

PLANT PROTECTION

Symptomatological Study in Different Wheat Varieties in Response to *Puccinia graminis tritici*

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ABSTRACT

Wheat is one of the essential cereals and is highest produced grain in the world after corn and rice. It is the second most important crop in India, next to rice. Wheat stem rust is the primary biotic constraint to wheat production, and it is essential to understand and analyse the disease in terms of pathological and biochemical responses. Formation of white flecks on the leaf surface seven to eight days post-inoculation was the first symptom of disease initiation. Number of pustules and uredia per centimeter square increased as time progressed along with the disease. The A-9-30-1, with a mean number of pustules and uredia of 25.25 and 6.45×10^4 per centimeter square, respectively, was highly susceptible to recording higher values during the entire study period, followed by Agra local (28.93 mean no. of pustules and 7.25×10^4 uredia per centimeter square) and Kharachia (31.55 mean no. of pustules and 7.4×10^4 uredia per centimeter square). No flecking, pustules, or uredia were observed in the three varieties *viz.*, GDW 1255, GW 496, and GW 451.

HIGHLIGHTS

- A-9-30-1 variety shows maximum susceptibility towards Puccinia graminis tritici.
- Three varieties show complete resistance to stem rust disease.

Keywords: Wheat, stem rust, uredia, pustules, resistant, susceptible

Wheat is one of the important cereals. It is the staple food for an estimated 35% of the world population. The world's top wheat producing regions are China, India, Russian Federation, United States, France, Canada, Ukraine, Pakistan, Germany, Argentina, Tukey, Australia, Iran, United Kingdom and Kazakhstan (FAO, 2020). These countries contribute about 76% of the total world wheat production. Diseases are the major threat to wheat production. Among various diseases, wheat rusts are the most important biotic constraints to wheat production. The rusts are the worldwide distributed diseases of wheat. The rust diseases are caused by fungi belonging to the class Basidiomycetes and order Pucciniales. There are three rust diseases of wheat: stem (or black) rust, leaf (or brown) rust and stripe

(or yellow) rust, caused by the pathogens *Puccinia* graminis f. sp. tritici, *P. triticina* and *P. striiformis* f. sp. tritici, respectively (Singh et al., 2011). Among all diseases, black or stem rust caused by *P. graminis* tritici is potentially most dreadful when the variety is susceptible and conditions are favourable for their development. It is known as 'killer' disease of wheat because it kills wheat plant. Stem rust occurs mainly on stems but can also be found on leaves, sheaths, glumes and awns. Initial symptoms appear as oval to elongate lesions with reddish-brown in colour. The initial macroscopic symptom is typically

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Sharma et al.

a small chlorotic fleck, which appears a few days after infection. As infected plants mature, uredinia change into telia, altering colour from red to dark brown to black, hence the disease is also called as black rust (Singh *et al.* 2012). Severe infection of stems interrupts nutrient flow to the developing heads, resulting in shrivelled grains, and stems weakened by rust infection are prone to lodging (Roelfs *et al.* 1992). Following research is based on symptomatologic study of different varieties of wheat against stem rust disease, which is important for planning any management strategies.

MATERIALS AND METHODS

Total six cultivars *viz.*, GW 451, GW 496 and GDW 1255, A-9-30-1, Kharachia and Agra local were grown. Standard agronomic practices were followed for the growing of the varieties. Artificial inoculation with *Puccinia graminis tritici* was done. For the successful development of infection is boot leaf stage i.e., at 30-35 days after sowing, hence, inoculation with uredospores of mixture of races 40 A, 11 and 117-6 of *P. graminis tritici* was made at this stage, with syringe inoculation method. Concentration of inoculation was maintained at 10⁶ uredospores/ml. In each plot of different varieties under study, ten plants were selected.

Disease initiation and symptomatology

The days to first appearance of stem rust symptom i.e., initiation of pustules was recorded for each variety.

Number of pustules/cm²

For each variety, 1.0 cm² area was marked on the two leaf halves, one on the right side and the other one on the left side. 10 observations were recorded for each variety. The number of pustules/cm² was counted.

Number of uredia/cm²

Leaf disc with pustules measuring 1.0 cm diameter was collected and transferred to test tubes with 10 ml sterilized distilled water. Uredospore counting was performed by haemocytometer under a microscope.

RESULTS AND DISCUSSION

After inoculation the development of stem rust

symptoms were carefully examined by randomly tagging ten plants in inoculated area of all varieties and observations were recorded periodically.

Disease initiation and symptomatology

The first symptom of stem rust development was observed as the formation of white flecks on the leaf surface, which was the indicator of stem rust disease initiation. Flecking symptoms (Fig. 1a) appeared eight days of post inoculation in three susceptible varieties. This was followed by the formation of tiny pustules (Fig. 1b) after few days. It took twelve days for the complete establishment of rust pustules (Fig. 1c) on the leaf surface. However, it was noticed that no flecking or pustules had appeared on the three varieties *viz.*, GW 451, GW 496 and GDW 1255.

Leonard and Szabo (2005) noted first macroscopic symptom as a small chlorotic fleck, which appeared a few days after infection in stem rust of wheat. Wheat varieties are characterized by various responses from small hypersensitive flecks to small to moderate size uredinia that may be surrounded by chlorotic or necrotic zones in leaf rust infected plants (Bolton *et al.* 2008). Thus, the recorded observations are in accordance with the previous studies made by various researchers.

Number of pustules/cm²

It was noticed that the number of pustules (per cm²) increased significantly from 15 to 60 days after the disease initiation in A-9-30-1, Kharachia and Agra local. There were no single pustule formation occurs in GW 451, GW 496 and GDW 1255 i.e., these are the completely resistant against stem rust disease.

The number of pustules (per cm²) recorded initially in all the three susceptible varieties were fewer. Later, the pustule numbers increased with progression of the disease along time. The highest number of pustules (per cm²) were recorded at 60 days after disease initiation, showing high rust severity. Thereafter, the pustules coalesced and had spread all the over the leaf surface, stem and earhead also (Fig. 2) without leaving any demarcation for the number to be counted and there by assuming the severe form.

At 15 days after the disease initiation in the variety Kharachia, eight plants showed 1-3 pustules per centimetre square out of ten observations taken



Fig. 1: Progress of symptoms of the stem rust disease



Pustules on leaf

Pustules on ear

Fig. 2: Coalescing of rust pustules on leaves, stem and ear

Pustules on stem

and two plants had not yet developed any pustules. In the next observation taken at 30 days after the disease initiation, the number of pustules had increased gradually by recording the values ranging from 9 to 21 per centimetre square. Slightly higher values for the number of pustules per centimetre square were observed at 45 days after the disease initiation. The number of pustules per centimetre square recorded was 25 to 37. In the last observation at 60 days after the disease initiation, the number of pustules per centimetre square was more compared to all other previous observations. Minimum of 45 and maximum of 60 pustules per centimetre square were observed (Table 1).

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	No. of pustules/cm ²												
Sl. No.	Kharachia					Ag	ra local		A-9-30-1				
	15 DAI	30 DAI	45 DAI	60 DAI	15 DAI	30 DAI	45 DAI	60 DAI	15 DAI	30 DAI	45 DAI	60 DAI	
1	2	12	26	58	1	11	34	52	0	24	35	59	
2	1	9	28	51	3	17	40	58	3	18	32	47	
3	3	18	36	48	3	18	29	55	0	25	39	56	
4	2	16	25	59	3	20	34	53	2	17	35	45	
5	0	12	34	52	2	26	41	56	2	27	40	60	
6	2	14	33	50	0	16	32	51	1	26	36	62	
7	3	18	37	55	3	20	44	57	4	29	49	78	
8	3	21	35	60	0	15	42	58	2	29	48	70	
9	0	11	30	45	0	13	48	65	2	18	46	64	
10	1	12	30	58	3	21	47	66	1	23	47	59	
Mean	1.70	14.30	31.40	53.60	1.80	17.70	39.10	57.10	1.90	23.60	40.70	60	

Table 1: Development of rust pustules/cm² in the wheat variety Kharachia, Agra local and
A-9-30-1 at 15, 30, 45 and 60 DAI

In the variety Agra local, the number of pustules per centimetre square recorded were ranged 1 to 3 during the first observation, and three plants showed no pustules. After 30 days of the stem rust initiation, minimum of 11 and maximum of 26 pustules per centimetre square were recorded. Increase in the values for the number of pustules per centimetre square was recorded at 45 days after the disease initiation during which the least number of pustules recorded was 29 and highest of 48 per centimetre square. In the final observation at the 60 days after disease initiation the number of pustules attained high status ranging from 51 to 66 per centimetre square (Table 1).

Similarly, stem rust pustules were recorded on eight plants out of ten, having 1 to 4 pustule per centimetre square, and the rest two none in A-9-30-1. Minimum of 17 pustules per centimetre square were recorded and maximum of 29, after 30 days of the disease initiation. The pustule numbers increased remarkably after 45 days of disease initiation by recording minimum of 32 to as high as 49 pustules per centimetre square. Increase in the formation of pustules after 60 days of disease initiation was ranged 45 to 78 (Table 1).

Mean number of pustules per centimetre square at fifteen days after disease initiation in the varieties Kharachia, Agra local and A-9-30-1 were 1.70, 1.80 and 1.90, respectively. Thirty days post disease initiation, the mean number of pustules increased moderately to 14.30, 17.70 and 23.60 per centimetre

square. Fourty five days after disease initiation, the mean number of pustules increased largely to 31.40, 39.10 and 40.70 per centimetre square. The highest mean number of pustules were recorded at sixty days post disease initiation and were found to be 53.60, 57.10 and 60 per centimetre square (Table 1).

A-9-30-1 recorded higher values with mean number of pustules of 31.55 per centimetre square over the study period and thereby proved comparatively more susceptible followed by Agra local (28.93 pustules per centimetre square). Kharachia recorded the comparatively less mean number of 25.25 pustules per centimetre square (Table 1).

No pustules were formed on the leaf surface and stem of any of the three resistant varieties *viz.*, GDW 1255, GW 496 and GW 451 during the entire period under study. This reflects the excellent builtin genetic resistance mechanism and their ability to withstand the artificial infection.

Leonard and Szabo (2005) noted that the pustules of stem rust of wheat were several millimeters long and a few millimeters wide formed by rupture of the host epidermis. Disease responses of barley genotypes to leaf rust pathogen *Puccinia hordei Otth*. investigated by Salih and Al-Hamdany (2012) revealed that pustule density per cm² area of flag leaf under heavy artificially epiphytotic conditions produced 15 in the mutant SA/12 to 127 pustules in cultivar Golden Melon and the naked barley strain Aamer. The pustules on moderately resistant genotypes caused by pea rust were small (1.5-1.7mm). Size of the pustules showed high variation of 1.3-4.4 mm in moderately susceptible genotypes whereas, the susceptible genotypes recorded pustule size of 2.9-4.8 mm. Largest pustule size of 4.2-4.6 mm was seen in highly susceptible genotypes (Upadhyay *et al.* 2017).

Thus, the results in the present investigation are in accordance with the findings of previous researchers.

Number of uredia/cm²

The number of uredia per centimetre square were recorded at 15, 30, 45 and 60 days after the disease initiation (DADI). It was found that the uredospore load significantly increased from 15 to 60 days after the disease initiation. Since three resistant varieties lacked formation of any pustules, the number of uredia/cm² could not be calculated in them.

The number of uredia/cm² initially recorded at 15 days after disease initiation were fewer in the genotype Kharachia. Out of ten plants under observation, eight showed pustules whose uredia/ cm² were ranged from 1×10^4 to 2×10^4 . Two plants recorded no pustule, hence the number of uredia/ cm² was zero. There was a slight increase in the number of uredia/cm² at 30 days after disease initiation which ranged from 1×10^4 to 6×10^4 . Gradual increase in uredospore load was evident at 45 days after disease initiation, by recording number of uredia/cm² ranging from 5×10^4 to 10×10^4 . The final observation taken at 60 days after disease initiation showed significant increase by recording 11×10^4 to 19×10^4 uredia/cm² (Table 2). In the variety Agra local, the number of uredia/ cm² initially recorded at 15 days after disease initiation were also fewer. Out of ten plants under observation, seven showed pustules whose uredia/ cm^2 were 1×10^4 to 3×10^4 , respectively. Three plants recorded no pustule, hence the number of uredia per centimetre square was zero. There was slight increase in the uredia/cm² at 30 days after disease initiation which ranged from 3×10^4 to 7×10^4 . Gradual increase in uredospore load was found at 45 days after disease initiation, by recording number of uredia/cm² ranging from 5×10^4 to 11×10^4 . The final observation at 60 days after disease initiation showed the significant increase in number of uredia by recording 11×10^4 to 20×10^4 per centimetre square (Table 2).

Many plants had developed pustules in the variety A-9-30-1. At 15 days after disease initiation whose number of uredia/cm² were ranged from 1×10^4 to 3×10^4 . Two plants recorded no pustule, hence the number of uredia per centimetre square was zero. Drastically increase in number of uredia/cm² varying from 3×10^4 and 7×10^4 was seen at 30 days after disease initiation. Hereafter, the number of uredia/cm² increased high by recording least of 7×10^4 and highest of 11×10^4 . There was a significant rise in uredospore load at 60 days after disease initiation which recorded 11×10^4 to 20×10^4 uredia/cm² (Table 2).

Mean number of uredia per centimetre square at fifteen days after disease initiation in the varieties Kharachia, Agra local and A-9-30-1 were 1.10 ×

Table 2: Number uredia/cm ² in the wheat variety Kharachia, Agra local and A-9-30-1 at 15, 30, 45 and 60 days after
disease initiation (DADI)

	No. of uredia/cm ² (in × 10 ⁴)											
Sl. No.		Khai	achia			Agra	local		A-9-30-1			
	15 DADI	30 DADI	45 DADI	60 DADI	15 DADI	30 DADI	45 DADI	60 DADI	15 DADI	30 DADI	45 DADI	60 DADI
1	1	5	8	14	1	5	9	15	0	4	10	16
2	1	3	6	12	2	4	7	18	2	5	5	11
3	2	6	10	19	1	7	11	13	0	3	6	12
4	1	4	8	15	2	5	9	15	1	4	9	13
5	0	3	7	16	2	6	11	20	3	5	9	15
6	1	3	8	16	0	4	10	15	2	6	11	20
7	1	4	9	14	3	4	9	13	3	4	10	16
8	2	1	5	11	0	5	5	11	1	5	9	15
9	0	2	6	12	0	3	6	12	1	4	7	13
10	2	2	5	13	2	4	7	14	1	7	11	17
Mean	1.10	3.30	7.20	14.20	1.30	4.70	8.40	14.60	1.40	4.70	8.70	14.80



 10^4 , 1.30×10^4 and 1.40×10^4 , respectively. Thirty days post disease initiation the mean number of uredia increased moderately to 3.30×10^4 , $4.70 \times$ 10^4 , 4.70×10^4 per centimetre square. Fourty five days after disease initiation the mean number of uredia increased largely to 7.20, 8.40 and 8.70 per centimetre square. The highest mean number uredia were recorded at sixty days post disease initiation and were found to be 14.20×10^4 , 14.60×10^4 and 14.80×10^4 per centimetre square. A-9-30-1 with mean number of uredia of 7.4×10⁴ per centimetre square, was superior recording higher values in the entire study period followed by Agra local $(7.25 \times 10^4 \text{ uredia per centimetre square})$. Kharachia recorded the comparatively less mean number of 6.45×10^4 uredia per centimetre square (Table 2).

No uredia were formed on the leaf surface and stem of any of the three resistant varieties *viz.*, GDW 1255, GW 496 and GW 451 during the entire time period under study as there were no pustules present of stem rust pathogen.

Salih and Al-Hamdany (2012) reported the high pustule density per cm² area of flag leaf under heavy artificial epiphytotic conditions produced in barley cultivar. Danelli and Reis (2016) reported that two soybean cultivars produced highest of 4,012.8 spores/ cm² and 7,348.4 uredospores/cm², respectively. The concentration of 4×10^4 uredospores/ml resulted in a disease intensity that allows safe differentiation between susceptible and resistant cultivars in the seedling stage (Turra *et al.* 2017). Hence, the results obtained in present investigations are more or less similar with the findings of previous researchers.

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