EFFECT OF PHOSPHORUS FERTILIZER AND WATER STRESS ON PROTEIN AND PHENOLIC CONTENTS IN COTTON (*GOSSYPIUM HIRSUTUM* L.)

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ABSTRACT:- Crop quality and production are affected by various fertilizers and water stress. In present research, the response of cotton variety CIM-496 to water stress and phosphorus fertilizer was investigated. Samples were collected after 90 days of planting. Kjeldahl method and thin layer chromatography (TLC) were used for the quantitative and qualitative analysis of total protein and phenolic compounds, respectively. Proteins were greatly affected by fertilizer treatment and water stress, but phenolic compounds remained unchanged upon fertilizer treatment. However, they were greatly affected by irrigation and water stress. Crop treated with 100 kg ha^{-1} P_2O_5 under water stress maintained high protein content as compared to unfertilized and no water stress treatments. However, phenolic compounds were found higher in fully irrigated plants as compared to water stress ones. Fertilizer treatments had no considerable effect on phenolic compounds.

Key Words: Cotton; Water Stress; Phenolic Contents; Proteins; Fertilizers; Pakistan.

INTRODUCTION

Cotton belongs to genus *Gossypium* and is placed in the Malvaceae family. It is found in abundance in tropical and subtropical areas of Asia, Africa, America and Australia. In Pakistan it is grown on large scale in Multan, D.G. Khan, Bahawalpur, Sahiwal, Sukkhur and Hyderabad Division. Probably two general centers of origin of the cotton plant are Indo-China and Tropical Africa in the Old World, and South and Central America in the New World (Gupta, 2003). Separate origins indicate the fact that consistently fertile hybrids have never been obtained from crosses between the 26-chromosome American cottons and the 13-chromosomes Asiatic cottons. Three distinct types of cotton grown in the United States, i.e., Upland, Sea Island and American-Pima are of American origin. Upland cotton assumed to have descended from Mexican cotton or from crosses of Mexican and South American species.

Cotton is a major cash crop of Pakistan and also for the farming community. It is dual purpose crop yielding both fiber and vegetable oil. It accounts for 8.2% of the value added in agriculture and about 2% to gross domestic production (GDP). It is realized that an increment of 14.0 million bales of cotton production in

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turn translated into 0.5% increase in GDP. As such this cash crop is believed to be the life time of national economy. In 1947-48, when Pakistan appeared on the world map, the county was producing 1.1 million bales and only 31 textile mills were in existence and now more than 1600 textile mills are working.

Besides fiber it yields food and feed in the form of oil and cotton seed cake for human and animal consumption. Cotton is not only an export earning crop but also provide the raw material to local textile industry. Cotton seed has 30% starch, 25% semi-dry oil and 16% protein (Cobley and Steele, 1976). The cotton hulls serve as roughage for livestock and linters (a fuzz of short seed hair) is used in the manufacturing of felts, twine, writing papers, plastics, carpets, rayon, explosives and cotton wool (Zakariya et al., 2006). It is also used in the manufacturing of medicinal supplies, tarpaulin, cordage and belting (Gibben and pain, 1985).

Cotton waste, trashes and other residues can be converted into ethanol without environment pollution (Showler and Moran, 2003). Glucose (9.4%) and reducing sugars (43%) have been obtained from powder cotton stalks (Meloni et al., 2003; John and Borah, 2012). Moreover, burying of cotton sticks @ 5 t ha^{-1} increased organic matter from 0.44 to 0.88% available phosphorus from 8.24 to 9.48 mg kg^{-1} and exchangeable potassium from 248 to 332 mg kg^{-1} in the soil (Ray and Tuker, 1999).

The low and fluctuating production of cotton crop as compared to the estimated production is caused due to poor quality seed, imbalance use of fertilizers, poor plant protection measures, pesticide hazard, resistance of pests, disturbance of biological equilibrium, residual toxicity, washing of pesticides by rains, mismanagement of irrigation water, etc. Water stress is the major environmental factor, which affects the crops (Howard et al., 2001). Plants remain under stress in arid and semi-arid environments. Cotton plant is considered a xerophytic plant but it requires supplemental irrigation for its growth and development (Riaz et al., 2013). The availability of water is declining in our country. Phosphorus is a major nutrient for crops and being used at large scale in cotton crop. Phosphorus is a part of DNA, RNA, ATP and cell nucleus. It is also a rich source of energy for plants which also play an important role in enzyme activation, photosynthesis, protein synthesis (Taize and Zeiger, 1991) and cell division; hence it has a stimulating effect on increasing the number of flowers and bolls per plant.

The objective of present study was to acertain the effect of phosphorus fertilizer and water stress on total protein and phenolic contents in cotton (*Gossypium hirsutum* L.) plant.

MATERIALS AND METHOD

Field experiment was conducted at farmers' field in Multan during 2013. The area lies in semi-arid region with scanty rainfall. The production of cotton depends upon the supplemental irrigation under these environments. The treatments were no water stress (< -1.6 MPa) and water stress (> -2 MPa) with three levels of phosphorus fertilizer [0, 50, 100 kg P_2O_5 ha^{-1}]. The design of experiment was split plot design with three replications. Cotton cultivar CIM-496 was used as a test crop. The

crop was sown during the 3rd week of May 2013. Distance from row to row and plant to plant was 75 cm and 30cm, respectively. Leaf samples were collected 4th from the top on the day 90 after planting. The samples were washed with distilled water and blotted. The material was oven dried at 400°C for 72h and ground for chemical analysis.

Protein was determined by Kjeldahl method which was developed by a brewer called Johann Kjeldahl in 1883. The material was digested with sulfuric acid in the presence of anhydrous sodium sulfate and copper sulfate as catalyst. Digestion converted any nitrogen (other than that which was in the form of nitrates or nitrites) into ammonia and other organic matter into CO_2 and H_2O. The nitrogen released was determined by titration techniques (Boric acid method). The amount of protein present was then calculated from the nitrogen concentration. A conversion factor of 6.25 (equivalent to 0.16 g N g^{-1} of protein) was used. This is only an average value and each protein has a different conversion factor depending upon its amino acid composition. The following equation was used to determine the nitrogen concentration of the sample.

$$\%N=\frac{\text{xmole}}{1000\text{cm}^3}X\frac{(Vs\text{-}Vb)\text{cm}^3}{\text{mg}}X\frac{14\text{g}}{\text{moles}}X\,100$$

Where Vs and Vb are the titration volumes of the sample and blank, 14g is the molecular weight of N. The N content was determined and converted to protein content by the following equation:

$$\%\ \text{Protein} = F \times \%\ N$$

Phenolic substances tend to be water soluble, since they most frequently occur combined with sugar as glycosides and they are usually located in cell vacuole.

The crushed cotton leaves (1 g) were soaked in 2 M HCl (25 ml) for 10 min and then refluxed for 30min in each case. After cooling, the solution was filtered, the residue was washed with absolute ethanol (5 ml) and the filtrate was extracted with ether (4 x 10 ml). The ether extracts were evaporated to remove the solvent; the residue was dissolved in absolute ethanol (1 ml) and preserved for the qualitative analysis of phenolic compounds.

Thin layer chromatography (TLC) was employed for the separation of phenolic compounds. The TLC plates (7.5cm x 20 cm) prepared of silica gel (60 F254) were used for this purpose. The samples were loaded and plates were developed using chloroform-acetic acid (9:1 v/v) as the solvent system.

RESULTS AND DISCUSSION

Data presented that nitrogen contents were affected by the imposition of water stress and addition of phosphorus fertilizer. Imposition of water stress had little effect on nitrogen concentration; however, the plant under water stress contained 1.6 % more nitrogen concentration as compared to that under no water stress (Table 1). The nitrogen contents were greatly affected by the application of phosphorus fertilizer. These increased by increasing phosphorus fertilizer. The maximum nitrogen contents were found in the crop treated with 100 kg ha^{-1} (P_2O_5) and the minimum in crop without any phosphorus fertilizer. The addition of 100 kg ha^{-1} (P_2O_5) caused an increase of 7.6% as compared to unfertilized

Table 1. Interactive effect of water stress and phosphorus fertilizer on percentage of nitrogen in leaves of cotton plant

(%)

Stress applied	P_2O_5 level (kg ha^{-1})	R_1 N	R_2 N	R_3 N	Mean N
No water stress	0	4.03	4.08	4.11	4.07
	50	4.25	4.28	4.25	4.28
	100	4.36	4.39	4.36	4.39
Water stress	0	4.08	4.11	4.14	4.11
	50	4.31	4.34	4.31	4.34
	100	4.42	4.45	4.42	4.45

Table 2. Interactive effect of water stress and phosphorus fertilizer on percentage of proteins in leaves of cotton plant

(%)

Stress applied	P_2O_5 level kg/ha	R_1	R_2	R_3	Mean
No water stress	0	25.20	25.55	25.72	25.49
	50	26.60	26.77	26.60	26.77
	100	27.30	27.47	27.30	27.47
Water stress	0	25.55	25.72	25.90	25.72
	50	26.95	27.12	26.95	27.12
	100	27.65	27.82	27.65	27.82

crop. The maintenance of high quantity of phosphorus under water stress improved the tolerance to drought stress.

Protein contents were appreciably affected by various water stress and fertilizer treatments. Crop under water stress maintained higher quality of proteins as compared to that under non-water stress conditions. The application of phosphorus fertilizer caused significant increase in protein content (Table 2). Higher quantity of protein was present in crop treated with 100 kg ha^{-1} (P_2O_5). To the contraries, minimum amount of protein content was found in unfertilized treatment. The maintenance of higher quantity of proteins by the crop under water stress and in that having received 100 kg ha^{-1} (P_2O_5) induced the plant to counterbalance the adverse effects of water stress.

Data indicated that phenolic compounds were influenced by water stress. Crop fully irrigated contained higher number of phenolic compounds as compared to that of stressed one (Table 3). However, numbers of phenolic compounds were not much affected by fertilizer treatments. Further study is needed to quantify the phenolic compounds under stressed environments.

Phosphorous is an essential plant nutrient and plays its specific role in plants (US Geological survey, 2005). Phosphorous is required for production of high quality seeds. It occurs as co-enzymes involved in energy transfer reactions. Energy is tapped in photosynthesis in the form of ATP and NADP. This is then used in photosynthetic fixation of CO_2 and the synthesis of essential organic compounds (Swan et al., 2006). Ibrahim et al. (2007) conform this study that the effect of foliar applica-tion of phosphorus and zinc on cotton plant increased the protein content in the plants. By the addition of phosphorous fertilizer, concentration of nitrogen as well as that of proteins remained high. It was also found that the crop under water stress maintained high quantity of protein as compared to that of non-stressed crops. The quantitative analysis of phenolics showed that various phosphorous applications had no considerable effect on phenolic compounds but water stress affected phenolic compounds to a certain extent. Fully irrigated crop main-

Table 3. Interactive effect of water stress and phosphorus fertilizer on percentage of nitrogen in leaves of cotton plant

Phenolic Compound α	(Rfx100) of α	Name of Standards β	(Rfx100) of β	No water stress			Water stress		
				0 P_2O_5 Kg ha^{-1} S_4S_{12} S_{18}	50 P_2O_5 Kg ha^{-1} S_4S_{12} S_{18}	100 P_2O_5 Kg ha^{-1} S_4S_{12} S_{18}	0 P_2O_5 Kg ha^{-1} S_4S_{12} S_{18}	50 P_2O_5 Kg ha^{-1} S_4S_{12} S_{18}	100 P_2O_5 Kg ha^{-1} S_4S_{12} S_{18}
A	6	Gallic Acid	5	+ - -	- - -	- + -	+ - -	+ - -	- - -
B	10	Un-Identified		+ - +	- + +	+ - -	- + +	- - +	- + -
C	14			- - -	+ - -	- - +	- - -	+ + -	- - -
D	19			- - +	- + -	+ + -	- + -	- - -	+ + +
E	29	Pyragallol	27	+ + -	+ + +	- - -	+ + +	- - +	+ - -
F	31	Hydroquinone	34	+ + -	- - -	+ - -	- - -	+ + -	+ + -
G	34			- - +	+ - -	- - +	+ - -	- - -	- - -
H	36			- - -	- + +	- + -	- + +	- - +	- - +
I	45	Un-Identified		- + -	+ - -	- - +	- - -	- - -	- - -
J	57			+ - +	- - -	- - -	- - -	- + -	+ - +
K	69	Phenol	70	- - +	- - -	- - +	- - -	- - -	- - -
L	84	2-Naphthol	84	- - -	- - -	- - -	- - -	- - -	- - -
M	92	o-Nitrophenol	92	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
Total spots				6 4 6	5 5 4	4 4 5	4 5 4	4 4 4	5 4 4

tained more phenolic compounds as compared to stressed crop. However, further study is needed to evaluate the effect of water stress and phosphorous on phenolic compounds in cotton plant.

It is thus concluded that fertilizer treatment and water stress can greatly affect proteins, but phenolic compounds remains unchange upon fertilizer treatment often, however, they affected by irrigation and water stress. Crop treated with 100 kg ha^{-1} P_2O_5 under water stress maintained high protein content as compared to unfertilized and no water stress treatments.

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AUTHORSHIP AND CONTRIBUTION DECLARATION

S. No	Author Name	Contribution to the paper
1.	Dr. Zafar Abbas	Conceived the idea, Data collection and write up introduction
2.	Dr. Ijaz Ahmad	Overall management of the article, Methodology and reference
3.	Mr. Adnan Shakeel	Did SPSS analysis
4.	Mr. Muhammad Abdullah	Wrote abstract
5.	Mr. Muhammad Islam	Data entry in SPSS
6.	Mr. Sadiq Muhammad	Results & discussion
7.	Ghulam Murtaza	Results & discussion
8.	Mr. Mushtaq Ahmad	Technical input and conclussion

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