

VARIABILITY IN MORPHOLOGY AND BIOCHEMICAL CONSTITUENTS IN WHEAT GERmplasm WITH SPECIAL REFERENCE TO SPOT BLOTCH

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Abstract

Variability among 11 wheat germplasms viz. KRL 283, KRL 330, KLP 402, WH 1112, DBW 111, NW 5055, RAJ 4270, KRL 327, KRL 331, KRL 210 and KHARACHIA 65 were studied considering their morphology and the presence of bio-molecules. The results revealed that all these germplasms possess variability in terms of the mentioned parameters. The germplasm, RAJ 4270 and KRL 331 were medium in plant height with synchronous tillering habit while the majority were dwarf in nature except KHARACHIA 65. The germplasm also showed different response to spot blotch at different stages of plant growth, reflecting 11.04 - 15.40% at booting, 21.65 - 28.75 at flowering and 30.5 - 40.78 % at milking stages. Among all the germplasms, NW 5055 showed minimum disease severity, representing 11.04, 21.65 and 30.5% at booting, flowering and milking stages, respectively. The possible mechanism of resistance revealed that the presence of higher amount of total phenol and soluble protein, resulted lower disease incidence. The germplasm NW 5055 contained maximum amount of soluble protein reflecting, 30.27 mg/g at seedling, 29.54 mg/g at booting, 28.48 mg/g at flowering and 28.25 mg/g at milking stage. Similarly, total phenol content was also found maximum 2.87 mg/g at seedling, 2.73 mg/g at booting, 2.50 mg/g at flowering and 2.35 mg/g at milking stage in the same variety. Correlation between total phenol and soluble protein with disease severity at different stages of plant growth showed that there was a negative correlation in almost all the germplasms.

Introduction

Wheat (*Triticum aestivum* L.) is the most important cereal crop of about 36% of the world population. It is grown in all the states in India. India ranks second in wheat production with a harvest of 93.50 million tonnes from 302.27 Lakh ha of area during the season 2015-16 followed by Russian Federation 56.24 million tonnes. However, China is on top in production with 117.41 million tonnes. However, as per productivity level, India is far behind than China, which is mainly due to diseases. Spot blotch of wheat has now been considered as one of the major constraints in wheat growing regions specially in South East Asia and Latin American countries where warm humid conditions persist during wheat crop season. Globally, an estimated 25 m ha of wheat area is affected by spot blotch (Van Ginkel and Rajaram 1998), about 40% of which is grown in the Indian sub-continent (Joshi *et al.* 2007), where the grain losses in wheat due to spot blotch have been estimated to be in the range of 24 - 27% in highly susceptible cultivar (Duveiller and Dubin 2002). Duveiller *et al.* (2005) found that spot blotch is the most serious disease of wheat in the warm plains of South Asia, and reported reduction of 20.5 and 15.5% in grain yield and thousand-kernel weight, respectively. However, the extent of losses caused by pathogen varies from variety

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to variety or genotypes to genotypes and even among the stages of crop growth of the same variety. Wheat is cultivated over a wide range of climatic conditions and therefore, understanding of genetics is of great value for genetics and plant breeding purposes.

Materials and Methods

The experiment was carried out during November-March, 2012-13, comprising 11 genotypes of wheat. The experimental materials constituted of 11 genotypes of wheat [*Triticum aestivum* (L.) Merriam-Webster] namely KRL 283, KRL 330, KLP 402, WH 1112, DBW 111, NW 5055, RAJ 4270, KRL 327, KRL 331, KRL 210 (C), and KHARACHIA 65 (C) collected from the Section of Rabi Cereals of Chandra Shekhar Azad University of Agriculture & Technology, Kanpur.

The experiment was conducted at Agriculture Research Farm of Chandra Shekhar Azad University of Agriculture & Technology, Kanpur - 208002 to evaluate the variability among different varieties of wheat with special reference to spot blotch pathogen, caused by *Drechslera sorokiniana*. The experiment was laid out Randomized Block Design (RBD) with three replications. Observations on morphology of wheat were taken at different stages of plant growth. The leaf samples were collected at different stages of growth for estimation of total soluble protein and total phenol content in wheat leaves. Disease severity was recorded at different stages i.e. seedling, booting, flowering and milk formation stages of plant. The yield (q/ha) and duration of crop was calculated after harvest of crop.

Disease observations were recorded at seedling, flowering, milk dough and hard dough stages of different genotypes of wheat. The total disease leaf area was calculated from 50 randomly selected leaves of disease plant individually. The sketch each leaf was drawn on a graph paper (mm) and area covered by squares was measured. The average of all readings of 50 leaves was calculated to get leaf area in cm². Similarly, disease area of total leaf area was calculated.

Leaves with no sign of infection received a score of zero, while those with highest received a score of IV. Similarly, leaves with 1-25, 26-50 and 51-75, area covered with spot blotch received a score of I, II, III respectively. The disease severity of individual plants was calculated by the following formula (Chenula and Singh 1964).

$$\text{Disease severity} = \frac{\text{Class rating} \times \text{Class frequency}}{\text{Total No. of leaves} \times \text{Maximum class rating}} \times 100$$

Variability based on bio-molecules

Soluble protein: The method developed by Lowry *et al.* (1951) was used with slight modification to determine the soluble protein content at different stages of wheat varieties.

Wheat leaves from different treatments were harvested, washed with distilled water several times and blotter dried before protein extraction. A quantity of 1 g of each leaf sample was cut into small pieces and grinded in pestle and mortar as 1 : 5 ratio of leaves extraction buffer. The suspension was centrifuged at 12000 rpm for 30 min at 4°C. The supernatant was collected and used for protein estimation. The working standard solution pipetted out 0.1, 0.2, and 4.0 ml of the solution was put into series of test tubes. A quantity of 0.1, 0.2, and 4.0 ml of the sample extract was also pipetted out and kept into two other test tubes. Then volume in all the tubes was made up to 1 ml with distilled water. A tube with 1 ml of water was served as the blank. Later on, 5 ml of solution C was mixed well and incubated at room temperature for 10 min thereafter; 0.5 ml of FCR was mixed well immediately and incubated at room temperature in dark for 30 min. The absorbance at 660 nm against the blank was read and standard graph was drawn to calculate the amount of protein in sample.

Total phenol: The accumulation of phenols in different wheat varieties was estimated following Bray and Thrope (1954) procedure. The total phenol estimation was carried out with Folin-Ciocaltu Reagent (FCR), which was measured at 650 nm calorimetrically. For estimations, 1 g of leaf samples of different wheat varieties was grind in pestle and mortar sequentially by adding in 10 times volume of 80% ethanol. It was then centrifuged to homogenate the suspensions at 10,000 rpm for 20 min. Supernatant was separated and the residue was re-extracted 5 times with volume of 80% ethanol, centrifuged and the supernatants were pooled. The supernatant was evaporated to dryness and residue was dissolved in 5 ml of distilled water. Different aliquots (0.1, 0.2, 0.4, 0.8 and 1.0 ml) were pipetted out into test tubes and the volume in each tube was made up to 3 ml with distilled water. Subsequently, 0.5 ml Folin-Ciocaltu Reagent (FCR) was added and after 3 min, 2 ml of 20% sodium carbonate (Na_2CO_3) in each tube was mixed thoroughly.

The tubes were placed in boiling water for one min and then cooled. Then absorbance at 650 nm against a reagent blank was measured using ultra violet visible (UV-VIS) spectrophotometer and the standard curve using different concentrations on catechol was prepared. From the standard curve the concentration of phenol in test sample was determined and expressed as milligram phenol per gm of sample materials.

Variability on correlation coefficient and regression equation: The biochemical analysis of wheat leaves under different growth stage and disease severity of the corresponding stage under field experiment showed that reduced disease severity was associated with increase soluble protein and total phenol content. However, to determine the level of association correlation coefficients (r) between soluble protein and total phenol and PDI were calculated. Simple regression equations ($Y = a + bx$) was also developed for both the variables (protein and phenol) separately to understand their relation with disease severity.

Results and Discussion

Morphological variation: Morphological characters like plant height, colour and tillering habit presented in Table 1 showed that wheat variety KHARACHIA 65 was tall in plant height with low tillering and red coloured grain, whereas RAJ 4270 and KRL 331 exhibited medium height with medium tillering with amber colour grains. Other genotypes like KRL 283, KRL 330, WH 1112 and KRL 327 were dwarf in nature. It was also found that out of four dwarf genotypes WH 1112 showed medium tillering, whereas KRL 283 and KRL 330 were high tillering in nature. Previously many workers also found the morphological variability among wheat varieties (Sahu and Biswas 2010 and Dwivedi and Biswas 2011).

The resistance could be assumed one factor for the reduction of disease severity. Severity of disease was recorded in field trial under natural condition. Data on disease severity are presented in Table 2. It was found that the maximum disease severity was in variety RAJ 4270 (15.40%) which was followed by KHARACHIA 65 with the value of 15.18% at booting stage, while the minimum disease severity occurred in NW 5055. In flowering stage maximum disease severity was found in variety KHARACHIA 65 with a value of 38.75%, while minimum disease severity occurred in NW 5055 (21.65%). Similar observation was also recorded at the milking stage of plant growth. From the Table 2 of disease severity, it is apparent that disease severity increased with age of plant. Stakman (1920) first reported the differences in susceptibility of wheat varieties to *H. sativum*. Sahu and Biswas (2010) also found the pathogenic variability among seven popular varieties of wheat. According to them, the variety, K 9107 represented minimum disease severity against all the varieties. Mishra and Singh (1969) also tested 72 wheat varieties and found variable response to *H. sativum*.

Table 1. Morphological variation among different wheat genotypes.

Sl. No.	Variety/germplasm	Morphological character
1	KRL 283	Dwarf in plant height, amber in colour, high tillering
2	KRL 330	Dwarf in plant height, amber in colour, high tillering
3	KLP 402	Medium in plant height, amber in colour, low tillering
4	WH 1112	Dwarf in plant height, amber in colour, medium tillering
5	KRL 210 (C)	Medium in plant height, amber in colour, high tillering
6	DBW 111	Medium in plant height, amber in colour, low tillering
7	KHARACHIA 65 (C)	Tall in plant height, red in colour, low tillering
8	NW 5055	Tall in plant height, amber in colour, medium tillering
9	RAJ 4270	Medium in plant height, amber in colour, medium tillering
10	KRL 327	Dwarf in plant height, low tillering
11	KRL 331	Medium in plant height, amber in colour, medium tillering

Table 2. Severity of leaf blight (spot blotch) on different genotypes of wheat.

Sl. No.	Variety	Leaf blight score in percentage		
		Booting	Flowering	Milking
1	KRL 283	13.95	26.80	36.81
2	KRL 330	13.15	25.42	34.02
3	KLP 402	13.84	25.98	35.74
4	WH 1112	14.12	27.05	39.50
5	KRL 210 (C)	15.10	28.15	40.25
6	DBW 111	13.25	25.84	34.25
7	KHARACHIA 65 (C)	15.18	28.75	40.78
8	NW 5055	11.04	21.65	30.50
9	RAJ 4270	15.40	30.50	41.72
10	KRL 327	13.10	24.78	33.45
11	KRL 331	14.02	26.95	37.14
	CD at 5%	1.05	3.05	4.0

Biochemical variation

Soluble protein: The results enumerated in Table 3 showed that the soluble protein content in different wheat varieties/germplasms was different and varied from stage to stage of plant growth. The maximum soluble protein with 30.27 mg/g of fresh leaf at seedling, 29.54 mg/g of fresh leaf at booting, 28.48 mg/g of fresh leaf at flowering and 28.25 mg/g of fresh leaf at milking stage was observed in the genotype NW 5055, which was followed by KRL 327, showing 29.67, 29.36, 27.25 and 26.0 mg/g soluble protein in fresh leaves at seedling, booting, flowering and milking stages, respectively. The statistical analysis of the data showed that the variety NW 5055 with KRL 327, DBW 111 with KLP 402, KRL 331 with WH 1112 and KRL 210 with KHARACHIA 65 at booting stage and KRL 327 with KRL 330, KLP 402 with KRL 283, KRL 331 with WH 1112 and KRL 210 with KHARACHIA 65 at milking stage were statistically at par in protein content. It was also found that the soluble protein content gradually decreased from seedling to booting and flowering to milking stages.

The genotype NW 5055 registered 2.41, 3.58 and 0.08% decreased from seedling to booting, booting to flowering and flowering to milking stage, respectively. The minimum content of soluble protein was found in variety RAJ 4270 with 22.84 mg/g of fresh leaf at seedlings, 21.76 mg/g of fresh leaf at booting, 20.47 mg/g of fresh leaf at flowering and 19.47 gm/g of fresh leaf milking stage. Boller (1985) reported that proteins were associated with defence response in plant against fungi and bacteria. Accumulation of increased amount of soluble protein in host plant due to infection by pathogen was reported by several workers (Hoj *et al.* 1989, Arzoo *et al.* 2012 and Biswas *et al.* 2012).

Table 3. Variation of soluble protein content in different wheat genotypes at different stages of growth.

Sl. No.	Variety	Total soluble protein (mg/g) of fresh leaf			
		Seedling	Booting	Flowering	Milking
1	KRL 283	28.76	26.83	26.55	22.81
2	KRL 330	29.25	28.50	27.25	25.97
3	KLP 402	28.80	27.50	27.05	22.81
4	WH 1112	27.83	25.17	22.99	22.24
5	KRL 210 (C)	26.75	22.85	22.35	20.95
6	DBW 111	29.25	27.50	27.10	25.35
7	KHARACHIA 65 (C)	23.83	22.77	21.55	20.95
8	NW 5055	30.27	29.54	28.48	28.25
9	RAJ 4270	22.84	21.76	20.47	19.47
10	KRL 327	29.67	29.36	27.25	26.00
11	KRL 331	27.83	25.17	22.99	22.24
	CD at 5 %	0.45	0.36	0.24	0.21

Total phenol: Phenols are well known antifungal, antibacterial and antiviral compounds. They are involved in the expression of disease resistance, in many ways like hypersensitive cell death, lignifications of cell walls etc. In the present study, total phenol contents in 11 wheat varieties/germplasm at seedling, booting, flowering, and milking stage were estimated (Table 4). The result showed that the total phenol content varied greatly among the genotypes. The maximum total phenol content 2.87 mg/g at seedling, 2.73 mg/g at booting, 2.50 mg/g at flowering and 2.35 mg/g at milking stage was found in the genotype NW 5055. It was also found that the total phenol content decreased with the increase in age of the plant. RAJ 4270 showed minimum amount of phenol content as 2.30 mg/g of fresh leaf at seedlings, 2.02 mg/g of fresh leaf at booting, 1.83 mg/g of fresh leaf at flowering and 1.65 gm/g of fresh leaf milking stage. The difference of the phenol content between RAJ 4270 and NW 5055 was 19.86, 26.0, 26.8 and 29.78% at seedling, booting, flowering and milking age of the plant growth, respectively.

Soluble protein and total phenol content are two important parameters for resistance response in plant. Gupta *et al.* (2004) reported that the high heritability on sedimentation value, protein content, phenolic reaction and yield per plant was found variable among different varieties of bread wheat. Shetty and Ahamad (1980) estimated the total phenolic contents at different growth stages in leaf and root tissue of resistant and susceptible maize and sorghum plants to downy mildew and reported faster accumulation of phenols in higher quantity in diseased area. Sahu and Biswas (2010) also reported that the content of phenol in leaves of wheat varieties varied from variety to variety and age of the plant.

Variation on correlation coefficient and regression equation: The correlation between disease severity and soluble protein content at different stages of wheat germplasm revealed a negative relationship (Table 5). Genotypes NW 5055 showed correlation values of 0.528, 0.965 and 0.994 at booting, flowering and milking stages, respectively. The regression equation of soluble protein and disease severity showed that higher the regression value, lower was the disease incidence. The genotype KRL 283 had the correlation coefficient (r) 0.758 at booting stage, 0.649 at flowering stage and -0.389 at milking stage, resulting response of resistance to spot blotch. The disease severity showed negative correlation with soluble protein ($r = -0.6364$) and total phenol ($r = -0.7653$) as reported by previous workers (Rajik and Biswas, 2012). Biswas *et al.* (2012) reported that there was a negative correlation $r = -0.6214$, -0.5867 and -0.5484 between disease severity and soluble protein content at 5, 10 and 15 days of imposition. They found that, total phenol content also showed negative correlation $r = -0.5370$, -0.5656 and -0.4225 with disease incidence.

Table 4. Variation in phenol content in different wheat varieties/germplasm at different growth stages.

Sl. No.	Variety	Total soluble phenol (mg/g) of fresh leaf			
		Seedling	Booting	Flowering	Milking
1	KRL 283	2.65	2.45	2.34	2.15
2	KRL 330	2.78	2.53	2.43	2.28
3	KLP 402	2.65	2.45	2.34	2.15
4	WH 1112	2.50	2.31	2.10	2.05
5	KRL 210 (C)	2.45	2.23	2.03	1.85
6	DBW 111	2.73	2.53	2.34	2.25
7	KHARACHIA 65 (C)	2.31	2.13	1.90	1.85
8	NW 5055	2.87	2.73	2.5	2.35
9	RAJ 4270	2.30	2.02	1.83	1.65
10	KRL 327	2.78	2.53	2.43	2.28
11	KRL 331	2.51	2.33	2.23	2.15
	CD at 5%	0.10	0.08	0.07	0.05

Correlation of disease severity with phenol content: Phenol is another important parameter for resistance response in plant. The high content of phenol indicates lower disease severity. There was also correlation between disease severity and total phenol content as presented in Table 5. The genotype NW 5055 showed correlation value 0.618, 0.950 and 0.990 at booting, flowering and milking stages, respectively. RAJ 4270 had the correlation coefficient (r) of 0.882 at booting, 0.661 at flowering and 0.996 at milking. The genotypes KRL 402 had statistically non-significant correlation at the flowering, dough and hard dough stages. KRL 327 is also showed negative correlation coefficient (r) -0.739 , -0.855 and -0.894 at booting, flowering and milking stage, respectively. Arzoo *et al.* (2012) also found that both soluble protein content ($r = -0.5995$) and total phenol content ($r = -0.5313$) showed a negative correlation with disease incidence.

It is observed that all tested germplasms possess variability in terms of morphology, diseases severity, and bio-molecules. Among all the germplasms, NW 5055 showed minimum disease severity, at booting, flowering and milking stages of plant growth. because the germplasm NW 5055 contained maximum amount of soluble protein relecting, booting, flowering and milking stage. Similarly, total phenol content was also found maximum at these stages. The possible

mechanism of resistance revealed that the presence of higher amount of total phenol and soluble protein, resulted lower disease incidence.

Table 5. Correlation of disease severity with soluble protein content in wheat genotypes.

Sl. No.	Variety	Stages	Correlation coefficient (r) with disease severity	Regression equation
1	KRL 283	Booting	0.758	$Y = 21.880 + 0.355 x$
		Flowering	0.649	$Y = 5.303 + 0.798 x$
		Milking	-0.389	$Y = 65.998 - 1.171 x$
2	KRL 330	Booting	0.769	$Y = 16.085 + 0.947 x$
		Flowering	0.953	$Y = 26.140 + 0.043 x$
		Milking	0.753	$Y = 20.475 + 0.165 x$
3	KLP 402	Booting	0.672	$Y = 18.810 + 0.624 x$
		Flowering	0.952	$Y = -165.290 + 7.405 x$
		Milking	0.903	$Y = -42.503 + 1.825 x$
4	WH 1112	Booting	0.986	$Y = 20.600 + 0.320 x$
		Flowering	0.956	$Y = -4.408 + 1.012 x$
		Milking	0.998	$Y = -8.264 + 0.773 x$
5	KRL 210 (C)	Booting	0.887	$Y = 3.870 + 1.258 x$
		Flowering	0.992	$Y = 10.363 + 0.427 x$
		Milking	0.753	$Y = -32.411 + 1.327 x$
6	DBW 111	Booting	0.892	$Y = -5.484 + 2.483 x$
		Flowering	0.844	$Y = 21.699 + 0.201 x$
		Milking	0.930	$Y = 10.702 + 0.425 x$
7	KHARACH IA 65 (C)	Booting	0.987	$Y = 13.943 + 0.580 x$
		Flowering	0.957	$Y = 4.210 + 0.600 x$
		Milking	0.802	$Y = -22.928 + 1.078 x$
8	NW 5055	Booting	0.528	$Y = 27.029 + 0.229 x$
		Flowering	0.965	$Y = 15.029 + 0.624 x$
		Milking	0.994	$Y = -8.988 + 1.227 x$
9	RAJ 4270	Booting	0.674	$Y = 1.749 + 1.293 x$
		Flowering	0.523	$Y = 6.515 + 0.456 x$
		Milking	0.993	$Y = -27.138 + 1.110 x$
10	KRL 327	Booting	0.995	$Y = 21.849 + 0.575 x$
		Flowering	0.997	$Y = -15.138 + 1.714 x$
		Milking	0.987	$Y = -10.663 + 1.090 x$
11	KRL 331	Booting	0.539	$Y = 20.043 + 0.368 x$
		Flowering	0.996	$Y = -5.756 + 1.033 x$
		Milking	0.936	$Y = 3.862 + 0.496 x$

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