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DISPARITY IN MINERAL CONTENTS OF RICE GENOTYPES INFECTED WITH BROWN LEAF SPOT

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Brown leaf spot (BLS), *Bipolaris oryzae* is one of the emerging threats to the rice crop. Its infection caused deficiency of minerals contents in rice crop. Therefore, current study was designed to determine the variations of ionic contents in rice crop after the attack of BLS disease. The leaf samples of resistant rice varieties (Kissan Basmati, Chenab Basmati, IRRI-6) and susceptible varieties (Basmati-2000, Basmati-515, KSK-133) were analyzed, and the significant alterations ($p \leq 0.05$) in the ionic contents were noted across treatments, types (un-inoculated and inoculated), groups (susceptible and resistant) and varieties of rice due to the infection of the pathogen. Nested structure analysis of variance revealed a significant difference in ionic status (N, P, K, Na, Ca, Cu, Fe, and Zn) of rice leaves. Resistant type of plants expressed 1.28% and 0.54% while susceptible type showed 1.22% and 0.43% difference in concentrations of N and K respectively. Moreover, resistant type expressed 7379.23, 2229.47, 5.02, 222.68, 28.94 and 662.29 ppm; while, susceptible type showed 7025.38, 1895.02, 4.15, 177.95, 22.17 and 520.71 ppm difference in concentrations of P, Na, Cu, Fe, Zn and Ca, respectively. It was accomplished that resistant variety stored ionic contents at higher concentrations than susceptible. The higher ionic contents concentration in resistant rice plants build up the physiological and biochemical processes of the rice plant, which help to avoid the spread of the pathogen.

Keywords: Brown leaf spot, *Bipolaris oryzae,* nested structure, ionic variations, rice.

INTRODUCTION

Rice (*Oryzae sativa* L.) is one of the most important cereal crops in the world which belongs to *Poaceae* family (Wang *et al*., 2014). Globally, it is cultivated in 112 countries (Khakwani *et al*., 2006). It is an export item having bigger share in economy of Pakistan (Salim *et al*., 2003). Its contributions are 3.2% in value added in agriculture and 0.7% in GDP of Pakistan. Pakistan occupied 11th position in terms of production and 4th in export of rice in the world (Shahzadi *et al*., 2018). About 3.5 billion people consume rice to obtain their daily calories more than 20% (IRRI Rice facts, 2012) because it has low fat and high contents of carbohydrates (Qudsia *et al*., 2017).Rice yield in Pakistan is low as compared to other countries because of biotic and abiotic factors, which causes losses about $ 5 billion per year (Asghar *et al*., 2007). Among biotic factors, fungus, nematodes, bacteria and viruses are reported to cause different diseases. Damage due to diseases depends upon the virulence of pathogens, the susceptibility of the host, plant growth stage, varietal resistance and environmental conditions (John and Fielding, 2014). Diseases cause alterations in normal physiological activities of the host plants and reduce the quality and quantity of the seed (Mueller*et al*., 2010). About 74 diseases on rice are reported in the world (Wubneh and Bayu, 2016),while 15 diseases are observed in Pakistan (Mustafa *et al*., 2013). Among fungal diseases, brown leaf spot (BLS) caused by *Bipolaris oryzae* (Quintana *et al*., 2017) is one of the potential threats to the successful production of rice (Aryal *et al*., 2016) and caused 16-43%yield reduction in Pakistan (Jatoi *et al*., 2016). When this disease appeared in epidemic form in Bengal in 1942, it caused 50-90% yield reduction and was the cause of 2 million people death (Zadoks, 2002). *Bipolaris oryzea* can attack coleoptile, leaf sheet, leaves, glumes and spikelet's. While its symptoms appeared in the form of the light reddish-brown area with grey center bounded by reddish to brown border with a bright-yellow halo (Quintana *et al*., 2017). Later on these spots merge and produce a bigger chlorotic lesions with yellow halo which destroyed the whole leaf blade (Dallagnol *et al*., 2009).The primary infection occurs through seed while secondary infection appears through wind. Other inoculums sources are weeds, soil and infested debris. Conidial germination occurred at 25-30°C and hyphal growth at 27-30°Coptimum temperature along high relative humidity over 90%. The disease wide spread occurred by continuous rain and cloudy weather with 25-30°C temperature along with leaf wetness of 8-24 (hours) (Percich *et al*., 1997).The maximum disease incidence is observed in the month of October ranging as 1.12-14.37% during 2014-2017. The relative humidity has a strong relationship with disease incidence (Choudhry *et al*., 2019).

Nutrients are vital for strengthening of plant cell walls and their normal growth which helps to reduce the disease severity (Huber and Graham, 1999). An appropriate availability of nutrients triggers the resistance mechanism of the host plants against pathogens; while, their deficiency makes the plant vulnerable to disease by changing the physiology and biochemistry of the plants. These changes are i.e. influence on growth pattern, chemical composition, decrease resistance and antioxidant defense capacity of plant (Hajiboland, 2012). Type of disease, amount of mineral elements, the form of elements and climatic 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 by disruption of metabolism process/ physiology of the plants by favoring plant pathogens or disturbing plant growth mechanism (Sahi *et al*., 2010).The plants which are scarce nutrients are more prone to disease as compared to nutrient deficient. The pathogen damage is compensating by a specific nutrient that reduces the disease through tolerance. The disease BLS incidence and severity influenced by different mineral nutrients i.e. Nitrogen, phosphorus, manganese, iron, and calcium (Ou, 1972).

Figure 1. Symptoms of brown leaf spot of rice.

The scarcity and surplus nitrogen enhance the level of BLS disease while in the form of ammonical, it decreases the disease severity if use it in the form of nitrate (Chattopadhyay and Dickson, 1960). Phosphorus(P) concentration threshold lies between 0.135 and 0.149% that limits brown spot disease development (Phelps and Shand, 1995). The quantity of P content high in soil correlated to decreased BLS incidence (Singh *et al*., 2005). Higher K and N levels lowered brown spot severity (Carvalho *et al*., 2010). Potassium in combination with zinc and iron were reported to bring about an increase in phenolic content which increased the incubation period and thus decreased sheath blight in rice (Prasad *et al*., 2010). Silicon (Si) also has a correlation with disease reduction in rice (Datnoff *et al*., 1997). Zinc (Zn) deficiency leads to the less vigorous growth of plants that are more susceptible to brown spot infection (Minnatullah and Jha, 2002).

Balance amount of nutrient develop optimal growth of plant as well as optimal for resistance. Although, resistance is controlled by genetically but it is considerably affected by environmental factors like mineral nutrition which can be easily controlled in agriculture system. In order to harmonize disease control methods, it is useful to recognize how mineral nutrients affect disease resistance in plants. Mineral nutrition can influence two prime resistance mechanisms: (a) the configuration of mechanical barriers, by development of thicker cell walls; (b) The synthesis of natural defense compounds i.e. flavanoids, phytoalexins and antioxidants which provide protection against pathogens (Schumann *et al*., 2017).

Different management strategies i.e. resistant varieties, fungicides, cultural and biological methods (Ribot *et al*., 2008) phyto extracts (Tripathi and Dubey, 2004) are utilized by researches and farmers against BLS disease of rice. Among these strategies some are practicable for farmers but are not eco-friendly while some are not economical for them. Like, fungicides is one of the effective ways to control the disease but their injudicious use cause environmental pollution (Dutta, 2015) produce resistance in pathogens (Gnanamangai and Ponmurugan, 2012). Among these tactics, strengthening of the host plant is the best one. That is why a contemporary study was designed to examine the alterations in N, P, K, Ca, Na, Cu, Fe and Zn in inoculated and un-inoculated rice plants from resistant as well as susceptible varieties. These alterations can be used as biochemical markers for identification of resistance source in rice germplasm and in development of environmentally safe approach towards BLS disease of rice and may help to strengthening the nutrition of the rice plants for better yield production.

MATERIALS AND METHODS

***Plant material*:** Three resistant (Kissan Basmati, Chenab Basmati, IRRI-6) and three susceptible varieties (Basmati-2000, Basmati-515, KSK-133) were selected on the basis of two-years (2018-19) BLS disease screening trials conducted in field.

***Establishment of a research trial under greenhouse conditions*:** Selected rice varieties were grown to establish a nursery in the greenhouse conditions with three replications in the field area (Plant Pathology Department, University of Agriculture, Faisalabad). Then these plants were transferred in the earthen pots($45×30\ cm^2$)filled with formalin sterilized loamy soil (2kg/pot) and divided into two groups as inoculated and un-inoculated group. After30 days of transplantation rice plants were inoculated with fungal spore culture @$1×10^5$ spores/ml of distill H_2O (by using Haemocytometer AP-131) by using a hand sprayer (Trigger Sprayer-SX-266) in the morning (Gupta *et al*., 2013).Humidity was maintained by spraying water daily. After 10-12 days of inoculation, the leaf samples of each variety (from inoculated and un-inoculated groups)were collected at three intervals (after 40, 60 and 80 days of sowing) (seedling, tillering and panicle stages) to observe alterations in mineral contents of leaves and their average were taken for analysis.

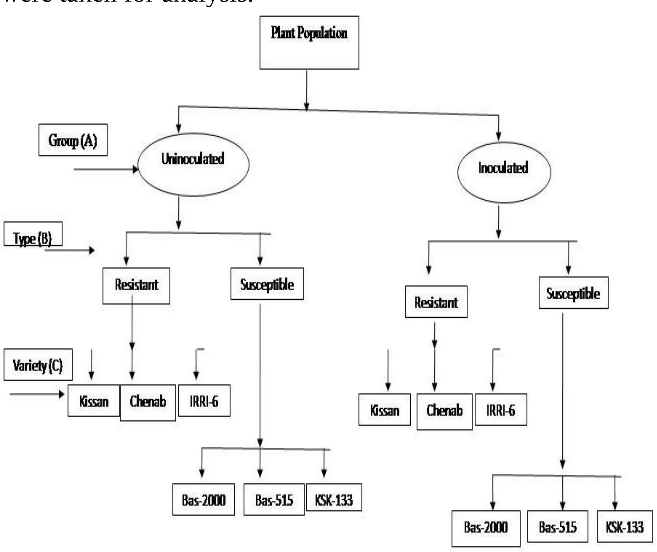

Figure 2. Layout of rice plant population classified under Nested structure design.

***Preparation of plant samples for determination of mineral contents in leaves*:** Sample of rice plant leaves (inoculated and un-inoculated groups)were collected from greenhouse and oven-dried at 70°C for two days, ground sample (100 mL), boiled in 10 mL (1.4N) HNO_3 by using Hot plate TH-550:AdvanTec, Tokyo, Japan at temperature 100°Cfor 30 minutes. Then these samples were further diluted about 250 times along with distill water and were used to determine the amount of N, P, K Na, Ca, Cu, Fe, Zn by following the protocol of Bhargava and Raghupathi (1993).

***Estimation of phosphorus*:** Sample solution of 0.1 mL was prepared through wet digestion and poured into volumetric flask (500 mL) along with 1 m L ammonium molybdate and 8.6 mL distilled water and stirred the whole solution thoroughly by adding 0.4 mL aminonaphthol sulphonic acid. Distilled water used as blank and absorbance was measured at 720 nm through spectrophotometer (BEL©, Model L.24) (Boltz and Mellon, 1948; Fiske and Subbarow, 1925).

***Determination of potassium and sodium*:** Amount of Potassium (K) and Sodium (Na) was determined by comparing with the standards of KCl and NaCl through flame photometer by using Helrich (1990) protocol. Before the use of standards, fresh working standards were prepared to obtain the K and Na standard curves through 10, 20, 30 and 40 ppm conc. for both.

***Estimation of calcium, copper, iron and zinc concentration*:** Concentrations of Ca, Cu, Fe and Zn were determined through AAS (Atomic Absorption Spectrophotometer) (Hitachi Polarized Zeeman AAS, Z-8200, Japan) by using the standards of Calcium chloride($CaCl_2$), Copper sulphate ($CuSO_4$), Iron sulphate ($FeSO_4$), Zinc oxide (ZnO and while standards curves determine through concentration of calcium (Ca) (10, 20, 40, 80, 100 ppm), copper (Cu) 0.1, 0.2, 0.5, 2 ppm, zinc (Zn) 0.2, 0.3, 0.5,2 ppm, iron (Fe) 1,2,3 ppm by obtaining their working standards immediately before use. Atomic absorption spectrophotometer (GA 3202 HGA) was used for the analysis of these minerals both from resistant and healthy rice varieties.

***Estimation of Nitrogen*:** Nitrogen contents from leaves of inoculated and un-inoculated groups were determined by using micro Kjeldahl method (Kjeldahl, 1883). Briefly, the samples were oven-dried (WI) and put intoa known amount of leaf sample into Kjeldahl flask. Then 5gm digestion mixture having K_2SO_4, $CuSO_4$ and conc. H_2SO_4 (25mL) wasadded. After that sample was placed in digestion hood and boiled ituntil become colorless. Then contents were cooled, filled in flask (volumetric), diluted with distilled water (up to 250mL). The 10 mL solution was placed in a distillation apparatus of micro Kjeldahl for distillation along with 10 M NaOH (40%) solution. Ammonia was formed and collected in a beaker having 10mL (2%) Boric acid and methyl red (two drops) use as an indicator. Then titrate it with 0.1N sulphuric acid as a standard until light pink color is obtained. Nitrogen (%) was estimated by using the formula:

$$\text{Nitrogen } \% = \frac{\text{mL } 0.1\text{ N } (H_2SO_4) \text{x } 0.0014 \text{x } 250 \text{ x } 100}{\text{WI x}100}$$

***Statistical analysis*:** Nested structured design was used as a standard analytic method for the determination alterations in the mineral contents (Gomez and Gomez, 1984). Data was statistically analyzed, and treatments means were compared using t-test by using Statistix 8.1 software.

RESULTS

Disease incidence observed as 8.70, 9.44 and 8.52% on three rice resistant varieties (Kissan Basmati, Chenab Basmati, IRRI-6); while, 22.62, 25.75 and 36.54% on three susceptible varieties (Basmati-2000, Basmati-515, KSK-133).

***Determination of Nitrogen, Phosphorus, Potassium and Sodium*:** A significant difference between both type inoculated (average 1.14%) and un-inoculated plants (average 1.37%) was observed for N, P, K, and Na. The 1.28% value was observed in the resistant group and (1.22%) in susceptible group, significant at $p \leq 0.05$. The components termed as group displayed total variance of 6.63%. Rice varieties displayed 1.9% of the total variability in nitrogen (N) contents elucidation (Table 1). The "IRRI-6" exhibited maximum concentration (1.30%) in the resistant group, while "KSK-133" displayed minimum (1.19%) in the susceptible group. About phosphorus contents, considerable variation was observed among un-inoculated and inoculated plants (averaging 7954.79 and 6449.82ppm, respectively) during disease stress with 93.28% of the total variance at $p \leq 0.05$. The total variance (4.64%) was observed in group with phosphorus concentration in resistant (7379.23 ppm) and susceptible (7025.38 ppm) varieties. IRRI-6 and Basmati-2000 showed maximum and minimum phosphorus concentration to the range of 7481.38 and 6836.77ppm, respectively (Table 1&2). Type and group exhibited 92.48% and 6.5% of the total variability of potassium contents (Table 1). Considerable variation was observed by susceptible and resistant varieties average 0.43 and 0.54% respectively in potassium. Significant variation (0.26 and 0.71%) was observed in inoculated and un-inoculated type, respectively. IRRI-6 showed maximum potassium fractions (0.57%) while minimum concentration was accumulated by KSK-133 (0.41) (Table 2). Significant variation was exhibited in sodium contents in type (averaging 1400.68 ppm in inoculated plants and 2487.02 ppm in un-inoculated plants) and total variance was accounted 98.89% (Table 1). In the same way, group displayed low variability of0.65% averaging 2229.47 ppm and 1895.02 ppm across the resistant and susceptible varieties, respectively. Varieties displayed significant variation as 0.45% of the total variance (Table 1). Sodium concentration was expressed 2075.85,

Table 1. Nested ANOVA for mineral contents (nitrogen, phosphorous, potassium, and sodium) of inoculated and un-inoculated leaves of rice plants

SOV	DF	SS	F value	Pr>F	MS	Variance Component	Total variance component (%)
Nitrogen (%)							
Type(A)	1	1.5265	26.052	0.036	0.3	0.027	91.12
Group(B)	2	0.1172	11.235	0.005	1.81	0.002	6.63
Variety(C)	8	0.0417	49.304	0.00	0.74	0.001	1.9
Error	96	0.0102	-	-	0.0001	0.00	0.35
Total	107	1.6956	-	-	0.34	0.03	100
Phosphorous (ppm)							
Type(A)	1	61141200	36.14	0.027	61141200	1100915.3	93.28
Group(B)	2	3383600	7.882	0.013	1691800	54709.1	4.64
Variety(C)	8	1717230	258.744	0.00	214654.22	23758.291	2.01
Error	96	79641.676	-	-	829.6008	829.601	0.07
Total	107	66321700	-	-		1180212.3	100
Potassium (%)							
Type(A)	1	5.4271	28.151	0.034	5.4271	0.097	92.48
Group(B)	2	0.3856	21.585	0.001	0.1928	0.007	6.5
Variety (C)	8	0.0715	98.178	0.00	0.0089	0.001	0.94
Error	96	0.0087	-	-	0.0001	0.00	0.09
Total	107	5.8928	-	-		0.105	100
Sodium(ppm)							
Type(A)	1	31861100	247.086	0.004	31861100	587631.91	98.89
Group(B)	2	257894.59	5.306	0.034	128947.3	3875.773	0.65
Variety(C)	8	194411.38	46087.25	0.00	24301.423	2700.1	0.45
Error	96	50.62	-	-	0.5273	0.527	0.00
Total	107	32313400	-	-		594208.31	100

1941.46 and 2671.1 ppm by Kissan Basmati, Chenab Basmati and IRRI-6 (resistant group); while 1899.43, 1889.70 and 1895.94 ppm concentration was displayed by varieties namely Basmati-2000, Basmati-515 and KSK-133 (susceptible group) respectively (Table 2).

***Determination of copper, iron, zinc and calcium*:** Copper concentration was observed in variation, both in the un-inoculated plant (3.19 ppm) and in inoculated plants (5.99 ppm) in disease pressure situation (Table 4) with 88.96% of the total variability (Table 3). Susceptible and resistant plants showed significant variation averaging 4.15 and 5.02 ppm, respectively.

Table 2. Amount of nitrogen, phosphorous, potassium and sodium in reaction types (un-inoculated and inoculated), groups (susceptible and resistant) and in varieties of rice plants.

Varieties (C)	Kissan Basmati		Chenab Basmati		IRRI-6		Basmati-2000		Basmati-515		KSK-133	
Group (B)				**Resistant**						**Susceptible**		
Type (A)	**I**	**UI**	**I**	**UI**	**I**	**UI**	**I**	**UI**	**I**	**UI**	**I**	**UI**
					Nitrogen (%)							
Amount of N in (C)	1.14	1.41	1.16	1.4	1.17	1.43	1.14	1.34	1.11	1.36	1.09	1.29
Av. amount of N in (C)	1.276		1.28		1.3		1.24		1.23		1.19	
Av. amount of N in (B)			R=1.28						S=1.22			
Av. amount of N in (A)			I=1.14						U=1.37			
					Phosphorous (ppm)							
Amount of P in (C)	6642.6	8174	6491	8005	6751.1	8212	6101.6	7572	6305	7785	6408.21	7981
Av. amount of P in (C)	7408.49		7247.82		7481.38		6836.77		7044.77		7194.6	
Av. amount of P in (B)			R= 7379.23						S=7025.38			
Av. amount of P in (A)			I= 6449.82						UI=7954.79			
					Potassium (%)							
Amount of K in (C)	0.33	0.75	0.3	0.73	0.38	0.77	0.23	0.7	0.18	0.67	0.16	0.66
Av. amount of K in (C)	0.54		0.51		0.57		0.47		0.43		0.41	
Av. amount of K in (B)			R=0.54						S=0.43			
Av. amount of K in (A)			I=0.26						UI=0.71			
					Sodium(ppm)							
Amount of Na in (C)	1530.4	2621	1402	2481	1420.4	2501	1360.8	2438	1351	2428.9	1340.55	2451
Av. amount of Na in (C)	2075.85		1941.46		2671.1		1899.43		1889.71		1895.94	
Av. amount of Na in (B)			R=2229.47						S=1895.02			
Av. amount of Na in (A)			I=1400.68						UI= 2487.02			

UI=Un-inoculated I= Inoculated S=Susceptible, R=Resistant

Table 3. Nested ANOVA for ionic contents (copper, iron, zinc and calcium).

SOV	DF	SS	F value	Pr>F	MS	Variance Component	Total variance component (%)
				Cu(ppm)			
Type(A)	1	210.4777	20.362	0.046	210.4777	3.706	88.96
Group(B)	2	20.6739	9.957	0.007	10.337	0.344	8.27
Variety(C)	8	8.3054	10478.75	0.00	1.0382	0.115	2.77
Error	96	0.0095	-	-	0.0001	0.00	0.00
Total	107	239.4665	-	-		4.166	100
				Fe(ppm)			
Type(A)	1	626531.77	21.974	0.043	626531.77	11074.441	88.59
Group(B)	2	57023.916	5.707	0.029	28511.958	870.965	6.97
Variety(C)	8	39967.213	9519.361	0.00	4995.9016	555.042	4.44
Error	96	50.3822	-	-	0.5248	0.525	0.00
Total	107	723573.28	-	-		12500.973	100
				Zn(ppm)			
Type(A)	1	16668.635	24.537	0.038	16668.635	296.098	91.08
Group(B)	2	1358.6677	13.822	0.003	679.3338	23.34	7.18
Variety(C)	8	393.2015	229.378	0.00	49.1502	5.437	1.67
Error	96	20.5705	-	-	0.2143	0.214	0.07
Total	107	18441.075	-	-		325.09	100
				Ca(ppm)			

Type(A)	1	1.05E+07	36.341	0.026	1.05E+07	188240	92.84
Group(B)	2	575253.83	8.65	0.01	287626.91	9421.374	4.65
Variety(C)	8	265998.52	21.16	0.00	33249.815	3519.831	1.74
Error	96	150847.99	-	-	1571.3332	1571.333	0.78
Total	107	1.14E+07	-	-		202752.53	100

Table 4. Quantity of copper, iron, zinc and calcium in reaction types (un-inoculated and inoculated), group (resistant & susceptible) in leaves of rice plants.

Varieties (C)	Kissan Basmati		Chenab Basmati		IRRI-6		Basmati-2000		Basmati-515		KSK-133	
Group (B)			**Resistant**						**Susceptible**			
Type (A)	**I**	**UI**	**I**	**UI**	**I**	**UI**	**I**	**UI**	**I**	**UI**	**I**	**UI**
					Copper(ppm)							
Amount of Cu in (C)	3.64	6.43	3.24	6.02	4.01	6.84	2.5	5.33	2.75	5.47	3.04	5.85
Av. amount of Cu in (C)	5.035		4.63		5.42		3.91		4.11		4.44	
Av. amount of Cu in (B)			R= 5.02						S=4.15			
Av. amount of Cu in (A)			I=3.19						UI= 5.99			
					Fe(ppm)							
Amount of Fe in (C)	146.72	309.06	140.73	301.5	136.22	302	130.7	294	80.35	220.7	110.3	232.26
Av. amount of Fe in (C)	227.89		221.09		219.06		212.11		150.51		171.25	
Av. amount of Fe in (B)			R=222.68						S=177.95			
Av. amount of Fe in (A)			I=124.15						UI=276.48			
					Zn(ppm)							
Amount of Zn in (C)	15.15	42.49	14.6	40.41	16.66	44.4	8.16	30.8	10.57	33.01	13.7	36.78
Av. amount of Zn in (C)	28.82		27.5		30.52		19.5		21.79		25.24	
Av. amount of Zn in (B)			R=28.94						S=22.17			
Av. Amount of Zn in (A)			I=13.14						UI=37.98			
					Ca(ppm)							
Amount of Ca in (C)	340.46	987.4	249.45	915.8	410.42	1070	225.4	865	216.2	774.9	240.5	802.17
Av. amount of Ca in (C)	663.93		582.64		740.31		545.29		495.53		521.31	
Av. amount of Ca in (B)			R= 662.29						S=520.71			
Av. Amount of Ca in (A)			I=280.40						U=902.60			

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

Varieties exhibited significant results, with 2.77% of the total variance. IRRI-6 (6.84 ppm) and Basmati-2000 (2.5ppm) displayed maximum and minimum concentration, respectively (Table 4). Un-inoculated and inoculated plants leave expressed significant variation of iron with 88.59% of the total variance (Table 3). Correspondingly, a considerable variation was observed in the leaves of resistant (222.68 ppm) and susceptible plants (177.95 ppm). Kissan Basmati and Basmati-515 retained 227.89 ppm (maximum) and 150.51 ppm (minimum) concentrations (Table 4) which accounted for 4.44% of the whole variability (Table 3). Zinc contents were exhibited significant variation in type (averaging 13.14 ppm in inoculated plants and 37.98 ppm in un-inoculated plants) (Table 3 and 4). While considerable variation (7.18% of total variance) was expressed by resistant and susceptible varieties averaging 28.94 ppm and 22.17 ppm, respectively. Varieties exhibited significant variation with 1.67% of the total variance. IRRI-6 and Basmati-2000 showed 30.52ppm and 19.5 ppm as maximum and minimum concentration, respectively (Table 3& 4). Type, group and varieties expressed 92.84, 4.65 and 1.74% of the total variability in calcium contents (Table 3). A significant difference was depicted in inoculated (280.40 ppm) and un-inoculated (902.60 ppm) type of plants. Minimum concentration of Ca was observed in case of susceptible group (520.71 ppm) as compared to resistant one (662.29) while in case of varieties maximum amount of Ca was exhibited by Kissan Basmati (740.31 ppm) while minimum by Basmati-515 (490.53 ppm), respectively.

DISCUSSION

In the present study, resistant type of plants expressed higher concentration while susceptible type showed minimum concentrations of minerals (phosphorus, sodium, calcium, nitrogen, potassium, zinc, copper and iron). Reduction in host mineral contents quantity was due to the exploitation of these nutrients by the fungal pathogen for its development and survival. Inoculated group of rice plants expressed less amount of nitrogen in susceptible types; while, un-inoculated and resistant plants contained more nitrogen contents. Similar results were reported by Jadon and Shah (2012) who reported that resistant varieties had higher quantity of nitrogen as compared to susceptible once upon pathogen infection and the combination of nitrogen and potassium in high rate contributed to decreased the incidence of BLS (Carvalho *et al*., 2010). Phosphorous is an essential element for the synthesis of nucleic acid and ATP (Schumann *et al*., 2017). It also has variable role in disease resistance by promoting the root growth and decreasing the disease incidence. Similarly, in present study, the phosphorous quantity was decreasing in inoculated type due to Infection of pathogen that promotes the disease. Like, Dorads (2008) reported about its low level that promotes the disease and balance level decreases the disease incidence. Analysis showed that higher content of phosphorus helped to reduce the BLS disease incidence (Singh *et al*., 2005). Potassium (K) is essential for the synthesis of proteins, starch, and cellulose in plants. Cellulose is a primary component of cell walls and its deficiency causes leakage of cell walls which enhances the chances of pathogen attack (Schumann *et al*., 2017). In contemporary study, reduction in K concentration was observed in inoculated plants which decreases the dry matter in the plant which support the pathogen to enhance the level of pathogen infection. Outcomes of the current study are supported by the work of (Ou, 1985) who reported decrease in dry matter contents due to imbalance amount of N and K as a result of *B. oryzae* attack (Ou, 1985). Current study results are also supported by the findings of Pandey (2018) who observed higher concentration of K in healthy leaves of rice as compared to those leaves which are affected by brown leaf spot. Similar results were reported by Pandey (2018) in case of sodium as were observed in the present study.

Copper has a key role in protein and carbohydrate metabolism (Imran and Gurmani, 2011).Its deficiency reduces production of defense compounds, accumulation of soluble carbohydrates, and lignification process. These factors increase the chances of disease by reducing the resistance of plants (Schumann *et al*., 2017). In present study, reduction in Cu contents was recorded in BLS affected leaves as compared to un-inoculated healthy leaves. These results are in similar with the work of (Liew *et al*., 2012) who reported increase in disease severity of BLS with the reduction in Cu contents and the foliar treatment had been found to effectively reduce disease incidence by 5% as it is an integral part of plant resistance towards plant diseases (Schumann *et al*., 2017).Iron (Fe)have a vibrant role in nucleic acid metabolism and is a compulsory part of chlorophyll and its low level can affect the chlorophyll contents of plants (Imran and Gurmani, 2011).When a pathogen attacks, it reduces level of iron as observed in present investigation. Decreased level of the Fe enhances the aggressiveness of a pathogen to cause maximum damage to the host plant. Results of the present study are in line with the findings of Dordas (2008) and Pandey (2018) who reported reduced quantity of Fe in the infected susceptible plants as compared to healthy resistant ones. Zn have a significant role as it is a vital part of metabolic, biochemical activities and in up taking of water. In study under consideration, reduction in Zn amount was observed due to BLS attack, which increases disease severity due to increase in reducing sugars and amino acids in rice plants as described by Dordas (2008). Reduction in Zn concentration is the cause of disintegration of plasma membrane and uplifts the process of pathogen infection as recorded by Mengel and Kirby (2001). Balanced amount of Ca is necessary for plants as it maintains resistance of plants towards pathogen infection and makes the plant to withstand disease severity. Deficiency of Ca boosts up the level of sugars and amino acids in both leaf and stem tissues which enable most of fungi to invade on the leaf surface by releasing enzymes, which dissolve the middle lamella. The activity of these enzymes is strongly inhibited by Ca, which further explains the close relationship between the Ca content of tissues and their resistance to fungal diseases (Schumann *et al*., 2017). Significant variability was observed in Ca contents of resistant cultivars of both groups (inoculated and un-inoculated) upon Infection with *B. oryzae* in present study. Findings of the current study are supported by the observations of Dordas (2008) who reported increase in disease intensity with the deficiency of calcium contents. Outcomes of the current study are also supported by the work of Pandey (2018) who declared higher amount of Ca in resistant plants as compared to infected susceptible rice plants which helps in disease progression

***Conclusion*:** Reduction in host mineral contents (phosphorus, sodium, calcium, nitrogen, potassium, zinc, copper and iron) was due to the exploitation of these nutrients by the fungal pathogen for its development and survival. Suitable application of these nutrients helps in strengthening of physiological and biochemical processes of the rice plants which ultimately help in increasing the resistance towards BLS of Rice.

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