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# Micro-morphological Studies of Potash Affected Cotton Leaf

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**Abstract:** A pot experiment was conducted at National Agricultural Research Center (NARC), Islamabad to study the effect of potash on internal leaf tissues of selected cotton varieties namely CIM443 CIM109, CIM446.Results of micro-morphological studies of cotton leaves showed that epidermal and mesophyll cells of leaves were more turgid, uniform, occupied more surface area, symmetrical and structurally improved with the addition of potash. Microscopic examination of thin cross sections of cotton leaves showed that there were a large number of starch grains present in cotton leaves without Potassium. The number of starch grains in leaves decreased with increasing $K_2O$ levels in the leaves of K treated cotton plants. The thickness of epidermal region (cuticle plus epidermis) was also increased significantly with the application of potash. The cotton leaf thickness was significantly increased with increasing K levels in all cotton varieties.

**Key words:** Potash, cotton leaf, micro- morphological studies

## Introduction

Increasing world population and pressures to produce abundant food and fiber have a natural tendency to increase land area devoted to agricultural production. If the Potassium (K) fertility is neglected in annual management programs, it can become a problem in either established fields or new land just brought into production. This dilemma is especially important for cotton producers because cotton appears to be more sensitive to K deficiencies than many other raw crops (Cope, 1981). Cotton may be more sensitive because the root system of cotton is less dense than that of other crops (Gerik *et al*., 1987). Potassium acting as a osmoticum to maintain tissue turgor pressure (Kaiser, 1982), regulating the opening and closing of stomates (Humble and Raschke, 1971) and balancing of charge of anions (Streeter and Barta, 1984) are the physiological functions of K in plant cells. Potassium and malate were the primary osmotica that produced turgor pressure in cotton leaf tissues and also needed for cotton fiber elongation (Dhindsa *et al*., 1975). Deficiency of Potassium in cotton plants increased susceptibility to infection by microorganisms (Reddy *et al*., 1996). Thimmaiash *et al*. (1994) have reported the anatomical differences between the leaves of insect pest resistant and susceptible genotypes of cotton. They observed that the insect pest resistant genotypes registered a reduced leaf thickness and reduced diameter of mid ribs as compared to susceptible cotton genotypes. Many of the aforementioned studies were conducted in growth chambers or greenhouses that may impose quicker and more drastic alternation of K level than would happen naturally under field conditions. Therefore, the objectives of present study were to determine the effect of potash on the internal leaf tissue ; to study the rate of translocation of starch grains from leaves to the other plant parts and to determine the thickness of cotton leaf and thickness of cotton leaf epidermal region (cuticle plus epidermis) in control and potash affected cotton leaves.

## Materials and Methods

A pot experiment was conducted in a glasshouse at National Agricultural Research Center (NARC), Islamabad from July 1999 to December 1999. Seeds of newly developed three cotton varieties namely CIM443, CIM109, CIM446 were grown in a sandy loam at a depth of 2.5 cm. Potassium was applied as Potassium chloride (KCl) @ 200 kg $K_2O$ $ha^{-1}$, Nitrogen (urea) was applied @ 150 kg N $ha^{-1}$ and phosphorus (Potassium dihydrogen phosphate) @ 75 kg $P_2O_5$ $ha^{-1}$. The amount of each fertilizer was calculated on the basis of 7 kg soil per pot. The calculated amount of each fertilizer grade by dissolving it in distilled water was applied to all pots. The calculated amount of Nitrogen and phosphorus was applied to all the pots including control (without potash) and treated pots (potash applied) and potash was applied only to treated pots. All the potash and phosphorus was applied at the time of sowing and Nitrogen was applied in four splits with the following schedule (1) at the time of sowing (2) 10 days after seeding (10 DAS) (3) 25 days after seeding (25 DAS) (4) at flower initiation stage (50 DAS). All other agronomic and cultural practices including plant protection measures were undertaken.

At boll formation stage (90 DAS) fully expanded young mature leaves of all the treatments for all the varieties were sampled. The central portion of leaf blades were cut into small pieces 1 to 2 cm long fixed in sera (FAA) for 4 to 5 h. The composition of FAA included formaline, acetic acid and absolute alcohol in 1:2:2 ratio, respectively.

Then the leaves were dehydrated in ascending grades of alcohol as mentioned below:

1. In 70% alcohol for 3 to 4 h
2. In 80% alcohol for overnight
3. In 90% alcohol for 4 h
4. In 100% alcohol for 4 h

After dehydration the leaves were transferred to cedar wood oil until they become clear and transparent. These leaves were then embedded in the paraplast wax at 60°C .The blocks were made and sectioned at a thickness of 10 $\mu$m by Richert Microtome and stretched at 60°C on fisher slide warmer. After deparatinizing, the slides were transferred to xylol for 15 min to remove remaining wax. The sections were hydrated in descending grades of alcohol and washed with distilled water. After that the sections were dehydrated in ascending grades of alcohol and stained with safranine and fast green stain (Mc Manus and Mowry, 1960). Finally the slides were microphotographed and a total of 270 microphotographs (x 225) were considered in determining the thickness of cotton leaf and structure of internal leaf tissue in both potash treated and control leaves.

By using ocular micrometer, approximately 10 microphotographs per treatment in all the varieties were used for measuring the thickness of leaf epidermal region and whole leaf thickness and averages were obtained. Mean data of leaf thickness and thickness of epidermal region were statistically analyzed by using "students" t test for comparison between treatment means in all the three varieties.

**Results and Discussion**

The micro-morphological observations of cotton leaves showed that the structure of internal leaf tissue was improved with the application potash. Potash acting as an osmoticum to maintain the tissue turgor pressure in the plant cells (Kaiser, 1982). The mesophyll cells, spongy parenchyma cells and epidermal cells were more turgid resulted to increase the surface area of these cells in plants receiving Potassium. The mesophyll cells were uniform, fully turgid, symmetrical and structurally improved in plant leaves with potash application (Fig. 2). Similar observations were made by Liang *et al*. (1992) that structure of potash affected leaf cells were improved. Fig. 1 showed that the internal leaf tissue cells were

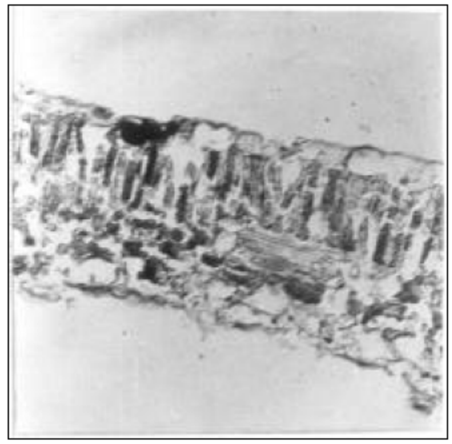

Fig. 1: Cross section of cotton leaf showing crumbled and asymmetrical cells without potash application (255 x)

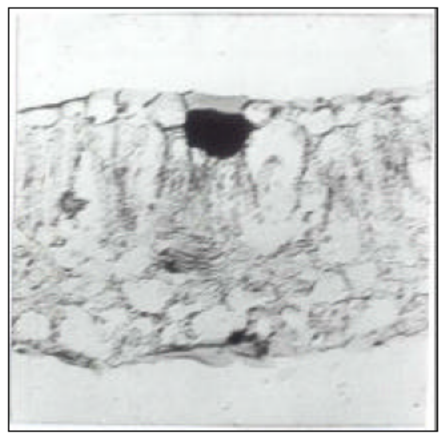

Fig. 2: Cross section of cotton leaf showing symmetrical, fully turgid internal leaf tissue with uniform margins after potash application (225 x)

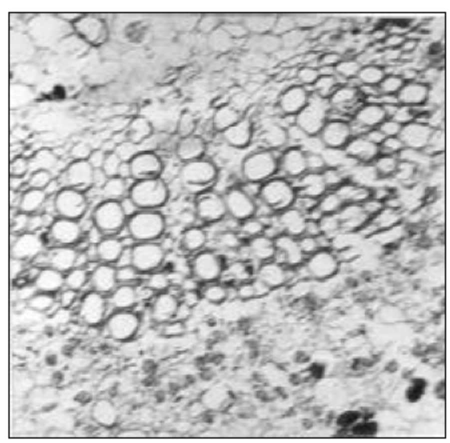

Fig. 3: Cross section of cotton leaf showing a large number of starch grains without potash application (225 x)

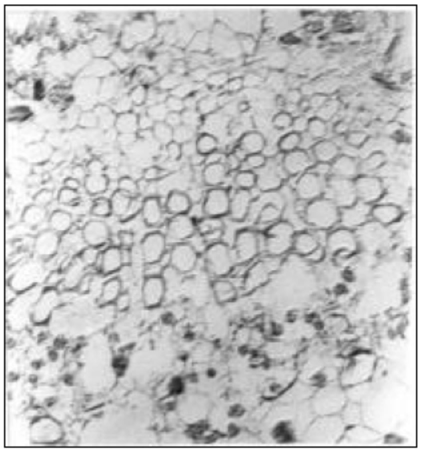

Fig. 4: Cross section of cotton leaf showing fewer starch grains after potash application (225 x)

Table 1: Effect of potash on cotton leaf thickness ($\mu$m)

| $K_2O$ kg $ha^{-1}$/Varieties | Cotton leaf thickness ($\mu$m) CIM443 | CIM109 | CIM446 | Mean |
|---|---|---|---|---|
| 0 | 135.0 | 178.0 | 206.0 | 173.0 |
| 200 | 198.0 | 229.0 | 245.0 | 224.0 |
| Mean | 166.5 | 203.5 | 225.5 | |
| K levels** | * Significant at 5% level of probability | | | |
| | ** Significant at 1% level of probability | | | |
| | N.S. Non Significant | | | |

Table 2: Effect of potash on thickness ($\mu$m) of epidermal region (cuticle+epidermis) of the cotton leaf

| kg $ha^{-1}$/Varieties | Thickness of cotton leaf epidermal region ($\mu$m)$K_2O$ CIM443 | CIM109 | CIM443 | MEAN |
|---|---|---|---|---|
| 0 | 14.0 | 19.0 | 22.0 | 18.33 |
| 200 | 26.0 | 29.0 | 32.0 | 29.0 |
| MEAN | 20.0 | 24.0 | 27.0 | |
| K levels** | * Significant at 5% level of probability | | | |
| | ** Significant at 1% level of probability | | | |
| | N.S Non Significant | | | |

crumbled and asymmetrical in cotton leaves when potash was not applied.

A large number of starch grains were accumulated in leaves of control plants (Fig. 3). After the potash application of 200 kg $K_2O$ $ha^{-1}$, the number of starch grains in leaf tissues were decreased (Fig. 4) due to translocation of these starch grains rather than accumulation in the leaves as Ashlay and Goodson (1972) have reported that K influence the rate of translocation of photoassimilates from the leaves to the other parts of the plant. Oosterhuis (1997) have 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. In present study it was observed that the structure of starch grains was not affected by potash but only the number of starch grains was decreased with the application of potash.

The results of the microscopic measurements indicated that cotton leaf thickness increased significantly with the application of 200 kg $K_2O$ $ha^{-1}$ as compared to control. Overall the cotton leaf thickness increased by 29.47% at 200 kg $K_2O$ $ha^{-1}$ as compared to control plants. The maximum leaf thickness of 224 $\mu$m was observed at $K_2O$ level of 200 kg $ha^{-1}$ (Table 1) and the minimum leaf thickness of 173 $\mu$m in the control plants. The mean minimum leaf thickness (225.5 $\mu$m) was found in variety CIM446 and the minimum mean leaf thickness (166.5 $\mu$m) in variety CIM443 (Table 1).

The increased turgidity of leaf cells in potash affected cotton leaves resulted to increase the surface area of cells which ultimately resulted to increase the thickness of cotton leaves after potash application (Liang *et al.*,1992). Similar observations were made by Dhindsa *et al.* (1975).

The effect of potash application on the thickness of epidermal region was highly significant (Table 2). The thickness of epidermal region increased by 58.21%with the application of 200 kg $K_2O$ $ha^{-1}$.The maximum epidermal region thickness (cuticle plus epidermis) of 29 µm was observed at 200 kg $K_2O$ $ha^{-1}$ and the minimum epidermal region thickness of 18.33 µm was observed in leaves of control plants .Mean maximum thickness of epidermal region (27 µm ) was found in variety CIM 446 and minimum mean thickness of epidermal region (20 µm) in variety CIM443 (Table 2). The thickness of epidermal region was increased significantly with the application of Potassium and maximum at 200 kg $K_2O$ $ha^{-1}$ in all the varieties.

In conclusion, the Potassium application was improved the structure of internal leaf tissue and increased the overall thickness of cotton leaves, which would result in the increased photosynthetic capacity per plant leaf and increased the rate of translocation of photoassimilates from leaves to other plant parts.

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