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Full Length Research Paper

Effect of potassium on micromorphological and chemical composition of three cotton (*Gossypium hirsutum* L.) genotypes

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An experiment was conducted at National Agricultural Research Centre (NARC), Islamabad, Pakistan to study the effect of potassium on yield and internal leaf tissues composition of cotton varieties, CIM-443, CIM-109 and CIM-446. Nitrogen (N) and phosphorus (P) were applied at 150 mg N/kg soil and 75 mg P_2O_5/kg soil to all the pots, while potassium (K) was applied at three levels; control (no K), 100 and 200 mg K_2O/kg soil. It was observed that epidermal and mesophyl cells were more turgid, uniform, flaccid, symmetrical and structurally improved with potassium application. Larger number of starch grains was observed in plant leaves grown without potassium application while in leaves supplied with K their number decreased. Thickness of the epidermal region (cuticle plus epidermis) increased significantly with potassium application and same was true for leaf thickness in all the varieties. Application of K decreased N content of leaves significantly while protein content increased. Protein contents were maximum (65 µg/g) at 200 mg K_2O/kg soil over that of control treatment. Phosphorus concentration also affected positively with potash application and it was maximum at 100 mg K_2O/kg soil. An increase of 15.63% in leaf chlorophyll content was recorded with K at 200 mg K_2O/kg soil. Seed cotton yield of all the varieties increased significantly with K application, maximum in case of CIM-446 followed by CIM-109 and CIM-443 variety. A strong positive correlation was observed among applied K, leaf thickness, epidermis layer, chlorophyll contents, protein and seed cotton yield.

Key words: Cotton, mesophyl cells, starch grains, protein, seed cotton yield, K applicatiion.

INTRODUCTION

Increasing world population and pressures to produce abundant food and fiber have a natural tendency to increase land area and use of high yielding varieties and high inputs devoted to agricultural production. This dilemma is important for cotton producers because cotton appears to be more sensitive to K deficiencies than many other row crops (Cope, 1981). Cotton may be more sensitive because the root system of cotton is less dense than that of cereal crops (Gerrik et al., 1987). Potassium acting as an osmoticum maintains tissue turgor pressure (Kaiser, 1982), regulate opening and closing of stomata (Humble and Raschke, 1971) and help balancing of charge of anions in plant system (Streeter and Barta, 1984). Potassium and malate are the primary osmotica that produced turgor pressure in cotton leaf tissue and are also needed for cotton fiber elongation (Dhindsa et al., 1975). Ashley and Goodson (1972) reported that K influenced the rate of translocation of photoassimilates from the leaves to the other parts of the plant. Oosterhuis (1997) reported that starch grains in cotton leaves were totally absent after the potash application due to increased rate of translocation of starch grains from the leaves to the other plant parts.

Potassium requirements of cotton depend mainly on available level of its cations in soil and fixation capacity of soil. Therefore, its amount required must be resident in the soil or be added through fertilizers. Approximately two-third of the total K uptake occurs during a 6-week

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Table 1. Characteristics of soil used for the experiment.

Characteristic	Result
Texture	**Loam**
pH	7.42
EC_e (dS/m)	1.01
CO_3^{2-} (me/L)	2.00
HCO_3^- (me/L)	3.33
Cl^- (me/L)	1.83
$Ca^{2+} + Mg^{2+}$ (me/L)	13.33
K (mg /kg)	70.00

period beginning at early flowering (Reddy et al., 1996). Potassium content of cotton leaves are highly correlated with soil extractable K.

Chlorophyll content of diagnostic leaves increased with the K application and its inadequate levels reduced total chlorophyll content in leaves (Oosterhuis, 1995 and 1997). Many of the afore-mentioned studies were conducted in greenhouses which may impose a quicker and more drastic alteration of the K level that would happen naturally under field conditions. In the field, adjustments in the K status is gradual and subtle because the available soil K level is a reflection of reversible equilibrium that exists between solution, exchangeable and non-exchangeable forms of K (Bertsch and Thomas, 1985). Keeping in view, the above mentioned role of potash in cotton plant, a study was carried out with the following objectives: a) to determine the effect of potassium on leaf morphology and translocation of photosynthates from leaves to the sinks, and b) to study the effect of potassium on mineral and chemical composition of cotton leaves and seed cotton yield.

MATERIAL AND METHODS

A pot experiment was conducted in the greenhouse at National Agricultural Research Centre (NARC), Islamabad, Pakistan during 1999-2000. Soil collected for the study belonged to Rasulpur soil series, sandy loam, mixed hyperthermic, udic, ustocherptic camborthids. The soil was normal with pH value 7.42 and electrical conductivity of soil saturation extract (EC_e) 1.01 dS/m. Soil had low plant available N and K (Table 1), $NaHCO_3$ extractable P was 5.3 mg /kg. Seven kilograms of soil was filled in each pot and three levels of potassium were applied as potassium chloride; control (no potassium application), 100 and 200 mg K_2O /kg soil. Nitrogen and phosphorus were applied at 150 mg N/kg soil and 75 mg P_2O_5 /kg soil in each pot in the form of urea and DAP (Diammonium phosphate) fertilizers. The experiment was laid out in Completely Randomized Design (CRD) with three replications. All the P and K were applied at the time of sowing and five seeds of three cotton varieties namely CIM-443, CIM-109 and CIM-446 were sown at a depth of 2.5 cm in respective pot. Only three cotton plants per pot were retained after establishment. Nitrogen was applied in four equal splits: at the time of sowing, 10 days after germination, 25 days after germination and 60 days after sowing (DAS), that is. at flower initiation stage.

Fully expanded diagnostic leaves were sampled at flowering stage (60 DAS). The central portion of leaf blades were cut into small pieces (1-2 cm) long and fixed in sera (FAA; formalin, acetic acid and absolute alcohol in 1:2:2 ratio) for 4-5 h. Then the leaf sections were dehydrated in ascending grades of alcohol; 1) in 70% alcohol for 3-4 h, 2) in 80% alcohol for overnight, 3) in 90% alcohol for 4 h, and 4) in 100% alcohol for 4 h. After dehydration the leaves were transferred to cedor wood oil until they became clear and transparent. These leaf sections were then embedded in paraplast wax at 60°C. The blocks made were sectioned at a thickness of 10 µm by Richert Microtome and stretched at 60°C on fisher slide warmer. After deparationizing, the slides were transferred to xylol for 15 min to remove wax. The sections were hydrated in descending grades of alcohol and washed with distilled water. After that the sections were dehydrated with the same ascending grades of alcohol and stained with safranine and fast green stain (McManus and Mowry, 1960). Finally the slides were microphotographed (X225) to determine the thickness of cotton leaf and structure of internal leaf tissue, in both, potassium treated and control leaves.

Diagnostic leaves were also sampled at flower initiation stage and washed with distilled water for chemical analysis. Leaf blades and petioles were separated, oven dried at 65°C, ground and analyzed for N, P and K concentration. Total N was determined in acid digested (H_2SO_4 + Se + H_2O_2) material by Kjeldhal method and acid molybdate/NSA using an auto analyzer system II respectively (Winkleman et al., 1990). For P and K analysis, dry ashing was done (Jones et al., 1991) and P was determined colorimetrically and K by flame photometer. Chlorophyll content was estimated by method of Arnon (1949), modified by Kirk (1968). Absorbance was recorded at 645 and 663 nm on spectrophotometer and total chlorophyll was calculated by equation given by Arnon (1949).

$$\text{Total chlorophyll (mg/L)} = (20.2 \times A645) + (8.02 \times A663)$$

Where A645 and A663 are the absorbance values at 645 and 663 nm wavelengths respectively.

Protein content of leaves was estimated by Lowry et al. (1951) method using Bovine serum albumin (BSA) as standard. Seed cotton yield was recorded in all varieties and averaged for a single plant. The data recorded were analyzed by using analysis of variance technique (Steel and Torrie, 1984). Means were separated by using LSD at 5% level of significance.

RESULTS

Micro-morphological characteristics of cotton leaves

The thickness of cotton leaves was maximum (224 µm) in the plants treated with K at 200 mg/kg (Table 2) and minimum (173 µm) in the control plants. As regards varieties, the maximum leaf thickness (225.5 µm) was recorded in CIM-446 and the minimum (166.5 µm) in variety CIM-443. The maximum thickness (29 µm) of epidermal region was observed where K was applied at 200 mg K_2O/kg and the minimum (18 µm) in K untreated leaves (Table 2). This increase in thickness of epidermal region was 58% with K application. In case of cotton varieties, maximum thickness of epidermal region (27 µm) was recorded for variety CIM-446 and minimum (20 µm) for CIM-443.

Microscopic study revealed that mesophyll tissues were uniform, symmetrical and structurally well developed,

Table 2. Effect of applied K on thickness of epidermal region (cuticle + epidermis) and leaves of cotton (µm).

K_2O treatment (mg /kg)	Cotton leaf thickness (µm)				Thickness of epidermal region (µm)			
	CIM-443	CIM-109	CIM-446	Mean	CIM-443	CIM-109	CIM-446	Mean
0	135 c	178 bc	206 b	173.0 B	14 d	19 cd	22 c	18.3 B
200	198 bc	229 ab	245 a	224.0 A	26 b	29 ab	32 a	29.0 A
Mean	166.5 C	203.5 B	225.5 A		20.0 C	24.0 B	27.0 A	

Cotton leaf thickness (µm)		Thickness of epidermal region (µm)	
C.V. (%)	5.86	C.V. (%)	3.86
LSD (0.05) Varieties**	22	LSD (0.05) Varieties**	0.94
K levels**	31	K levels**	0.29
Interaction**	52	Interaction**	0.50

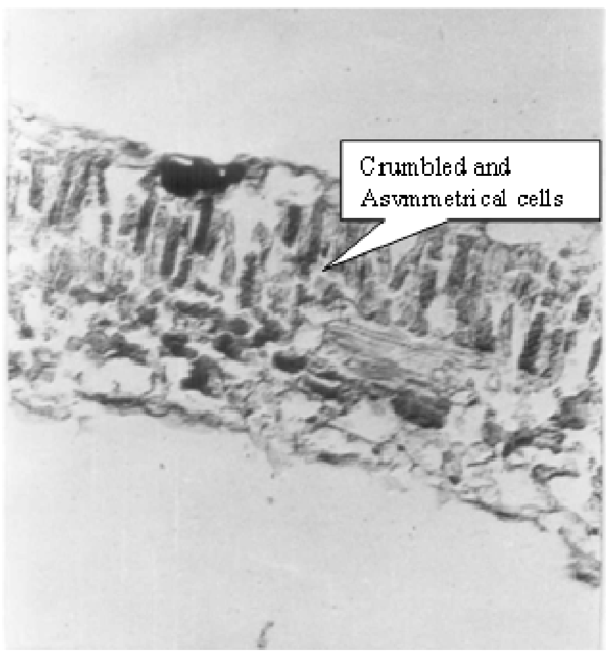

Figure 1. Cross section of cotton leaf showing crumbled and asymmetrical cells without potash application (225 X).

Figure 2. Cross section of cotton leaf showing symmetrical, fully turgid internal leaf tissue with uniform margins after potash application (225 X).

while the plant grown without application of K showed reverse of these characteristics; that is, the cotton leaves were crumbled and cells were asymmetrical (Figures 1 and 2). A large number of starch grains accumulated in the leaves of plants of control treatment where only N and P was applied while in plants treated with K at 200 mg K_2O/kg soil, the number of starch grains decreased in leaf tissues without altering the structure of leaf tissues (Figures 3 and 4).

Nutrient concentration in cotton leaves

Significant decrease in N concentration of leaves with K application was noted (Table 3). The maximum N (3.2%) was observed in cotton leaves grown without K application and the minimum (2.6%) in plants treated with K at 200 mg K_2O/kg soil at flowering stage. The decrease in N was 20.5% with K application at 200 mg K_2O/kg soil over that of control treatment. As regards varieties, maximum N content (3.2%) was recorded in variety CIM-443 and minimum (2.6%) in CIM-446. The interaction for N concentration in leaves between varieties and K levels was also highly significant. It was maximum in variety CIM-443 at control treatment followed by the leaves of variety CIM-109 at 100 mg K_2O /kg soil and at control. The minimum N concentration (2.27 %) was in case of variety CIM-109 at 200 mg K_2O/kg soil.

The P concentration of diagnostic leaves increased significantly with the application of potash (Table 3). The

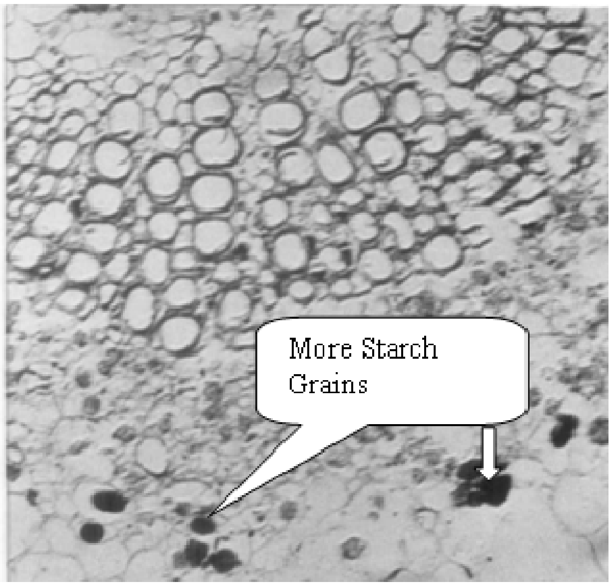

Figure 3. Cross section of cotton leaf showing a large number of starch grains without potash application (225 X).

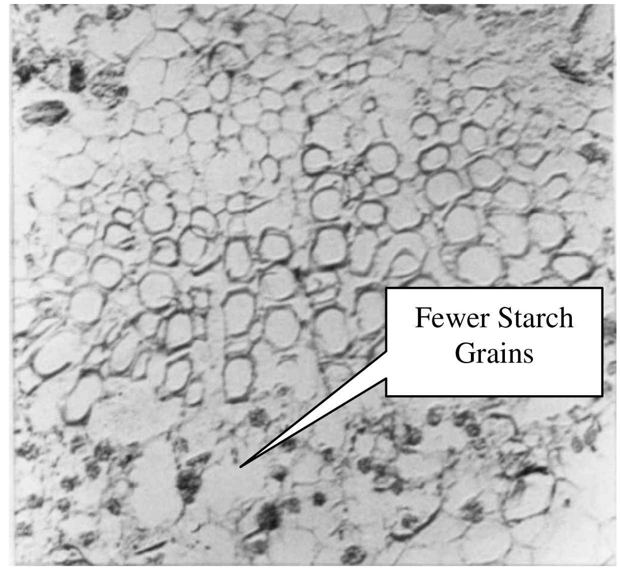

Figure 4. Cross section of cotton leaf showing fewer starch grains after potash application (225 X).

leaves P contents were maximum (0.31%) at 100 mg /kg K application and minimum (0.28%) in the control plants. The maximum P content (0.32%) was observed in variety CIM-109, while the minimum (0.28%) in variety CIM-443. Phosphorus content in variety CIM-446 increased with increasing K levels. However, in other varieties, P content increased only when K_2O was applied at 100 mg/kg soil. The interaction between varieties and K application was highly significant for P content in leaves.

Potassium content increased significantly and on overall basis it increased by 135% with its application at 200 mg/kg (Table 3). The maximum potassium content of 1.81% was recorded in plants supplied with K at 200 mg K_2O/kg soil and minimum (0.77%) in the control treatment. The K content was more (1.59%) in CIM-109 followed by CIM-446 (1.45%) and CIM-443 had the minimum (1.14%). The interaction among varieties and K levels was highly significant for potassium content of leaves. Plants supplied with K at 200 mg /kg had the maximum K content (2.29%) in variety CIM-446 and the minimum (0.68%) in varieties CIM-109 and CIM-446 of the control treatment.

Protein and chlorophyll content of leaf tissues

Leaf protein content increased in plants treated with K. On overall basis it increased by 37% in plants treated with K at 200 mg K_2O/kg soil over the control treatment where it was maximum (65.0 µg/g). The minimum protein contents were recorded in the leaves of control plants (Table 4). The maximum protein content (63.4 µg /g) was observed in variety CIM-109 and minimum (44.38 µg /g) in CIM-446. The interaction between varieties and K levels was highly significant for leaf protein content. Application of K at 200 mg/kg soil resulted in maximum leaf protein content of 77 µg/g in variety CIM-109 and the minimum was in the control treatment of the same variety. Similar trend was observed in the other two cotton varieties.

There was a non significant effect of K on total leaf chlorophyll content (Table 4). Plants treated with K at 200 mg K_2O/kg soil, increased chlorophyll content by 15.6% as compared to control ones. The maximum of 26.6 mg/L was recorded in plants supplied with K at 200 mg K_2O/kg soil and the minimum (23.0 mg/L) in the control. Significant differences in chlorophyll content amongst cotton varieties were observed. The interaction between varieties and K level in this regard was non-significant.

Effect of potassium on seed cotton yield

Seed cotton yield per plant increased significantly with K application. Seed cotton yield increased by 38% with K application at 200 mg K_2O/kg soil. Seed cotton yield in this treatment was 8.42 g/plant while it was minimum (6.08 g/plant) in the treatment without K application (Figure 5). As far as varieties are concerned, maximum seed cotton yield of 8.65 g/plant was produced by CIM-443 variety and the minimum (5.85 g/plant) by variety CIM-109. Seed cotton yield increased significantly with increasing K levels in all the varieties and it was maximum where K was applied at 200 mg K_2O/kg soil.

Correlation studies

Multiple correlation analyses were run to find out relation-

Table 3. Effect of potash application on N, P and K concentration of cotton leaves.

K_2O (mg/kg)	Leaf N content				Leaf P content (%)				Leaf K content (%)			
	CIM 443	CIM 109	CIM 446	Mean	CIM 443	CIM 109	CIM 446	Mean	CIM443	CIM 109	CIM4 46	Mean
0	3.92 a	3.32 b	2.43 g	3.22 A	0.24 d	0.33 ab	0.27 c	0.28 B	0.96 de	0.68e	0.68e	0.77C
100	2.93 c	3.33 b	2.73 e	2.99 B	0.32 b	0.33 ab	0.28 c	0.31 A	1.24 cd	2.16ab	1.39 c	1.59B
200	2.83 d	2.27 h	2.58 f	2.56 C	0.28 c	0.29 c	0.34 a	0.30 A	1.21 cd	1.92 b	2.29a	1.81A
Mean	3.23 A	2.97 B	2.58 C		0.28 C	0.32 A	0.29 B		1.14 B	1.59 A	1.45A	

Leaves N contents		Leaves P contents		Leaves K contents	
C.V. (%)	0.44	C.V. (%)	5.45	C.V. (%)	14.04
LSD (0.05)Varieties**	0.04	LSD (0.05) Varieties**	0.01	LSD (0.05) Varieties**	0.22
K levels**	0.01	K levels**	0.01	K levels**	0.20
Interaction**	0.02	Interaction**	0.02	Interaction**	0.35

Table 4. Effect of K application on protein and chlorophyll contents of cotton leaves.

K_2O (mg /kg)	Leaf protein contents (µg/g)				Leaf chlorophyll contents (mg/L)			
	CIM-443	CIM-109	CIM-446	Mean	CIM-443	CIM-109	CIM-446	Mean
0	51.28 e	50.17 e	40.80 h	47.42 C	26.72	20.13	22.22	23.02
100	66.40 c	63.03 d	44.13 g	57.85 B	29.58	21.96	22.14	24.56
200	69.77 b	77.0 a	48.20 f	64.99 A	30.14	23.31	26.42	26.62
Mean	62.48 A	63.4 A	44.38 B		28.81 A	21.80 B	23.59 AB	

Leaf protein contents (µg/g)		Leaf chlorophyll contents (mg/L)	
C.V. (%)	1.84	C.V. (%)	20.63
LSD (0.05) Varieties	1.73	LSD (0.05)Varieties	5.61
K levels	1.07	K levels	N.S.
Interaction	1.86	Interaction	N.S.

ship among the applied K and thickness of epidermis, whole leaf thickness, leaf N, P, K contents and seed cotton yield (Table 5). The results showed a positive correlation between applied K and leaf P, K, chlorophyll and protein content, and seed cotton yield in variety CIM-443 except for N that showed strong negative correlation with r = -0.905. Similar trend was observed in case of CIM-109 regarding applied K leaf K, chlorophyll content, protein content and seed cotton yield while N and P were negatively correlated. In case of CIM-446, all the parameter studied were positively correlated.

DISCUSSION

The internal structure of leaf tissues improved with the application of potassium. Potassium acted as an osmoticum to maintain turgor pressure of plant tissue (Kaiser, 1982). The mesophyll, spongy parenchyma and epidermal tissues were more turgid and had increased surface area of cells in the plants receiving external potassium (Figures 1 and 2). The accumulation of greater number of starch grains in non-K applied plants can be attributed to slow translocation of starch to the sinks hence stored in the leaves for longer time. The reverse of this phenomenon was observed in K-treated plants (Figures 3 and 4).

The thickness of the epidermal region of cotton leaves increased with K application. Increase in the thickness of epidermal and mesophyll tissues indicated that their water holding capacity increased with K treatment which helped in enhancing their metabolic activities and increased production of photosynthates like carbohydrates, proteins and their translocation to respective sinks. It can be deduced from the Figures 1 to 4 that K application accelerated the translocation of starch grains from leaves to the sinks as in K treated plants leaves had less starch grains than the non-K treated plants. In the study, it was also observed that the structure of starch grains was not affected by potassium but number of starch grains decreased in the plants treated with K. Liang et al. (1992)

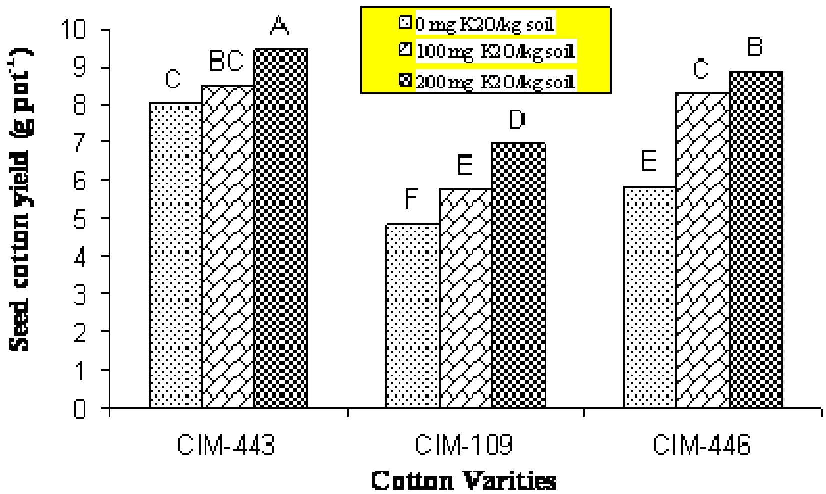

Figure 5. Seed cotton yield of different genotypes as affected by K application.

Table 5. Correlation between applied K and N, P, K, chlorophyll, protein contents and seed cotton yield in different cotton genotypes.

CIM-443	Applied K	N	P	K	Chlorophyll	Protein
N Concentration (%)	-0.905**	-				
P Concentration (%)	0.500 N.S.	-0.822*	-			
K Concentration (%)	0.813*	-0.984**	0.911**	-		
Chlorophyll contents (mg/L)	0.932**	-0.998**	0.780*	0.969	-	
Protein contents (Ug/g)	0.939**	-0.996**	0.768*	0.964	0.999**	-
Seed cotton yield (g/pot)	0.981**	-0.805*	0.322 N.S.	0.685 N.S.	0.844*	0.854*
CIM-109						
N Concentration (%)	-0.862*	-				
P Concentration (%)	-0.866*	0.999**	-			
K Concentration (%)	0.781*	-0.356 N.S.	-0.363 N.S.	-		
Chlorophyll contents (mg/L)	0.996**	-0.815*	-0.819*	0.832*	-	
Protein contents (µg/g)	1.000**	-0.874*	-0.878*	0.765*	0.994**	-
Seed cotton yield (g/pot)	0.996**	-0.905**	-0.908**	0.720*	0.984**	0.998**
CIM-446						
N Concentration (%)	0.500 N.S.	-				
P Concentration (%)	0.924**	0.132 N.S.	-			
K Concentration (%)	0.998**	0.440 N.S.	0.948**	-		
Chlorophyll contents (mg/L)	0.858*	-0.016 N.S.	0.989**	0.891*	-	
Protein contents (µg/g)	0.998**	0.449 N.S.	0.945**	0.999**	0.886*	-
Seed cotton yield (g/pot)	0.939**	0.768*	0.736*	0.913**	0.628 N.S.	0.917**

* Significant at 5% level of probability.
** Significant at 1% level of probability.
N.S.= Non Significant.

and Dhindsa et al. (1975) reported that the increased cells turgidity of leaf in K treated cotton leaves increased the cells surface area, which ultimately resulted in increased thickness of cotton leaves. Hence the internal leaf structure and tissues improved with potassium application. It also acts as an osmoticum to maintain turgor pressure of tissue in the plant (Kaiser, 1982). Ashlay and Goodson (1972) reported that K influenced the rate of translocation of photoassimilates from the leaves to the other parts of the plant. Oosterhuis (1997) reported that starch grains in cotton leaves were totally absent after the potash application due to increased rate of translocation of starch grains from the leaves to the other plant parts.

Significant decrease in N concentration of leaves with K application was noted. The maximum N was in plants without K application and the minimum in the leaves of plants treated with K at flower initiation stage. These results are in line with those of Pettigrew and Meredith (1997), who reported decreased nitrogen content in the leaf tissues of K, fertilized plants as compared to control plants. The P concentration of diagnostic leaves increased significantly with the application of potash (Table 3). The results are in line with those reported by Liang et al. (1992). They reported that phosphorus content increased by 28% in cotton supplied with K fertilization. Potassium content in our study also increased significantly and on overall basis it increased by 135% with its application at 200 mg K_2O/kg soil (Table 3). The maximum potassium content of 1.8% was recorded in plants supplied with K at 200 mg K_2O/kg soil. Similar results have been reported by Hsu et al. (1978), Liang et al. (1992) and Pettigrew and Meredith (1997). Different cotton genotypes had also varying ability to absorb K and other nutrients like P and N from soil to fulfill their requirements for carrying on metabolic activities. These results are in line with the findings of Oosterhuis (1995 and 1997).

Leaf protein content increased in plants treated with K (Table 4). On overall basis it increased by 37% in plants treated with K at 200 mg/kg soil. The nitrogen contents decreased while protein content of leaves increased with K application (Tables 3 and 4) which indicated that potash application increased N use efficiency by cotton. Blevins (1985) reported that potassium played a significant role in protein metabolism of plants.

Significant increase in seed cotton yield with increasing K levels in all the varieties indicated that application of appropriate K dose is required to obtain optimum seed cotton yield along with optimal N and P levels. These results can be supported by the findings of Wahdan et al. (1994); Abdel Malak and Mukrum (1996); Reddy et al. (2000).

A positive correlation between applied K, thickness of epidermis, leaf N, P, K contents and seed cotton yield was present except for N concentration in leaves which showed strong negative correlation (Table 5). This again indicated the translocation of photosynthates from leaves to different sinks and their transformation in to different metabolites in the presence of sufficient level of K. This indicated that K plays a key role in the synthesis of carbohydrate and protein.

Conclusion

The potassium fertilization improved the internal structure of cotton leaf tissues and also increased the thickness of cotton leaves which increased photosynthetic capacity of cotton plant. Increased rate of translocation of photo-assimilates from source (leaves) to the sinks resulted in higher production of seed cotton in all the varieties under investigation.

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