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ALTERATION IN IONIC CONTENTS PROFILING OF COTTON DUE TO BACTERIAL BLIGHT DISEASE CAUSED BY *Xanthomonas citri* pv. *malvacearum*

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The current research was conducted to find out modification in mineral profiling of cotton after the attack of bacterial blight disease caused by *Xanthomonas citri* pv *malvacearum*. Leaves of three susceptible and resistant (un-inoculated and inoculated) cultivars were collected for the determination of alteration in ionic status. The remarkable variation ($p \leq 5$) in the ionic contents was observed across treatments, groups (un-inoculated and inoculated), types (susceptible and resistant) and varieties of cotton plants due to the infection by *X. citri* pv *malvacearum*. Nested random's effect analysis of variance revealed significant difference in ionic status (Ca, N, K, P, Mg, Zn, Cu and Fe) in leaves of cotton. Resistant type of plants expressed 2.40% and 0.19% while susceptible type showed 2.17% and 0.16% difference in concentrations of P and N respectively. Moreover, resistant type expressed 408.3, 310.2, 21.1, 2.9, 1.83 and 1.61 ppm while susceptible type showed 378.8, 270.2, 14.6, 2.4, 1.75 and 1.35 ppm difference in concentrations of Ca, K, Zn, Mg, Cu and Fe respectively. It was accomplished that resistant cultivars accumulated these ions at higher concentrations than susceptible varieties. These increased ionic contents in resistant plants strengthen the biochemical and physiological processes of the host plants which help to avoid the spread of pathogen.
Keywords: bacterial blight, cotton, ionic contents, *Xanthomonas citri* pv *malvacearum (Xcm).*

INTRODUCTION

Cotton (*Gossypium hirsutum*) is the most important fiber crop of Pakistan which plays a significant role in the economy of country. It belongs to family Malvaceae and genus *Gossipium* which is closely related to okra, jute and mallow. Cotton is unique among agricultural crops as it provides food, edible oil, fiber and other byproducts for livestock. It is grown in temperate and subtropical regions of the world including Pakistan (Smith, 1999) cultivated on an area of 33.1 million hectares in the world while on 3.0 million hectares in Pakistan during 2013-14 with the production of 116.7 and 9.5 million bales respectively (Johnson *et al.,* 2014).

Bacterial blight of cotton (BLB) caused by *Xanthomonas citri* pv. *malvacearum (Xcm)* is one of the serious diseases of cotton (Saha *et al.,* 2001). The pathogen of this disease is gram negative and aerobic bacterium with motile single flagellum. This bacterium enters in healthy plants through stomata or wounds. Typical symptoms of BLB including small, irregular and dark water socked spots on lower epidermis of leaves that later becomes dark brown (Liberato *et al*., 2007), water soaked abrasions on bolls, early stem and leaves senescence and stunted growth of infected plants (Rungis *et al*., 2002).

Nutrients are essential for normal plant growth by strenghtening cell wall which simultaneously reduce disease severity (Huber and Graham, 1999). An aprropriat density/availibility of nurtietns trigger the resistance mechanism of plants agaisnt pathogen while their deficiency makes the plant valunerable to disease and change the physiology and biochemistry of the plant (Hajiboland, 2012). Type of disease, amount of mineral elements, form of elements and weather conditions are also important factors to determine their effects on the disease development. Minerals are the crucial part of plant nutrition and their deficiency/ excessive amount cause certain types of maladies either through disturbing metabolism or physiology of the plants by favoring plant pathogens or discouraging plant growth (Sahi *et al.,* 2010). Similarly, the pathogen of bacterial blight also reduces the up-taking efficiency of the plant and the infected plants fail to optimize macro and micro contents i.e. N, P, K, Ca, Mg, Zn, Cu and Fe etc in their body and plants become

susceptible to disease. The plants deficient in these nutrients provide favourable conditions for the pathogen establishment and disease development. Application of nutrients in the rhizosphere is an important cultural control to prevent the plant from disease which consequently increases agricultural production. The availability of nutrients through inorganic fertilizers provides the better means for reducing the severity of many diseases of crop plants (Savant *et al*., 1997). Therefore, it is need of the hour to find an alternative approach for artificial manipulation/ provision of ionic contents to diminish disease severity and ultimately enhanced crop production Thus the present research was planned to determine the impact of in ionic contents such as N, K, P, Mg, Ca, Zn, Na, Cu and Fe etc. in cotton leaves in resistant/ susceptible cultivars after inoculation and compare it with un-inoculated plants.

MATERIALS AND METHODS

***Collection of germplasm and establishment of experiment*:** Seeds of six varieties/ advanced lines (Bt-MNH 886, Bt-FH 177, Bt-ASO1, Bt-FH 142, Bt-FH 182 and Bt-FH 169) were collected from Department of Plant Breeding and Genetics (PBG) Faisalabad, Ayub Agricultural Research Institute (AARI), Faisalabad and Central Cotton Research Institute (CCRI) Multan for determination of biochemical/physiological changes due to attack of bacterial blight disease in cotton leaves. Plants of cotton varieties/ advanced were sown in pots (45 × 30 cm^2) filled with loamy soil (2kg/pot) and transferred to greenhouse. Plant population was composed of two groups i.e. un-inoculated and inoculated; each group was consisted of two reaction types (Susceptible and resistant). Resistant reaction type contained Bt-MNH 886, Bt-FH 177 and Bt-ASO1while susceptible reaction type contained three varieties/lines viz. Bt-FH 142, Bt-FH 182 and Bt-FH 169. Each treatment was replicated five times. At the age of 6-7 weeks plants were inoculated by using syringe method (Weindling, 1948) and injected 20 µl (local isolates) bacterial suspensions (10^7cfu ml^{-1}) while un-inoculated plants served as control. In green house, humid condition was maintained by spraying water on daily basis. After 6 to 8 days of inoculation, typical symptoms (water soaked lesions, 72.70-79.20% disease incidence) of bacterial blight appeared on leaves.

***Determination of ionic status in cotton cultivars*:** To remove dirt leaves of susceptible and resistant cotton varieties/cultivars were collected and washed in 0.2% detergent solution, to remove metallic contaminants washing in 0.8% HCl following de-ionized water to remove earlier solutions. Samples were dried, placed in paper bags and oven dried (Heaes D650 Hanau) at 70°C for 72 hours to get constant weight. Dried samples were grounded with mortar and pestle. Grounded samples (100 mg) were boiled in 10 ml of 1.4N HNO_3 on hotplate (TH-550; Advantec, Tokyo, Japan) at 100 °C for 30 minutes. After cooling, suspension was diluted 250 times with distilled water, followed by analysis for the determination of ionic contents following Bhargava and Raghupathi, (1995) method. Nitrogen and phosphorous contents were recorded on percent basis while contents of other elements were recorded as ppm (parts per million).

***Determination of phosphorus from leaves of un-inoculated and inoculated cotton plants*:** A 0.1 mL of sample solution, already prepared by wet digestion method, was taken in a volumetric flask (ASTM- E288). Then 8.6 ml of distilled water was added along with 1mL of ammonium molybdate reagent ($[NH4]_6Mo_7O_{24}.4H_2O$). After swirling the flask to mix solution, 0.4 mL of amino nephthol sulphonic acid ($C_{10}H_9NO_4S$) was added. Absorbency was measured using distilled water as reagent blank in place of sample solution at 720 nm on a spectrophotometer (Hitachi U-2001, model 121-003). Phosphorus concentration was determined by comparing the absorbency to a previously prepared standard curve (Fiske and Subbarow, 1925; Bolts and Mellon, 1948) by Atomic absorption spectrometer (Hitachi Polarized Zeeman).

***Determination of potassium contents from leaves of un-inoculated and inoculated cotton plants*:** Potassium was measured by flame photometer (Janway, PFP-7). For the measurement of potassium, KCl was used as standard. Standard curves for K was prepared by using 10, 20, 30 and 40 ppm concentrations. Fresh working standards were prepared immediately before use.

***Determination of calcium, magnesium, copper, iron and zinc from leaves of un-inoculated and inoculated cotton plants*:** Calcium, Magnesium, Copper, Iron and Zinc were determined by using spectrophotometer (Hitachi U-2001, model 121-003). For the determination of these ions, Calcium chloride($CaCl_2$), Copper sulphate ($CuSO_4$), Magnesium sulphate ($MgSO_4$), Iron sulphate ($FeSO_4$) and Zinc oxide (ZnO) were used as standards respectively and their standard curves were obtained by using 10, 20, 40, 80, 100 ppm for Ca; 5, 10, 15 and 20 ppm for Mg; 2, 2.5, 3 and 3.5 ppm for Cu; 1,2,3 ppm for Fe and 0.2, 0.3, 0.5 and 2 ppm for Zn, respectively. These working standards were arranged as fresh just before exercise.

***Determination of total nitrogen from leaves of un-inoculated and inoculated cotton plants*:** Total nitrogen in a sample was determined by following micro Kjeldahl method (46MC; Quickfit, England). (Kjeldahl, 1983). Measured amount of oven (D6450 Hanus; Heraeus) dried sample (WI) was taken in a long neck Kjeldahl flask. Five gram of digestion mixture containing $CuSO_4$ and K_2SO_4 and 25 mL of concentrated sulphuric acid (H_2SO_4) were added. Digestion hood was used to boil the sample (KB8S Kjeldatherm), at the start at low temperature and then at vigorous boiling till the contents became clear. After cooling, the contents present in flask were diluted with distilled water in a 250 mL volumetric flask (ASTM- E288). 10 mL of this solution was transferred

to the micro Kjeldahl distillation apparatus (VAP20.Gerhardt) and was distilled in the presence of 10 mL of 40 percent NaOH solution. The ammonia (NH_3) so produced was collected in a beaker containing 10 mL of two percent of boric acid (H_3BO_3) solution having two drops of methyl red as an indicator. The distillate was titrated against standard 0.1 N sulphuric acid (H_2SO_4) to light pink point. The percentage of nitrogen was calculated according to the following formula.

$$\text{Nitrogen } \% = \frac{0.1\ \text{NH}_2\text{SO}_4\ \times\ 0.0014\ \times\ 250}{\text{WI}\ \times 100} \times 100$$

***Statistical analysis*:** To estimate the ionic contents standard analytical methods were adapted by using Nested Design (Gomez and Gomez, 1984). By using PROC MIXED procedure of the Statistical Analysis System (SAS) version 2009, statistical analysis was performed. Data were analyzed statistically and treatments means were compared.

RESULTS

***Determination of Nitrogen, Phosphorus, Potassium and Calcium) from un-inoculated and inoculated cotton plants*:** Significant variation was observed between inoculated (averaging to 1.79% across the inoculated group) and un-inoculated plants (averaging to 2.78% across the un-inoculated group) representing that Nitrogen contents were seemed to affect metabolic processes as a result of disease outcome. The 2.40% value was observed in resistant type and 2.17% in susceptible significant at $p \leq 0.05$ (Table 2). The components termed as type uttered total variance of 4.93%. Varieties exhibited their natural tendencies with respect to N concentration explaining 0.50% of total variability (Table 1). Maximum concentration was exhibited by variety named "Bt-ASO 1" to the extent of 2.41% and minimum by "Bt-FH 142" to the tune of 2.11% (Table 2). About P contents, considerable variation was observed among un-inoculated and inoculated plants (averaging 0.14 and 0.22% respectively) during disease stress with 90.48% of the total variance at $p \leq 0.05$. While 8.51% total variance was observed between resistant (0.19) and susceptible (0.16) and 0.95% of the total variance was shown by the varieties in their natural tendencies with respect to phosphorus concentration. Bt-ASO 1and Bt-FH 142 displayed maximum and minimum concentration of P to the extent of 0.21 and 0.15% respectively (Table 2). Group and type exhibited 98.19 and 1.63 of the total variability of potassium contents (Table 1). Considerable variation was observed by susceptible and resistant cultivars/varieties averaging 408.3 and 378.8 ppm respectively. Significant variation (512.1. and 276.6 ppm) was observed in un-inoculated and inoculated group respectively. Bt-ASO1 showed maximum potassium fractions (416 ppm) while minimum concentration was accumulated by Bt-FH 142 (372.5 ppm) (table 2). Significant variation was exhibited in calcium contents in group (averaging 191 ppm in inoculated plants and 412.3 ppm in un-inoculated plants) as shown in (Table 2) and total variance was accounted 95.62%. In the same way types were found possessing low variability of

Table 1. Nested ANOVA for ionic contents (Nitrogen, Phosphorous, Potassium, Calcium and Magnesium)

SOV	DF	SS	F. Value	Pr>F	MS	V. component	Total variance component %
Nitrogen (%)							
Group-A	1	26.235	26.235	0.025*	37.906	0.473	94.30
Type-B	2	1.384	0.692	0.022*	28.751	0.025	4.93
Variety-C	8	0.192	0.024	0.000*	18.604	0.003	0.50
Error	96	0.124	0.001			0.001	0.26
Total	107	27.936				0.502	
Phosphorous (%)							
Group-A	1	0.353	0.353	0.044*	21.497	0.006	90.48
Type-B	2	0.033	0.016	0.000*	27.632	0.001	8.51
Variety-C	8	0.005	0.007	0.000*	137.250	0.000	0.95
Error	96	0.0004	0.000			0.000	0.06
Total	107	0.391				0.007	
Potassium (ppm)							
Group-A	1	1.498	1.498	0.008*	118.85	27509.796	98.19
Type-B	2	25208.66	12604.3	0.000*	41.394	455.549	1.63
Variety-C	8	2436.000	304.500	0.008*	16.150	31.738	0.11
Error	96	1810.000	18.854			18.854	0.07
Total	107	1.527				28015.938	
Calcium (ppm)							
Group-A	1	1.1077	1.108	0.021*	46.544	20073.620	95.62
Type-B	2	47601.66	23800.8	0.000*	77.317	870.111	4.14
Variety-C	8	2462.667	307.833	0.000*	19.115	32.414	0.15
Error	96	1546.000	16.104			16.104	0.08
Total	107	1.159				20992.250	

Table 2. Amount of Nitrogen, Phosphorous, Potassium and Calcium in reaction groups (un-inoculated and inoculated), types (susceptible and resistant) and in varieties/ advanced lines of cotton plants.

Varieties-C	Nitrogen (%)											
	Bt-MNH 886		Bt-FH 177		Bt-ASO 1		Bt-142		Bt-FH 182		Bt-FH 169	
Type-B	Resistant								Susceptible			
Group-A	I	UI	I	UI	I	UI	I	UI	I	UI	I	UI
Quantity of N in C	1.86	2.85	1.90	2.92	1.94	2.98	1.63	2.60	1.69	2.65	1.74	2.73
Av. quantity of N in C	2.35		2.41		2.46		2.11		2.17		2.23	
Av. quantity of N in B	R = 2.40								S = 2.17			
Av. quantity of N in A	I= 1.79								UI = 2.78			
Phosphorous (%)												
Quantity of P in C	0.125	0.250	0.133	0.269	0.141	0.271	0.102	0.206	0.111	0.214	0.223	0.119
Av. quantity of P in C	0.18		0.20		0.21		0.15		0.16		0.17	
Av. quantity of P in B	R= 0.19								S = 0.16			
Av. quantity of P in A	I = 0.14								UI =0.22			
Potassium (ppm)												
Quantity of K in C	286	521	292	527	298	534	256	491	262	497	266	503
Av. quantity of K in C	403.5		409.5		416		372.5		379.5		384.5	
Av. quantity of K in B	R = 408.3								S = 378.8			
Av. quantity of K in A	I = 276.6								UI=512.1			
Calcium (ppm)												
Quantity of Ca in C	205	410	210	416	217	534	165	365	171	371	178	378
Av. quantity of Ca in C	307.5		313		325.5		265		271		274.5	
Av. quantity of Ca in B	R = 310.2								S = 270			
Av. quantity of Ca in A	I = 191								UI=412.3			

Inoculated= I; Un-Inoculated= UI; Resistant= R; Susceptible=S; N= Nitrogen; P= Phosphorus; K= potassium; Ca=Calcium

Table. 3 Nested ANOVA for ionic contents (Magnesium, Copper, Zinc and Iron).

SOV	DF	SS	F value	Pr>F	MS	V. component	Total variance component %
Magnesium (ppm)							
Group-A	1	43.141	5.393	0.000*	8.364	61.075	75.01
Type-B	2	1046.054	523.027	0.000*	96.990	19.172	23.55
Variety-C	8	3821.090	3821.090	0.114^{ns}	7.306	0.528	0.65
Error	96	61.897	0.645			0.645	0.79
Total	107	4972.183				81.419	
Copper (ppm)							
Group-A	1	70.374	70.374	0.040*	23.392	1.248	91.40
Type-B	2	6.017	3.008	0.000*	43.318	0.109	7.97
Variety-C	8	0.556	0.069	0.000*	69.595	0.008	0.56
Error	96	0.096	0.001			0.001	0.07
Total	107	77.042				1.365	
Zinc (ppm)							
Group-A	1	0.519	0.065	0.000*	36.852	0.007	67.58
Type-B	2	8.606	4.303	0.000*	66.327	0.157	30.70
Variety-C	8	22.963	22.963	0.147^{ns}	5.337	0.346	1.37
Error	96	0.169	0.002			0.002	0.34
Total	107	32.257				0.511	
Iron (ppm)							
Group-A	1	25.667	25.667	0.035*	27.064	0.458	92.56
Type-B	2	1.897	0.948	0.000*	55.623	0.034	6.98
Variety-C	8	0.136	0.017	0.000*	38.604	0.002	0.37
Error	96	0.042	0.0004			0.000	0.09
Total	107	27.742				0.495	

4.14% averaging 310.2 ppm across the resistant plants and 270.2 ppm across the susceptible plants. Varieties uttered significant variation as 0.15% of the total variance (Table 1). Calcium concentration was expressed 307.5, 313 and 325.5 ppm by Bt-MNH 886, Bt-FH 177 and Bt-ASO 1 (resistant type), while 265, 271 and 274.5 ppm concentration was displayed by varieties namely Bt-FH 142, Bt-FH 182 and Bt-FH 169 (susceptible type) respectively. (Table 2).

***Determination of Magnesium, Copper, Zinc and Iron from un-inoculated and inoculated cotton plants*:** Magnesium

Table 4. Quantity of Magnesium, Copper, Zinc and Iron in reaction groups (un-inoculated and inoculated), types (susceptible and resistant) and in varieties/lines of cotton plants.

Varieties C	Bt-MNH 886		Bt-FH 177		Bt-ASO 1		Bt-142		Bt-FH 182		Bt-FH 169	
Type-B	Resistant								Susceptible			
Group-A	I	UI	I	UI	I	UI	I	UI	I	UI	I	UI
Magnesium (ppm)												
Quantity of Mg in C	14.97	28.16	15.54	29.07	16.02	30.02	10.46	20.63	11.25	21.60	11.81	22.53
Av. quantity of Mg in C	21.5		22.3		23		15.5		16.4		17.2	
Av. quantity of Mg in B	R = 21.1								S = 14.6			
Av. quantity of Mg in A	I = 13.3								UI = 25.3			
Copper (ppm)												
Quantity of Cu in C	1.98	3.63	2.08	3.73	2.17	3.81	1.53	3.14	1.63	3.21	1.73	3.29
Av. quantity of Cu in C	2.8		2.9		3		2.3		2.4		2.5	
Av. quantity of Copper in B	R = 2.9								S = 2.4			
Av. quantity of Copper in A	I = 1.85								UI = 2.87			
Zinc (ppm)												
Quantity of Zinc in C	1.05	2.14	1.19	2.26	1.24	2.37	0.70	1.47	0.79	1.56	0.85	1.67
Av. quantity of Zinc in C	1.59		1.72		1.80		1.08		1.17		1.26	
Av. quantity of Zinc in B	R= 1.83								S= 1.75			
Av. quantity of Zinc in A	I = 1.09								UI= 1.91			
Iron (ppm)												
Quantity of Iron in C	1.09	2.05	1.13	2.09	1.15	2.15	0.81	1.8	0.86	1.84	0.90	1.88
Av. quantity of Iron in C	1.57		1.61		1.65		1.30		1.35		1.39	
Av. quantity of Iron in B	R = 1.61								S = 1.35			
Av. quantity of Iron in A	I = 0.99								UI= 1.96			

Inoculated= I; Un-Inoculated= UI; Resistant= R; Susceptible=S

contents were found in clear variation, both in the un-inoculated plant (25.3 ppm) and in inoculated plants (13.3 ppm) in disease pressure situation (Table 4) with 75.01% of total variability (Table 3). Susceptible and resistant plants showed significant variation (23.55%), averaging 21.1 ppm and 14.6 ppm respectively. Varieties exhibited non-significant results with 0.65% of the total variance which exhibited their natural trends regarding magnesium concentration. Bt-ASO1 (23 ppm) and Bt-FH 142(15.5ppm) displayed their maximum and minimum concentration respectively (Table 4). Un-inoculated and inoculated plants leaves expressed significant variation of copper with 91.40% of total variance (Table 3). Correspondingly, considerable variation of about 7.97% was observed in the leaves of resistant (2.9 ppm) and susceptible plants (2.4 ppm). Bt-ASO 1 and Bt-FH 142 retained 3 ppm (maximum) and 2.3 ppm (minimum) concentrations (Table 4) which accounted for 0.56% of the whole variability (Table 3). Zinc contents were found possessing significant variation in groups (averaging 1.09 ppm in inoculated plants and 1.91 ppm in un-inoculated plants) (Table 3 and 4). While considerable variation (30.70%) was observed by susceptible and resistant cultivars/varieties averaging 1.83 ppm and 1.75 ppm respectively. Varieties exhibited non-significant variation as 1.37% of total variance. Bt-ASO 1 and Bt-FH 142 showed 1.80 ppm and 1.08 ppm maximum and minimum concentration respectively (Table 3 and 4). Group, type and varieties expressed 92.56, 6.98 and 0.37% of the total variability in Iron contents (Table 3). Bt-FH 142 (1.30 ppm) and Bt-ASO (1.65 ppm) displayed their minimum and maximum concentration respectively.

DISCUSSION

***Biochemical changes induced by bacterial blight in cotton leaves*:** In plants deficiency/excessive quantity of nutrients cause different maladies which are affected by quantity of elements, form of elements, type of disease and environmental conditions affect the appearance of disease. Elements i.e. Carbon, Hydrogen, Oxygen, Nitrogen, Phosphorus, Potassium, Calcium, Magnesium, Iron, Manganese, Zinc, Copper, Boron, Molybdenum and Chlorine are necessary for the growth of plants and completion of their life cycle. Because nutrients expressed a variety of effects on disease incidence in different plant species that simplification/ generalization becomes difficult. So it is possible that different types of nutrients may affect the resistance status of the host as well as virulence of the pathogen. Plants obtaining well balanced nourishment, with all necessary elements easily available in proper amount undergo a smaller amount of disease and get protection from upshots of fresh infection and expressed pronounced growth, development and yield (Mishra *et al*., 2005). A makeable effect of bacterial blight disease was observed on the nutritional status of leaves in cotton.

Nitrogen is an essential nutrient for all plants obtained from soil and decaying organic matter. A huge amount of N is required by the plant because it is essential to stimulate

different types of enzymes, proteins and structures. Its balanced quantity plays pivotal role to enhance resistance against bacterial blight of cotton. That is why plant expressed pronounced effect towards its application. Its excessive application errand some plant diseases while plants deficient in N contents favor some other types of symptoms. In present study decrease in N level was observed and decrease in nitrogen quantity favor the incidence of bacterial blight of cotton and adequate application along with K activate defense system and create resistance against pathogen attack (Chase, 1989; Vidhyasekaran, 1988; Agrios, 2005; Dordas, 2008).

Phosphorus is also utilized by plant for development of essential molecules i.e. DNA and RNA synthesis, activation of transcription and translation, phospholipids, coenzyme NAD, NADP, ATP and other high energy compounds (Devlin and Witham, 1983). However, its role in resistance is variable. It can decrease incidence of different plant diseases by promoting root growth but in present study decrease in P quantity was observed which facilitate the growth of *Xcm* and enhanced the disease incidence. Similar results were reported by Dordas (2008) who also observed that low level of P favor the development of bacterial blight and balanced application of P reduced disease incidence of bacterial blight (Huber and Graham, 1999; Kirkegaard *et al.,*1999; Reuveni*et al.,* 2000).

Potassium play crucial role in metabolism of carbohydrate due to production of enzyme. Beside metabolism they also play an important role in photosynthesis and stomatal opening (Salisbury and Ross, 1992). In contemporary study, decrease in K amount was noted due to bacterial blight disease. Outcome the present study is privileged by the work of Mishra *et al.,* (2005) and Dordas (2008) who decrease in K enhanced the severity of bacterial blight by favoring the growth of *Xcm* (Sharma *et al.,* 2005).

Plants absorb calcium as a Ca^{+} cation. It helps in stimulation of leaf and root development, uptake of nutrients and microbial activity. It prevents the penetration of pathogens and develops resistance in host plant which strengthens the plant structure (Mishra *et al*., 2005). In infected leaves of cotton there was a reduction in calcium concentration which was observed both in susceptible and resistant cultivars which enhanced disease (Marschner, 1995; Mishra *et al.,* 2005). Comparable results were reported by Dordas (2008) that there was decrease in calcium contents which enhanced the bacterial blight infection.

Magnesium plays a significant role in the synthesis of chlorophyll contents, photosynthesis and carbohydrate metabolism (Devlin and Withman, 1983). In view of the fact that Mg is a vital element of structural tissues and take part in different biochemical and physiological processes. In current study level of Mg was decreased in cotton leaves due to attack of bacterial blight disease. Conclusion of present study is supported by the work of Batson (1971) and Huber and Jones (2012) who also observed decrease in Mg amount in cotton leaves due to attack of *Xcm*.

Copper is an important component of lignin and have a key role in protein and carbohydrate metabolism and acts as catalyst in different metabolic activities of the plant (Imran and Gurmani, 2011). In contemporary study decrease in Cu concentration was observed. Outcome of the present study is supported by the fact that when plant became infected, its defense system activated and starts secreting certain types of phenolics and flavonides both at the infection site and away from the site. Production of these substances is controlled by nutrients of the plant. Therefore, shortage of elements like Cu, Fe, K, Mn and Zn takes place at the infection site and copper application reduce the intensity of bacterial diseases (Marschner, 1995).

Zn is an important micronutrient in cotton plants; it plays a vital role in uptake and efficient use of water and work as a catalyst in different metabolic and biochemical processes. In present study Zn concentration was decreased in cotton leaves due to attack of bacterial blight disease. Upshot of the current study is verified by the work of Marschner (1995); Dordas (2008) who also reported decrease in concentration of Zn due to disease. Low level of Zn increase severity of disease due to accumulation of amino acids and reducing sugars, which help in disintegration of plasma membrane and increase pathogenesis (Grewal *et al.,*1996; Mengeland Kirby, 2001).

Iron is the foremost component of chlorophyll and has vital role in nucleic acid metabolism and its deficiency can reduce chlorophyll contents of plants (Imran and Gurmani, 2011). In present study it was concluded that Fe concentration was decreased due to bacterial attack. It may due the fact that plant pathogens generally have higher requirement of Fe and act as virulence factor during the course of disease development because Fe activate enzymes which are involved in the infection process of the host by the pathogen (Graham and Webb, 1991; Dordas, 2008).

***Conclusion*:** Reduction in host ionic contents (Phosphorus, Calcium, Nitrogen, Potassium, Magnesium, Zinc, Copper and Iron) quantity was due to the exploitation of these nutrients by the bacterial pathogen for its development and survival. Suitable application of these nutrients help in the host plants in strengthening of its physiological and biochemical processes, which ultimately help in increasing the resistance against bacterial blight disease of cotton.

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