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EVIDENCES OF VARIABLE RESPONSE TO SPOT BLOTCH IN DIFFERENT WHEAT VARIETIES

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ABSTRACT
Comparative studies on seven wheat varieties *viz*. K 65, K 9107, K 8027, PBW 343, HUW 234 and PBW 443 against spot blotch pathogen have been revealed that K 9107 variety was comparatively resistant against spot blotch pathogen, showing minimum per cent disease index (PDI) with 33.2 % . Biochemical analysis of this wheat variety with respect to N %, crude protein, soluble protein and total phenol content revealed that the less affected varieties possessed higher level of soluble protein and total phenol content, whereas, susceptible variety had higher level of crude protein and N %. The maximum soluble protein (41.45 mg/g of fresh leaf) was recorded in the variety K 9107, which was followed by K 8027 (39.23 mg/g of fresh leaf). The maximum total phenol content 3.02 mg/gm of fresh leaf at 5 days, 2.88 mg/g of fresh leaf, at 10 days, 2.65 mg/gm of fresh leaf at 15 days, and 2.85 mg/g of fresh leaf at 20 days was found in the resistant variety K 9107 at seedling stage. The highest phenol content with 3.92mg/g of fresh leaf was found in variety K 9107 which was followed by K-65 (3.71mg/g of fresh leaf) at vegetative stage. The variable amount of crude protein, soluble protein and total phenol content resulted different types of disease response to spot blotch in wheat.

INTRODUCTION

Wheat (*Triticum aestivum* L.**)** is one of the most important crops of the world, occupying a premier position among cultivated food cereals at national and international level. Over 150 high yielding varieties of wheat have been developed during last three decades in India. But production and productivity in India are remaining stagnant for last few decades which solely have been attributed due to number of diseases. Spot blotch caused by *Dreshslera sorokiniana* [(Sacc.) Subram and Jain] is one of the major yield and quality reducing factors of wheat. The disease occurs throughout the world, but severe losses occur in Bangladesh, Bolivia, Brazil, Paraguya and Zambia. In India, spot blotch has been a serious problem in Northern region, but in recent years, it has been assumed significance in North-Western region of the country (Singh and Srivastava, 1997). The yield losses due to disease are about 29.4% in India (Singh *et al*., 1999). The pathogen affects all aerial parts of plant but the extent of damage may vary from variety to variety. High humidity along with increase in temperature favours the severe outbreak of disease (Ragiba *et al*., 2004). The economically and environmentally safe method for sustainable disease control is the use of resistant varieties. The mechanism of disease resistance is a complex phenomenon and in response to invasion by a disease causing organism, plant produces various kinds of biochemical reactions. It is well known that in several pathosystems the phenolic compounds like phytoalexins or phytoanticipins, or physical barrier, *i*.e. lignins can play an important role in disease resistance, thus preventing plant tissue colonization (Nicholson and Hammerschmidt, 1992). In general, the infection of pathogen brings about lot of changes in respiratory pathway and photosynthesis which is vital processes taking place inside the plant leading to wider fluctuations in biochemical components *viz*., phenols and sugars. Rapid accumulation of phenols slows the growth of the pathogen and allows activation of phytoalexins or other stress related substances at the infection site (Klement and Goodman, 1967). The high phenolic content in resistant genotypes may be due to more sugar as it acts as a precursor for synthesis of phenolics, phytoalexins, lignin and cellulose which plays an important role in defence mechanism of plants against invading pathogens. The resistant and susceptible reactions against pathogens are played by biochemical compounds. Therefore, analysis of different biochemical response in selected resistant and susceptible cultivars to spot blotch disease was carried out to understand their role in resistance/susceptibility of wheat genotypes. The present studies were undertaken to find out the biochemical evidence of variable response to spot blotch in different wheat varieties.

MATERIALS AND METHODS

The experiment was conducted at Agricultural Research Farm of C. S. Azad University of Technology Kanpur-208002 to find out the biochemical evidence of variable in response to spot blotch among different varieties of wheat. Seven popular wheat varieties *viz*. K 65, K 9107, K 8027, PBW 343, HUW

234, Sonalika and PBW 443 were taken to conduct this experiment. The experiment was laid out RBD with three replications. Recommended agronomical practices were followed. Biochemical analysis with respect to soluble protein and total phenol content was carried out using leaves of different wheat varieties at seedling (5, 10, 15 and 20 days old) and vegetative stage (60 days age) of plant. The crude protein, N- estimation and disease severity was recorded at 60 days age of plant.

Soluble protein

The method developed by Lowery *et al*. (1951) was used with slight modification to determine the soluble protein content in leaves. Wheat leaves from different varieties were harvested, washed with distilled water several times and dried with blotting paper before protein extraction. A quantity of 1gm of each leaf sample was cut into small pieces and grinded in pestle and mortar using 1:5 ratio of leaves and extraction buffer. The suspension was centrifuged at 12000 rpm for 30 minutes at 4ºC. The supernatant was collected and used for protein estimation after adding with sample buffer. The working standard solution was pipette out as 0.1, 0.2, 0.4, 0.8 and 1.0 ml in series of test tubes. Similarly, 0.1, 0.2, 0.4, 0.8 and 1.0 mL of sample extract was also pipette out and kept into other test tubes. Then volume in all the tubes was made up to 1mL with water. A tube with 1.0 mL of water was served as the blank. Later on, 5ml of solution C was mixed well and incubated at room temperature for 10 minutes. Thereafter, 0.5mL of Folin-Ciocalteu Reagent (FCR) was mixed well immediately and incubated at room temperature in dark for 30 minutes .The absorbance at 660 nm against the blank was measured by Ultra Violate- Visible Spectrophotometer (UV-VIS) and standard curve using different concentrations of Bovine serum albumin (BSA) was prepared. From the standard curve, the concentration of soluble protein in test sample was determined and expressed as mg soluble protein per gram of sample.

Phenol estimation

The accumulation of total phenols in seedlings of different varieties was estimated following Bray and Thorpe (1954) procedure with slight modification. One gm of leaf sample from each varieties of wheat was ground in pestle and mortar separately in 10 times volumes of 80% ethanol. It was then centrifuged to homogenate the suspension at 10,000 rpm for 20 minutes. Supernatant was separated and the residue was re-extracted with five times volumes of 80% ethanol and centrifuged. The supernatants were evaporated to dryness and residue was dissolved in 5mL of distilled water. Different aliquots (0.1mL, 0.2 mL, 0.4mL, 1.0 mL, 1.4 mL and 1.8mL) were pipette out into test tube and the volume in each tube was making up to 3mL with water. Subsequently 0.5 mL of FCR was added and after 3 minutes, 2 mL of 20% Na_2Co_3 solution in each tube was thoroughly mixed. The tubes were placed in boiling water for one minute and then cooled. The absorbance at 650 nm against a reagent blank was measured using Ultra Violet Visible (UV-VIS) spectrophotometer. The standard curve was prepared by using different concentrations of Catechol. Form the standard curve, the concentration of phenol in the test sample was determined and expressed as mg phenol per gm of sample material.

Nitrogen estimation

The nitrogen content in different varieties of wheat leaves was estimated by Micro-Kjeldahl method (A.O.A.C. 1970). This method essentially involves digestion of the sample to convert N-compound into NH_4 form. The Nitrogen content of the sample was calculated by the used of following formula:-

Nitrogen % (in 100gm) = 1.4 x N x V X 100

W

N = Normality of HCl

V = Titrate value of sample – Titrate value of blank

W = Weight of the sample

The estimated value of N was multiplied by 6.25 to obtain % of crude protein available in leaf samples.

Measurement of per cent disease index (PDI)

Disease observations were recorded at 60 days age of seedlings, showing spot blotch lesions by using five point scale (O - IV) (Chenula and Singh, 1964). Fifty leaves of wheat were randomly selected from each sample and Per cent disease index (PDI) was calculated. Leaves with no sign of infection received a score of zero while those with highest infection (*i.e.* with the 76 or above leaf blighted) received a score of IV. Similarly, leaves with 1-25, 26-50 and 51-75, area covered with spot blotch lesions received a score of I, II, III, respectively. The per cent disease index (PDI) was calculated by the following formula.

$$PDI = \frac{\sum \text{Class rating x class frequency}}{\text{Total no. of leaves x maximum class rating}} \times 100$$

Correlation co-efficient (r) of per cent disease index (PDI) with soluble protein and total phenol

Correlation coefficients (r) between soluble protein and PDI and between total phenol and PDI were calculated by standard statistical calculation. Simple regression equations (Y = a + bx) were also developed for both the variables (Protein & phenol) separately to understand their relation with per cent disease index.

RESULTS AND DISCUSSION

At seedling stage

Soluble protein content

The result enumerated in Table 1 showed that the soluble protein content in different wheat varieties is different and also varies with the ages to age of seedling in the same variety. The maximum soluble protein with 30.11mg/g of fresh leaf at 5days, 29.00 mg/g of fresh leaf at 10 days, 27.54 mg/g of fresh leaf at 15 days and 29.23mg/g of fresh leaf at 20 days age of seedling were found in the variety K-9107. It is also found that the soluble protein content was gradually decreased after 5 days to 15 days but it was further increased at 20 days age of seedling. The variety K-9107 registered 3.68% and 7.44% decreased from 5 - 10 days and 10 - 15 days age of seedling respectively, but 6.13% increased from 15 to 20 days. The minimum content of soluble protein has been found in variety

Table 1: Variation in soluble protein and total phenol content in different varieties of wheat seedling stage

Variety	Soluble protein (mg/g of fresh leaf)				Total phenol (mg/g of fresh leaf)			
	5 days	10 days	15 days	20 days	5 days	10 days	15 days	20 days
K 65	29.24	27.38	25.22	27.25	2.78	2.61	2.38	2.65
K 9107	30.11	29.0	27.54	29 23	3.02	2.88	2.65	2.85
K 8027	30.0	28.88	26.75	28.76	2.98	2.78	2.59	2.81
PBW 343	23.41	21.88	20.01	22.34	2.69	2.41	2.20	2.50
HUW 234	23.10	21.72	20.30	24.41	2.70	2.47	2.23	2.60
Sonalika	26.42	24.24	22.18	24.30	2.66	2.39	2.15	2.32
PBW 443	28.78	23.79	22.01	24.24	2.70	2.45	2.19	2.42
CD at 5 %	0.61	0.83	1.04	0.72	0.10	0.11	0.12	0.12

Table 2: Biochemical variation in mature leaves of different wheat cultivars and it response to PDI per cent.

Variety	N (%)	Total protein (%)	Total soluble protein (mg/g)	Total phenol (mg/g)	PDI (%)
K 65	2.55	15.93	35.54	3.71	58
K 9107	2.5	15.62	41.45	3.92	33.2
K 8027	2.52	15.75	39.23	3.68	48
PBW 343	2.56	16	35.42	3.53	63.5
HUW 234	2.64	16.5	32.79	3.54	73.7
Sonalika	2.68	16.75	32.54	3.23	83.5
PBW 443	2.64	16.5	35.39	3.58	68.2
CD at 5%	0.07	0.31	0.07	0.09	9.66

HUW 234 which was 23.10mg/g of fresh leaf at 5 days, 21.72 mg/g of fresh leaf at 10-days, 20.30 mg/g of fresh leaf at 15 days and 24.41mg/g of fresh leaf at 20 days age of seedlings. The observation on total soluble protein content in different wheat varieties at 20 days age of seedling revealed that the variety K-9107 and K-8027 are statistically at par whereas, HUM 234 and PBW 343 are also statistically at par up to 15 days age of seedling but at 20 days, its make difference to each other. Brady and Scott (2006) reported that the development of the second leaf of wheat, the contents per lamina of fraction 1 protein and total soluble protein increased for 6 days after leaf emergence and contents of cytoplasmic and chloroplast rRNA for 5 days. They also found that total soluble protein and chlorophyll content changed as a result of nitrogen supply and carboxylation efficiency in both winter and spring wheat. Mishra *et al*. (2011) also found that variable resistance response to Alternaria blight by different varieties of wheat at different growth stages of plant.

Total phenol content

Phenol is well known antifungal, antibacterial and antiviral compounds. They are involved in the expression of disease resistance, in many ways like lignifications of cell wall. The result presented in the table 1 showed that the total phenol content in different wheat variety was varied from each other. The maximum total phenol content 3.02 mg/gm of fresh leaf at 5 days, 2.88 mg/g of fresh leaf, at 10 days, 2.65 mg/gm of fresh leaf at 15 days, and 2.85 mg/g of fresh leaf at 20 days was found in the variety K 9107. Data from the table also revealed that the total phenol content was decreased at 5 - 15 age of seeding but it was further increased after 15 days. The variety Sonalika, showed minimum amount of phenol content as 2.66 mg/g of fresh leaf at 5 days, 2.39 mg/g of fresh leaf at 10 days, 2.15mg/g of fresh leaf at 15 days and 2.32 mg/g of fresh leaf at 20 days age of seedling. At 20 days of observation on total phenol content in different varieties of wheat revealed that varieties K9107 with K8027, HUW 234 with PBW 343 K65 and PBW 443 with sonalika were statistically at par. The varieties K 9107 and K 8027 had significant difference with rest of all these varieties. Gupta *et al*. (2004) reported that the high heritability on sedimentation value, protein content, phonolic reaction and yield per plant were found variation among different varieties of bread wheat. Shetty and Ahamad (1980) estimated that the total phenolic content in resistant and susceptible maize and sorghum plants to downy mildew and reported faster accumulation of phenol on higher quantity in disease area. Singh (2010) also reported that resistant variety of wheat content higher amount of total phenol content as compare to susceptible variety.

At vegetative stage

Nitrogen content

The result of total nitrogen in wheat leaf presented in table 3 indicated that total nitrogen content was maximum (2.68%) in leaves of sonalika, while minimum with 2.5% in variety K 9107, followed by variety K 8027. Varieties, K 65, K 9107, K 8027 and PBW 343 showed non significant difference with 2.55%, 2.50%, 2.52% and 2.56% nitrogen in wheat leaves. On the other hand, HUM 234, Sonalka and PBW 443 are statistically at par. Jalan and Sindhan (1988) observed that low content of nitrogen, manganese and iron and high content of phosphorus, potassium, zinc and copper were found in resistant variety as compared to susceptible cultivars of wheat.

Total protein content

The experimental results present in table-2 showed that the crude protein content in wheat leaves ranges from 15.62 - 16.75 per cent. The maximum with 16.75 percent protein was observed in the variety Sonalika followed by PBW 443 and HUW 234 with the value of 16.50%and 16.50%, respectively. The minimum protein percent was found in variety K 9107 (15.62%) which was statistically at par with K

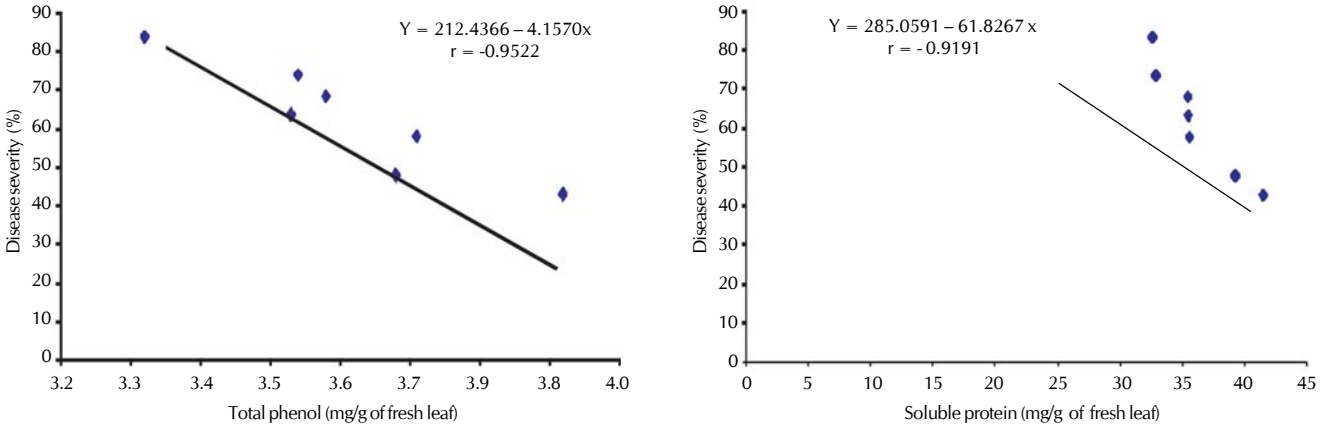

Figure 1: Correlation between Per cent disease index with total phenol and soluble protein content in different wheat varieties

8027. Lokendrajit *et al.,* (2013) found that total protein content of the Purple or red fleshed local potato cultivar (*Solanum tuberosum* Linn) was evaluated from the four districts of Manipur and found to be very high as compared to the other genotype.

Soluble protein content

Biochemical analysis of mature leaves of different wheat varieties revealed that the amount of soluble protein content varied to each others, resulted different resistant response among the varieties (Table 2). The maximum (41.45mg/g of fresh leaf) soluble protein was recorded in the variety K 9107, which is followed by variety K 8027 (39.23mg/g of fresh leave).The experimental finding also indicated that all the varieties are significantly difference to each other in respect of soluble protein content. The PBW 443 and PBW 343 varieties were statistically at per but significantly different from rest of the varieties. The higher concentration of soluble protein provided defense response in plant was reported by several workers (Biswas *et al*., 2011; Mishra *et al*. 2011). Boller (1985) opined that protein form of chitinase and â-1,3-glucanase may be involved in defense of plants against fungi and bacteria.

Total phenol content

Estimation of total phenol revealed that total phenol content in all the variety varies from 3.23 – 3.92 mg / g of fresh leaf (Table 2). The highest phenol content with 3.92mg/g of fresh leaf was found in nature leaf of variety K 9107 which was followed by K-65. The variety K-65 also showed statistically at par with K 8027. Statistical analysis of data revealed that wheat varieties PBW 443, HUW 234 and PBW 343 showed non-significant difference to each other in respect of total phenol content which was 3.58, 3.54 and 3.53 mg/g of fresh leaf. From the table, it is also cleared that the variety K 9107 had significantly difference from rest of the varieties including sonalika which represented lowest (3.23 mg/g of fresh leaf) amount of phenol in leaf. The total phenolic content of the Purple or red fleshed local potato cultivar (*Solanum tuberosum* Linn) was evaluated from the four districts of Manipur and found to be very high (Lokendrajit *et al.,* 2013). Shetty and Ahamad (1980) estimated the total phenolic content at different growth stages in leaf and root tissue of resistant and susceptible maize and sorghum plant to downy mildew and reported faster accumulation of phenol in higher quantity in disease area.

Per cent disease index (PDI)

Resistance could be assumed to be one factor for the reduction of disease severity. Data on disease severity presented in table -2 found that the sonalika variety showed maximum (83.5%) disease severity followed by HUW 234 (73.7%), indicating more susceptible variety to spot blotch disease. The minimum disease severity with 33.2% was recorded in K 9107, showing comparatively resistant among all the tested varieties. Mohanty and Manna (1972) tested 216 wheat varieties to foliar infection of the pathogen under natural conditions and found 40 verities to be resistant, 64 moderately resistant and rest were susceptible. Swati *et. al.,* (2014) studied on impact of resistance in minimizing the yield loss in bread wheat (*Triticum aestivum* L.) against karnal bunt and reported that maximum percent infection was recorded in the susceptible parent, WL 711, which resulted maximum grain yield loss, while minimum infection and yield loss was recorded in the resistant plant.

Co-relation analysis

The correlation analysis between PDI with soluble protein and total phenol content showed that decreasing PDI with increasing soluble protein and total phenol content in leaf. There was a negative co-relation (r), showing -0.9191 between PDI and soluble protein and -0.9522 between total phenol and PDI (Fig. 1). Similar observations were also found in in tomato against Fusarium wilt (Raziq, 2010; Arzoo, 2010).

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