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PHYSIOLOGICAL AND BIOCHEMICAL CHANGES ASSOCIATED WITH THE DEVELOPMENT OF BROWN SPOT DISEASES IN RICE LEAVES

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ABSTRACT: ***The healthy and diseased leaves of twelve rice samples collected from six local and six improved cultivars, were assessed for physiological and biochemical changes during brown spot infection. The photosynthetic related parameters like chl a, chl b and carotenoids were studied using spectrophotometry and changes in ionic composition like sodium, potassium, magnesium, calcium and iron were evaluated using flame photometry. The result reveals reduction in calcium, potassium, iron, chl.'a', chl.'b' and total chlorophyll and increase in magnesium, sodium, total soluble sugar and carotenoids contents after brown spot incidence. The study concludes that understanding of host-plant interaction for developing a relevant defense mechanism must be given priority and use of resistance cultivars by local growers is highly recommended.***
Keywords: brown spot, physiological changes, biochemical changes.

INTRODUCTION:

Brown spot of rice, caused by *Bipolaris oryzae*, has increased recently under the impact of global warming due to relatively high temperature (around 30 °C) (Mizobuchi *et al.*, 2016). The yield losses due to the disease in India vary from 4 to 52 % (Savary et al., 2000a). The disease is a seed borne and shows maximum infection during crop harvest and seed storage condition (Pandey, 2015). In Central India in Kymore plateau region the disease occurrences is common mainly due to conducive weather conditions and dominance of local land races (Pandey *et al.*, 2008). The disease becomes severe under heavy rainfall, soils with low pH and potassium along with scarcity of essential and trace elements (Chakrabarti, 2001). The optimal temperatures in the range of 25–30 °C favours conidial and 27–30 °C favors hyphal germination of the pathogen (Barnwal *et al.*, 2013). The management of disease using botanicals (Pandey *et al.*, 2008), plant extract (Pandey, 2015), or fungicides (Pandey, 2015) is even not preventing the disease prevalence and every year shows impaired crop yield. During initial stage the infection causes decline in phenolic contents and even in more advanced stage peroxidase and phenylalanine-

ammonia lyase activities also decreases, releasing toxin in the tissues and suppressing plant defence system (Vidhyasekaran *et al*, 1992).

The information and understanding of physiological and biochemical changes during the infection process of a pathogen helps to predict the effects of the disease on growth and yield of the crop (Bastiaans, 1993). The disease infection mainly affects carbon assimilation (Resende *et al.*, 2012; Debona *et al.*, 2014), transpiration and changes in ionic composition. Brown spot inoculated leaves shows changes in leaf physiology damaging the cells, upto membrane level and causes increased malondialdehyde acid (MDA) concentration and high electrolyte leakage (EL) and reduction in transpiration, photosynthesis, stomatal conductance, mesophylly potentiality and light harvesting capacity (Dallagnol *et al.*, 2011). The proper understanding of the physiological changes mainly photosynthesis mechanism and ionic composition during disease infection helps to provide crucial information in combating the disease. Therefore this investigation was designed to estimate possible changes in physiological and biochemical changes during brown spot infection in healthy and diseased leaves in local and improved rice cultivars.

MATERIALS AND METHODS

The experimental trials were conducted at Rewa (24'18 and 25'12 north latitudes and 81'2 and 82'18 east longitudes), Madhya Pradesh. Twelve rice cultivars were used in this investigation, out of these six were local viz Indrajal, Gurmatia, Dehula, Aajaan, Lochai and Newari, and six were improved cultivars viz. Vandana, Basmati, Govinda, Jaya, Kalinga, and IR-64. The experiment was laid down in random block design with three replicates. The leaf samples of healthy and diseased leaves of the cultivars were collected after 45 days of sowing.

Physiological studies:

The leaf samples of healthy and diseased leaves of the cultivars were subjected for further study. The cell sap was prepared in distilled water using 5 ml/gm of leaf sample for physiological studies.

Determination of Chlorophyll and Carotenoids:

For determination of chlorophyll content 500 mg of fresh leaf samples were finely chopped and kept in 50 ml conical flask containing 25 ml of 80% acetone. These flasks were cork-tighted and kept in dark for 24 hours. After 24 hrs this mixture was then centrifuges at 3000 rpm for 15 minutes. The volume of the supernatant after decantation was made up to 40ml with 80% acetone. The spectrophotometer was used to measure optical densities of chlorophyll extracts at 480, 510, 645 and 663 mm wave length.

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The amount of chlorophyll 'a' 'b' and carotenoids in term of mg pigment per gram fresh leaf were calculated using following formula:

$$Chl'a' = \frac{12.4 \times 663 - 0.86 \times 645 \times v}{d \times 1000 \times w}$$

$$Chl'b' = \frac{19.3 \times 645 - 3.6 \times 663 \times v}{d \times 1000 \times w}$$

$$Carotenoids = \frac{7.6 \times 480 - 1.49 \times 510 \times v}{d \times 1000 \times w}$$

Where,

V = volume of chlorophyll extracts in acetone.
D = length (cm) of light path
W = fresh weight (in gms) of leaves (Holden, 1965)

Determination of ionic pigments:

The leaf samples of the twelve tested cultivars were collected from the field. The collected samples of healthy and diseased leaves were dried in the oven and analyzed for Na, K, Mg, Ca and Iron by flame Photometry method (Jackson, 1967).

Determination of sugar content:

The Phenol reagent method was utilized for determination of sugar content (Dubois et al., 1951). The 0.1 gm of fresh leaf was homogenized in 80% dissolute alcohol (1 ml) and centrifuged for 15 minutes, 1 ml extract + 1 ml phenol (5%) and 5 ml conc. H_2So_4 were added and optical density of the mixture was measured using spectrophotometer at 480 mm. The concentration was quantified based on a standard curve prepared using glucose solution.

RESULTS

Chlorophyll and Carotenoids

The investigation of chlorophyll ‘a’, chlorophyll ‘b’ and carotenoids differed significantly among the tested cultivars in healthy and diseased condition (Table 1). Among the cultivars, chl 'a' content was maximum in improved and minimum in traditional cultivars under healthy conditions. The observation of the diseased leaves reveals that chl‘a’ content decreases with the disease incidence in the order improved to local cultivars.

Similar pattern was also noticed in case of chl 'b' content which also shows high content under healthy conditions but as the disease progresses the chl ‘b’ concentration decreases in all the cultivars. As regard to carotenoids all the cultivars revealed a high content under healthy condition, but with the incidence of the disease the carotenoid concentration decreases in the tested samples.

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Table 1 Photosynthetic pigments in Healthy (H) and Diseased (D) leaves after Brown spot infection in rice varieties

Variety	Chlorophyll 'a' (mg/g)		Chlorophyll 'b' (mg/g)		Carotenoids (mg/g)		Total pigments (mg/g)	
	H	D	H	D	H	D	H	D
Indrajal	2.10	1.46	0.43	0.31	1.76	1.62	4.29	3.39
Vandana	2.32	1.56	0.59	0.46	1.81	1.71	4.72	3.73
Gurmatia	2.38	1.66	0.47	0.30	1.78	1.67	4.63	3.63
Basmati	2.35	2.29	0.58	0.46	2.18	2.04	5.11	4.79
Govinda	1.91	1.84	0.59	0.47	2.12	2.02	4.62	4.33
Jaya	1.87	1.63	0.58	0.42	2.20	2.01	4.65	4.06
Kalinga	1.94	1.71	0.58	0.41	2.07	1.96	4.59	4.08
Dehula	1.69	1.21	0.50	0.34	1.92	1.80	4.11	3.35
IR-64	2.45	2.39	0.59	0.49	2.31	2.13	5.35	5.01
Ajaan	2.21	1.50	0.48	0.35	1.88	1.76	4.57	3.61
Lochai	2.44	1.55	0.57	0.46	1.82	1.79	4.83	3.8
Newari	2.44	1.77	0.56	0.44	1.86	1.83	4.86	4.04
SEm ±	0.020	0.006	0.008	0.006	0.006	0.019		
CD (5%)	0.065	0.021	0.025	0.019	0.019	0.060		

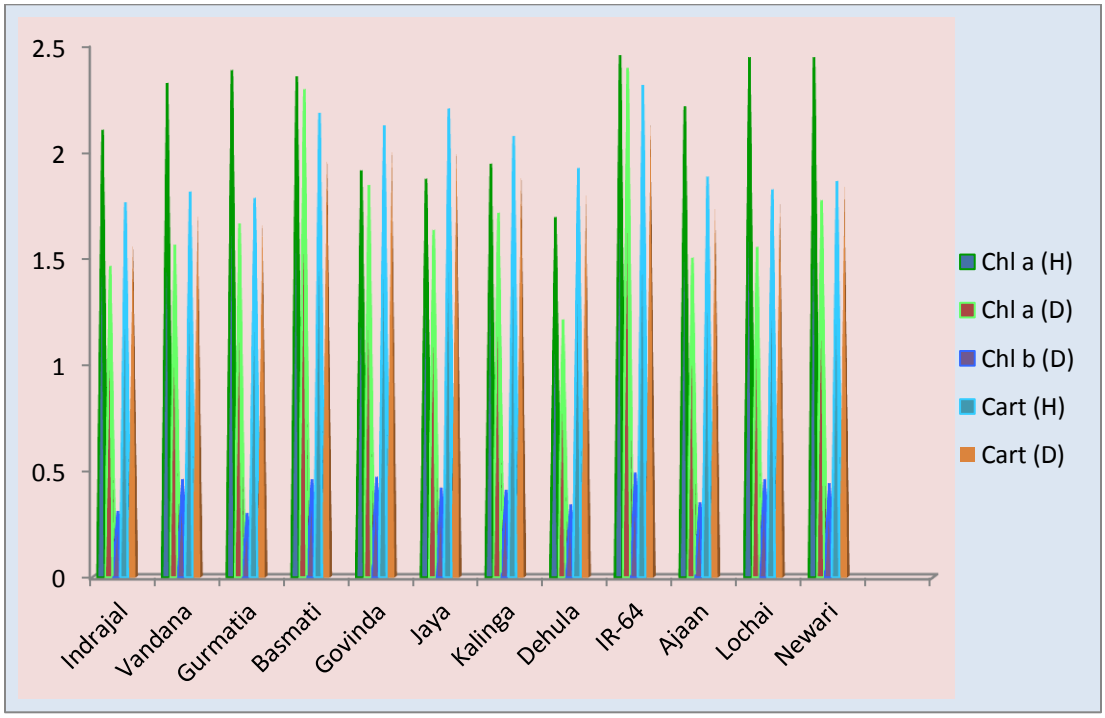

Fig. 1: Changes in photosynthetic pigment of rice leaves after brown spot infection

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Ionic pigments

The data presented in Table 2 reveals that sodium content was reported to be high in healthy condition in all the cultivars. Among the cultivars susceptible cvs. Gurmatia, Indrajal and Dehula shows high content whereas improved cvs. Govinda, Kalinga and IR-64 exhibit low sodium content. It is evident from the data that sodium content decreased significantly after disease development; however difference in the values were noted in cv. Basmati. The remaining cultivars had also exhibited decrease in sodium concentration in diseased conditions. The data on potassium content in healthy and diseased leaves shows a remarkable variation. Under healthy condition the potassium content was recorded to be high in all the cultivars starting from improved to local susceptible cultivars in descending order. After disease incidence the potassium content decreases significantly in most of the tested cultivars and was reported minimum in local and susceptible cv. Gurmatia. Similarly, the analyses of magnesium content observed a rise in all cultivars under healthy condition. As the disease progresses, the magnesium content gradually reduces in the cvs. however, the susceptible cvs. Gurmatia and Dehula exhibited higher magnesium content compared to other culivars.

The data on calcium content presented in Table 2 reveals that under healthy conditions, the calcium content was found high in improved cvs. IR-64 and Govinda whereas, traditional cvs. Gurmatia and Dehula shows least calcium content. The calcium content decreases with the disease progression and was reported to be least in local cv. Gurmatia. Similarly, the iron content of healthy and diseased leaf samples shows significant variation. It was observed that improved as well as local susceptible cultivar exhibited minimum value of iron content in healthy conditions. It was reported that the iron content reduced significantly with the disease development in all the tested cultivars. The data on total soluble sugar (TSS) reveals that in healthy condition, the TSS value decreases in the sequence local to improved cultivars. It was observed that as the disease progresses the TSS content indicated a significant decrease in all the cultivars.

Table 2 Ionic pigments in Healthy (H) and Diseased (D) leaves after Brown spot infection in rice varieties

Variety	Sodium content (mg/g)		Potassium content (mg/g)		Magnesium content (mg/g)		Calcium Content (mg/g)		Iron content (mg/g)		Total Soluble Sugar (% dry wt.)	
	H	D	H	D	H	D	H	D	H	D	H	D
Indrajal	7.52	7.39	1.76	1.62	7.52	7.39	4.78	4.73	6.86	6.83	6.80	6.13
Vandana	7.39	7.28	1.81	1.71	7.39	7.28	5.04	5.00	7.11	7.06	6.48	5.92
Gurmatia	7.67	7.58	1.78	1.67	7.67	7.58	4.72	4.66	6.93	6.80	6.80	6.41
Basmati	7.24	7.12	2.18	2.04	7.24	7.12	5.06	5.04	7.09	7.07	5.62	4.90
Govinda	7.40	7.25	2.12	2.02	7.40	7.25	5.08	5.03	7.05	7.03	5.73	5.03
Jaya	7.31	7.26	2.20	2.01	7.31	7.26	4.82	4.77	7.01	6.99	5.50	4.90

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Kalinga	7.28	7.19	2.07	1.96	7.28	7.19	4.91	4.87	7.00	6.98	6.44	4.96
Dehula	7.58	7.48	1.92	1.80	7.58	7.48	4.72	4.67	6.82	6.78	6.82	6.07
IR-64	7.14	7.06	2.31	2.13	7.14	7.06	5.10	5.06	7.15	7.08	5.70	5.10
Ajaan	7.43	7.32	1.88	1.76	7.43	7.32	4.76	4.71	6.71	6.69	6.59	6.36
Lochai	7.41	7.30	1.82	1.79	7.41	7.30	4.80	4.76	6.90	6.83	6.38	4.89
Newari	7.47	7.37	1.86	1.83	7.47	7.37	4.80	4.75	6.88	6.81	6.68	6.02
SEm ±	0.018	0.005	0.007	0.009	0.013	0.009	0.010	0.012	0.007	0.005	0.007	0.007
CD (5%)	0.058	0.018	0.024	0.029	0.043	0.029	0.032	0.039	0.024	0.018	0.025	0.024

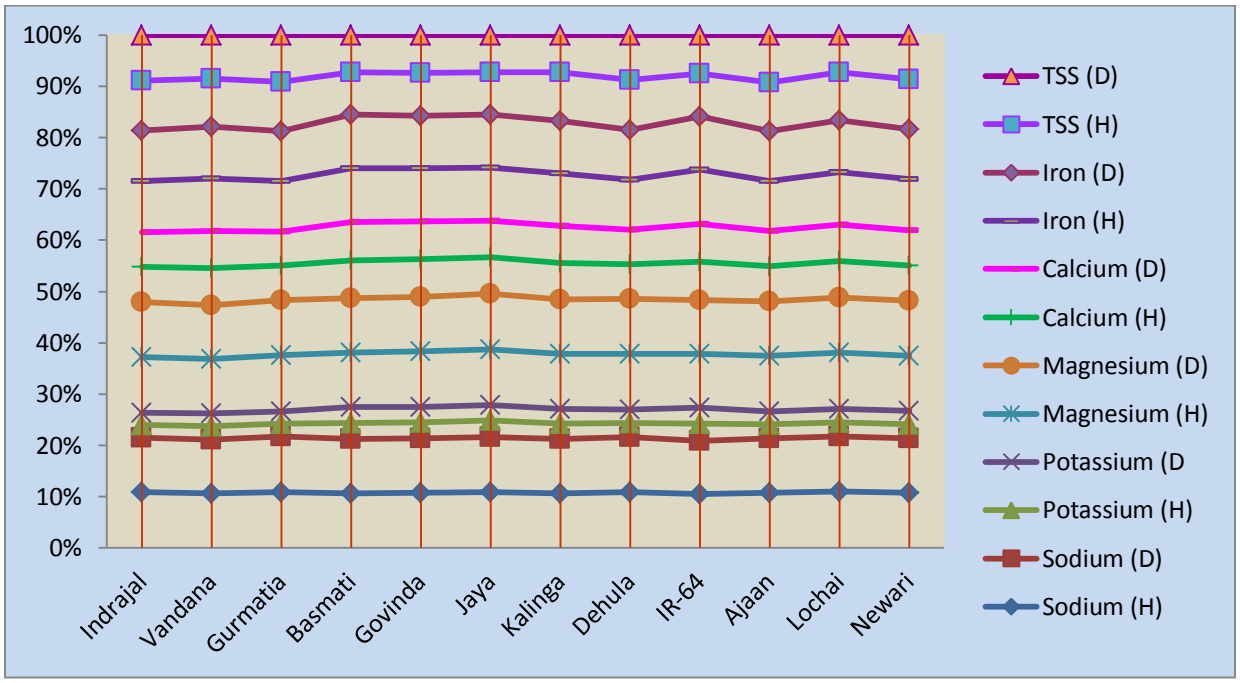

Fig. 2: Changes in ionic pigment of rice leaves after brown spot infection

DISCUSSIONS

Brown spot infection modifies plant physiology with adverse impact on some photochemical and biochemical steps of photosynthesis and reduces rate of carbon assimilation, stomatal functioning, concentration of CO_2 and transpiration along with reduction in chlorophyll *a* fluorescence, photochemical, PSII electron transport yield and apparent electron transport rate (Dallagnol *et al.*, 2015). *H. oryzae* pathogen produces ophiobolin toxin resulting in loss of electrolytes from coleoptiles, roots and causing plasmolysis in leaves (Chattopadhyay and Samaddar, 1976). Low disease incidence results increase in total phenol and soluble protein and protein banding pattern is an important factor for developing resistance against brown spot pathogen (Bisen *et al.*, 2015).

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Brown spot infection causes decrease in Chlorophyll (Chl), total carotenoids (Car), and Chl *a* + *b*/Car ratio (Dallagnol *et al.,* 2011), which was also observed during this investigation. A significant reduction was noted in biochemical contents like sodium, potassium, magnesium, calcium, iron and total soluble sugar after the incidence of diseases. It was noted that sodium, magnesium, calcium and total soluble sugar was higher in local susceptible cultivar viz. Gurmatia, Indrajal and Dehula, whereas potassium and iron content was more in improved varieties which may induced the resistance against the diseases. Higher calcium content helps in combating with the diseases in resistant compared to susceptible cultivars (Mukherjee and Ghosh, 1972). However, Kaur *et al.,* (1986) found negative correlation of calcium content with disease severity and positive correlation with Potassium and Iron. The biochemical contents are thought to restrict the RNA synthesis thus inhibiting the protein synthesis process and resulting in the increase of the susceptibility. The moderate susceptible cultivars viz. Ajaan, Lochai and Newari shows more magnesium content than improved varieties.

Glufosinate ammonium a herdicide reduces brown spot incidence in transgenic rice by causing slight chlorosis and decrease in chlorophyll content, but under malnutrition conditions this effect is nullified (Ahn, 2008). In vitro studies of rice leaves collected from the plot treated with abscisic acid (ABA) reveals that the hormonal activity does not depend on enhancing expression of jasmonic or salicylic acid, or even callose dependent resistance mechanisms but it requires a functional Ga-protein or Ga signaling and MAPK gene OsMPK5. The study also report that ABA shows positive effect on brown spot resistance by affecting ethylene (ET) response pathway. The application of ethephon increases susceptibility, whereas genetic disruption of ethylene signaling makes plant less vulnerable to pathogen attack. However, Ga-mediated signaling affects of abscisic acid induced resistance remaisn to be clearified (Vleesschauwer *et al.,* 2010). Similarly, silicon nutrition delays fungal penetration causing decrease in lipid peroxidation and electrolyte leakage and increase in total soluble phenolics and lignin (Dallagnol *et al.,* 2011), and prevents the pathogen from hijacking ethylene pathway (Bockhaven *et al.,* 2015), thus acting as a barrier in disease development. But in order to understand silicon-mediated disease resistance response, special emphasis must be given to decipher the genes and molecular pathways (Bockhaven *et al.,* 2013).

CONCLUSION

Thus it may be concluded that there was positive association of brown spot disease with the biochemical components like magnesium, sodium, total soluble sugar and carotenoids and negative correlation with calcium, potassium, iron, chl.'a', chl.'b' and total chlorophyll. This study also reveals that local and improved cultivars show a significant brown spot incidence and thus there is a need of assessing host pathogen interaction to develop a noble defense mechanism among the cultivars.

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