, 1986; Rao & Terry, 1989; Jacob & Lawlor, 1992; Sawada *et al.*, 1992). However, net CO₂ assimilation rate in S-12 diseased plants were higher as compared to S-12 healthy and those of CLCuV resistant CIM-448 at the lower P levels of the growth medium. This can be related to its higher P accumulation at these levels, which might have enhanced the carboxylation capacity (De Groot *et al.*, 2003b), which in turn might have increased its resistance to disease at the lowest P level.

In conclusion, the results for this study clearly show that low P levels had a significant effect on disease resistance in S-12. Low P of the growth medium had a significant effect on growth of two cotton cultivars differing in resistance to CLCuV. High biomass production in CIM-448 was associated with high rate of photosynthesis, high accumulation of free amino acids and Mg²⁺, whereas increase in disease resistance in S-12 at low P regime was associated with its high accumulation of P and high photosynthetic rate.

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