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Pre-existing defense mechanism for stem rust resistance in hexaploid wheat (*Triticum aestivum* L.)

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Abstract
The resistance stability of bread wheat cultivars against *Puccinia graminis* f. sp. *Tritici* was studied. Present investigation was conducted to investigate the anatomical basis of defense mechanism against the stem rust and to estimate the heterosis for yield and yield contributing characters in 9 genotypes of Wheat. The material was evaluated in Randomized Block Design with three replications during *Rabi*, 2019 at PG research farm R.C.S.M, College of Agriculture, Kolhapur. Study of the various quantitative and anatomical characters contributed to wheat resistance. The studies on stem anatomical parameters indicated epidermis thickness, phloem tissue thickness, vascular thickness, ground tissue thickness was found to be more in resistant genotypes (cross: SRRSN 6038 x GW-16-750) than susceptible ones. The average mean performance of F_1 was higher than parents in desired direction for many of the characters suggesting existence of heterotic effects. Cross SRRSN 6038 x GW-16-750 showed maximum ground tissue thickness, numerical superior epidermis thickness, highly significant phloem tissue thickness, highly significant vascular bundle thickness. Only one cross K1314 x SRRSN6038 showed highly significant xylem vessels diameter. The extent and magnitude of various heterotic effect in percentage over mid parent (RH) better parent (HB) and over check hybrid (SH) varied with character to character and cross to cross. Cross SRRSN6038 x GW-16-750 showed best heterotic effect over standard check (SH) for all 9 qualitative and 5 stemanatomical characters. Cross SRRSN 6038 x GW-16-750 showed best heterotic effect over mid parent (RH) as well as over better parent (HB) while, cross SAWYT-HT-74 x SRRSN 6070 showed best heterotic effect over mid parent (RH) as well as over better parent (HB). These results were ensured with the anatomical investigations in which proved that the genotype K 1314 (5R; TR), GW 16-750 (5R; TR), SRRSN 6070 (5R; TR) and cross SRRSN 6038 x GW 16-750 (10R; TR) was resistant at both the locations and were found resistant anatomically for stem rust.

Keywords: *Triticum aestivum* L., Pgt: *Puccinia graminis* f. sp. *tritici.* Stem rust

Introduction
Globally, wheat (*Triticum aestivum* L.) is grown on more area than any other crop, and is ranked second to rice, corn in total production. It is one of the main staple foods in both developed and developing countries. Wheat is cultivated since pre-historic times in the world. In India dwarf wheat is introduced by Dr. N. E. Borlaug form Mexico, hence called Mexican dwarf wheat and was responsible for green revolution. (Anon, 2021) [2].

Biotic and Abiotic stresses are responsible for significant reduction in crop production throughout the world. Biotic stresses like diseases, weeds and insects can destroy about 31 to 42% of all crops annually. Losses due to diseases are approximately 14%, which amount to about US$220 billion per year. (Agrios, 2005) [1]. Likewise, wheat is attacked by pathogen such as Parasitic Fungi, Nematodes, Viruses and Bacteria. These all are capable of reducing yield significantly. Among these, the rusts are the most important diseases of cereals causing major crop losses globally (Singh *et al.* 2005; Park 2008; Vurro *et al.* 2010; Fetch *et al.* 2011) [12, 9, 13, 5]. Black stem rust is most important and destructive disease through the world where ever wheat is grown. The rust epidemics of 1946-47 in MP, Maharashtra, Rajasthan and UP destroyed over two million tons of grain. In 1956-57 rust was severe in WB, Bihar and Eastern parts of UP causing heavy damage. The present investigation was undertaken in order to reduce economic losses due to rust pathogens, scientists need to know anatomy, cultivar susceptibility, epidemiology of pathogens and knowledge of resistant gene available and potential sources of resistance for deployment. Therefore, there is an urgent need to improve the existing varieties against leaf and stem rust by imparting the resistant genes from the available source. It is also necessary to investigate the different defense mechanisms which will help to select resistant lines from segregating generations.

Heterosis leads to superiority in adaptation, yield, quality, disease resistance, maturity and general vigour over its parent.

Heterosis reported in wheat for the first time by Freeman (1919) [6]. Briggle (1963) [3] reported presence of heterosis in considerable quantity for grain yield components in various F_1 wheat crosses. The present investigation was undertaken with 9 genotypes with following the main objective of the study is 1. To investigate the anatomical basis of defense mechanism against the leaf rust. 2. To estimate the heterosis for yield and yield contributing character.

Material and Methods

The present research work entitled "Pre-existing defense mechanism for rust resistance in hexaploidy wheat (*Triticum aestivum* L.)" was carried out at PG farm R.C.S.M, College of Agriculture Kolhapur during *Rabi* 2019.

Plant materials: 9 genotypes (3 F_1 + 5 Parents + 1 Check) of bread wheat (*Triticum aestivum* L.). Three hybrids developed by using three susceptible and two resistance genotypes were collected from Agriculture Research Station, Niphad. District Nashik and used for research work. 3 F1: SAWYT-HT-74 X SRRSN 6070, K 1314 X SRRSN 6038, SRRSN 6038 X GW-16-750; 5 Parents: SAWYT-HT-74, K 1314, SRRSN 6038, SRRSN 6070, GW-16-750; 1 Check: Phule Samadhan.

Experimental design: The experiment was laid out in randomized block design with three replications. The experimental material *i.e.*, 9 genotypes were randomly planted in three replications. Each entry was represented by single rows of 5M length spaced at 22.5 cm between 2 rows and 10 cm between two plants.

Disease severity and response: Disease severity was recorded four times, every 10 days intervals, during growing season. Stem rust severity (percentage) and response of plans to disease were also assessed using a modified Cobb's scale (Peterson *et al*, 1948) [10]. *i.e.*, O – No visible infection on plant, R – Resistant, MR – Moderately resistant, MS – Moderately susceptible, S – Susceptible, X – Intermediate. Reading of severity and reaction are recorded with Final rust severity (FRS %).

Anatomical study: Specimens were taken from center of the internode of stem from apex. The following observations were recorded after 80 days from sowing *vi* Epidermal thickness (µ), Phloem tissue thickness (µ), Vascular bundle thickness (µ) Xylem vessels diameter (µ) and Ground tissue thickness (µ). Sample were cleaned with tap water cut into suitable specimens (5mm in length). Specimens were fixed in70% ethyl alcohol. A free hand sections taken with the thickness 15-18 microns and were fixed on slides with glycerin, stained with safranin-light green combination. Slides were observed under 10X x 20X magnification by using trinocular microscope (Leica make) with software facility. (Plate-1)

Statistical Analysis: Randomized Block Design with three replications was followed with the mean values of ten randomly selected observational plants for 14 different traits were used for statistical analysis. The analysis of variance was done as suggested by Panse and Sukhatme (1985) [8].

Heterosis: The heterosis can be classified into three types on the basis of estimation. They are relative heterosis over mid-parent, heterobeltosis and standard heterosis over commercial hybrid/variety and calculated as per Rai (1979) [11].

Results and Discussion

The stem anatomical structures among 9 cultivars and heterosis were summarized in (Table 1 and 2) and their transverse sections illustrated in Plates 1 and 2. Studies on the anatomical characteristics of wheat leaf and stem rust in relation to rust are very meager and inconclusive. Similarly, the identification of differences in histological events, between resistant and susceptible genotypes is a prerequisite for understanding the host pathogen interaction. Screening of wheat genotypes to rust carried out by earlier workers indicated that some genotypes have shown resistance, partial resistance and some have shown susceptible reaction to rust (Draz *et al*., 2015) [4]. So, it was presumed that some anatomical factors could be involved in disease resistance or susceptibility. Anatomical investigations in which proved that the thickness of epidermis, ground tissue and phloem thickness (µ) were increased in resistant cultivars compared with susceptible. Consequently, grain yield were increased in resistant cultivars. (Khaled *et al.*, 2018) [7]. Epidermis thickness increased in resistant genotype while, reduced in susceptible genotypes. Similar results were depicted by Khaled *et al.*, (2018) [7]. Phloem tissue thickness increased in resistant genotype while, reduced in susceptible genotypes showed minimum phloem tissue thickness (table1 and Plate 2). Similar results were observed by Khaled *et al.*, (2018) [7]. The vascular bundle consists of xylem and phloem. Vascular bundle thickness increased in resistant genotype while, reduced in susceptible genotypes. Similar results were depicted by Khaled *et al.*, (2018) [7]. The vascular bundle were collateral and distributed in ground tissues. Ground tissue thickness increased in resistant genotype while, reduced in susceptible genotypes. Similar results were depicted by Khaled *et al.* (2018) [7].

Severity of stem rust in field condition: 9 Bread Wheat cultivars were evaluated at adult stage to study the response of infection for *Puccinia graminis* f. sp. *Tritici*, during *rabi* season 2019. Disease severity was recorded four times at every 10 days intervals, during each of the two successive growing seasons, expressed as the percentage coverage stem area of wheat plants with rust pustules, according to the method described by Peterson *et al.* 1948 [10].

Table 1: Mean performance of 9 genotypes for 5 stem anatomical characters in wheat.

Sr. No.	Genotype	Epidermal thickness(µ)	Phloem tissue thickness(µ)	Vascular bundle thickness (µ)	Xylem vessels diameter (µ)	Ground tissue thickness(µ)
1.	SAWYT-HT-74	8.23	41.66	133.88	33.26*	216.86
2.	SRRSN6070	16.36	33.24	83.71	16.33	225.72
3.	K 1314	25.11	50.24	166.69	25.17	291.82
4.	SRRSN 6038	7.89	31.39	165.73	32.85*	290.81
5.	GW-16-750	8.72	50.35	133.07	33.11*	333.88**
6.	SAWYT-HT-74 x SRRSN 6070	8.96	42.17	166.33	33.98*	335.64**
7.	K 1314 x SRRSN 6038	8.74	33.47	166.48	42.26**	250.41
8.	SRRSN 6038 x GW-16-750	25.32	53.51**	208.29**	42.68**	350.68**

9.	*Phule Samadhan (C)*	*25.02*	*50.90*	*183.75*	*25.86*	*316.85*
	Mean	14.62	42.99	156.43	31.72	290.29
	S. E.	0.15	0.32	0.26	0.33	0.50
	CD 5%	0.47	0.98	0.80	1.02	1.52
	CD 1%	0.65	1.34	1.10	1.40	2.10
	CV	1.83	1.31	0.29	1.85	0.30

*, ** significant at CD 5 % and CD 1% respectively.

Table 2: Estimates of relative heterosis, heterobeltosis and standard heterosis for 5 Stem Anatomical Characters.

Sr. No.	Crosses	Epidermis thickness (µ)			Phloem tissue thickness(µ)			Vascular bundle thickness (µ)			Xylem vessels diameter (µ)			Ground tissue thickness(µ)		
		Heterosis over			Heterosis over			Heterosis over			Heterosis over			Heterosis over		
		MP/RH	BP/HB	SC/SH	MP/RH	BP/HB	MP/RH	BP/HB	MP/RH	BP/HB	BP/HB	MP/RH	BP/HB	BP/HB	MP/RH	BP/HB
1	SAWYT-HT-74 x SRRSN 6070	-27.12	-45.23	-64.18	12.60	1.22	-17.15	45.67	28.64	63.41	53.64	24.35	-9.39	15.21	15.01	5.93
2	K 1314 x SRRSN 6038	-47.03	-65.19	-65.06	-17.99	-33.37	-34.24	37.04	2.16	31.39	0.07	-0.21	-0.21	13.15	10.93	-20.96
3	SRRSN 6038 x GW-16-750	204.87	190.36	1.19	30.92	6.27	5.12	29.41	28.90	65.04	39.41	25.68	25.68	12.27	5.03	10.67
Minimum		-47.03	-65.19	-65.06	-17.99	-33.37	-34.24	29.41	2.16	31.39	0.07	-0.21	-9.39	12.27	5.03	-20.96
Maximum		204.87	190.36	1.19	30.92	6.27	5.12	37.04	28.90	65.04	53.64	25.68	25.68	15.21	15.01	10.67

Table 3: Response of wheat cultivars against stem rust infection.

Genotype/cross	Rust reaction at Agricultural Research Station, Niphad.	Rust reaction at PG farm R.C.S.M, College of Agriculture Kolhapur	Anatomical rust reaction
K 1314	5R	TR	R
K 1314 x SRRSN 6038	5MR	5R	R
SRRSN 6038	10MR	TMR	R
SRRSN 6038	10MR	TMR	R
SRRSN 6038 x GW 16-750	10R	TR	R
GW 16-750	5R	TR	R
SAWYT -HT-74	20S	5S	S
SAWYT -HT-74 x SRRSN 6070	5MR	5R	R
SRRSN 6070	5R	TR	R
Phule Samadhan	5MR	TR	R

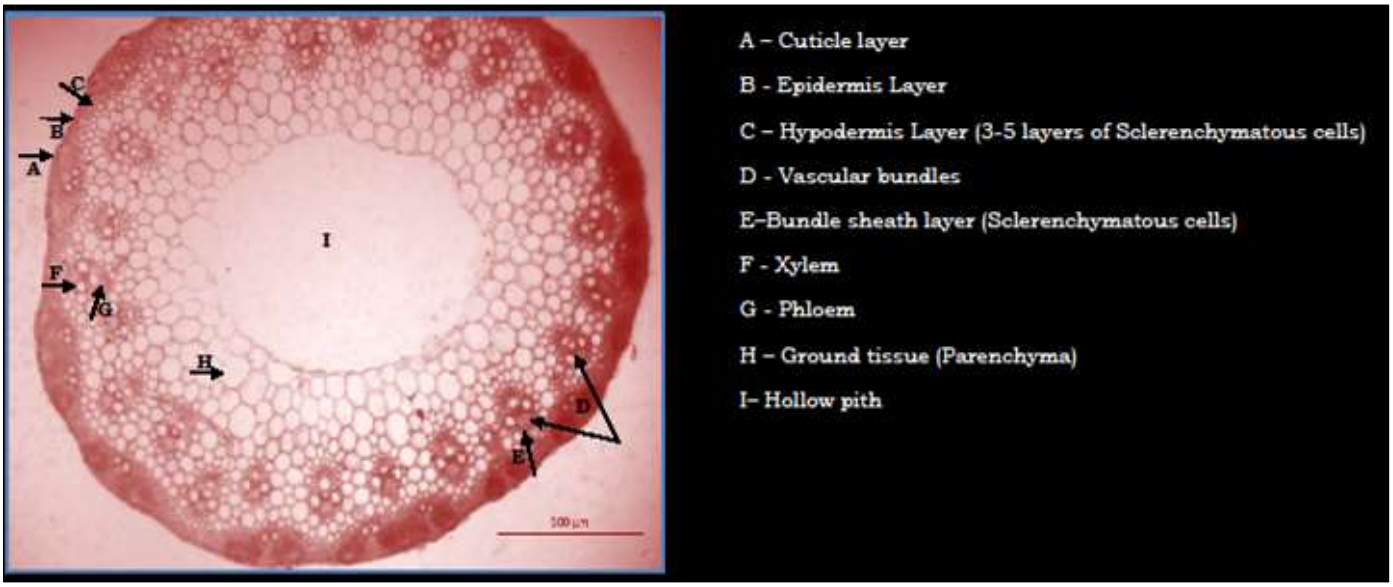

Plate 1: Transverse section of wheat stem var. Phule samadhan

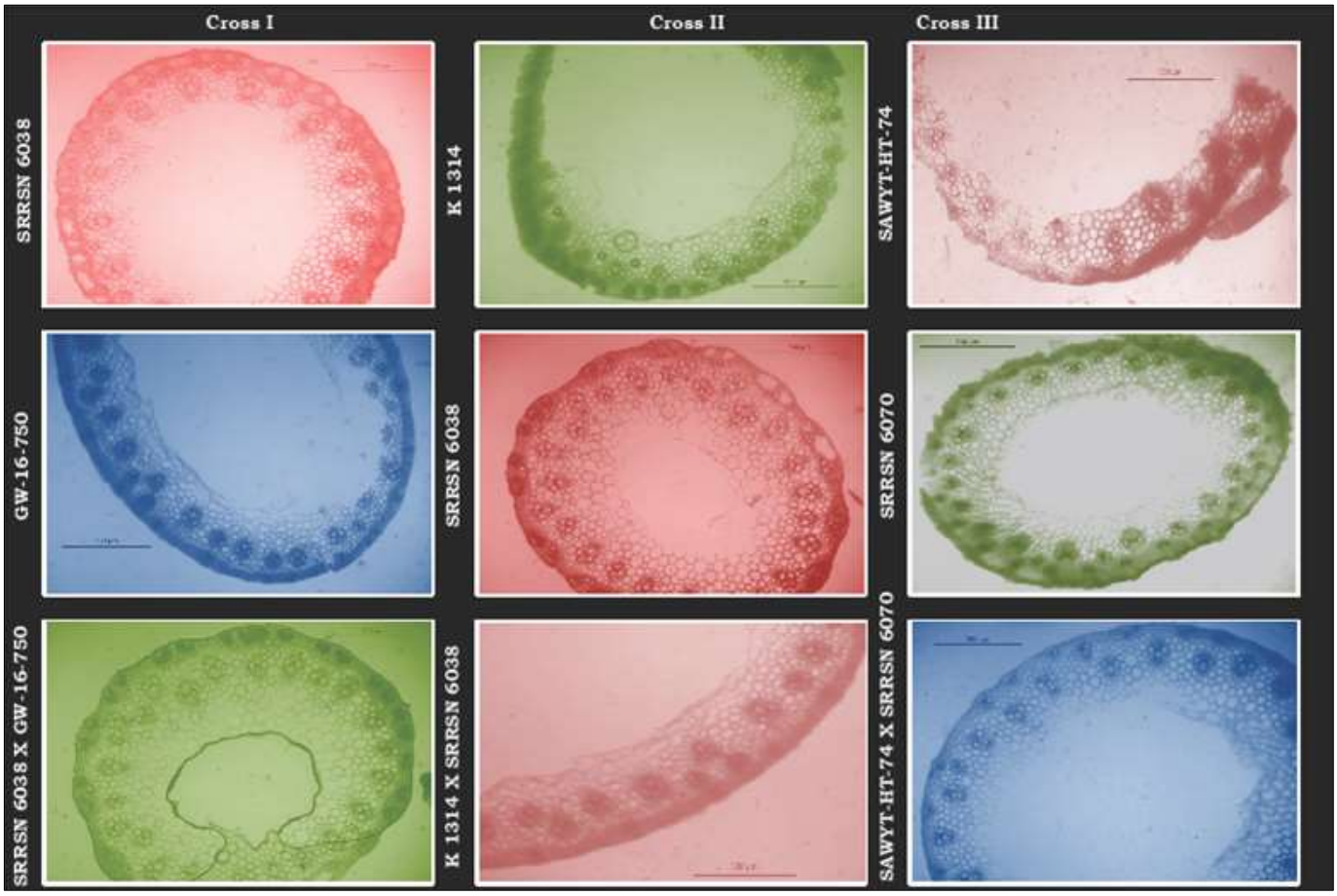

Plate 2: Stem anatomy of wheat present and hybrid

Conclusion

Stem anatomical character: It has been concluded that among parents, Genotype K 1314 showed numerical superior epidermis thickness (25.11µ) and maximum vascular bundle thickness (166.69µ), genotype GW-16-750 showed numerical superior phloem tissue thickness (50.35µ) and showed highly significant ground tissue thickness (333.88µ). Genotypes SAWYT-HT-74 (33.26µ) followed by genotype GW-16-750 (33.11µ) showed significant xylem vessels thickness. Among all 3 F_1, cross SRRSN 6038 x GW-16-750 showed maximum ground tissue thickness (350.68µ), numerical superior epidermis thickness (25.32µ), highly significant phloem tissue thickness (53.51µ), highly significant vascular bundle thickness (208.29µ). Crosses SRRSN 6038 x GW-16-750 (42.68µ) and K 1314 x SRRSN 6038 (42.26µ) showed highly significant xylem vessels diameter, Heterosis: The extent and magnitude of various heterotic effect in percentage over mid parent (RH) better parent (HB) and over check hybrid (SH) varied with character to character and cross to cross. Cross SRRSN 6038 x GW-16-750 showed best heterotic effect over standard check (SH) for all 9 qualitative and 5 stemanatomical characters. Cross SRRSN 6038 x GW-16-750 showed best heterotic effect over mid parent (RH) for epidermal thickness (µ) and phloem tissue thickness (µ) while, cross SAWYT-HT-74 x SRRSN 6070 showed best heterotic effect over mid parent (RH) for vascular bundle thickness (µ), xylem vessels diameter (µ), ground tissue thickness (µ). Cross SAWYT-HT-74 x SRRSN 6070 showed best heterotic effect over better parent (HB) for ground tissue thickness (µ). Cross SRRSN 6038 x GW-16-750 showed best heterotic effect over better parent (HB) forstem epidermal thickness (µ), phloem tissue thickness (µ), vascular bundle thickness (µ), xylem vessels diameter(µ) while, cross SAWYT-HT-74 x SRRSN 6070 showed best heterotic effect over better parent (HB) for ground tissue thickness (µ).

Severity of stem rust in field condition: Genotype SAWYT-HT-74 (20S; 5S) showed susceptible reaction at both the locations at field condition similar reaction was also observed in anatomical characters. Genotype K 1314 (5R; TR), GW 16-750 (5R; TR), SRRSN 6070 (5R; TR) and cross SRRSN 6038 x GW 16-750 (10R; TR) was resistant at both the locations and were found resistant anatomically. Genotype SRRSN 6038 (10MR; TMR) was moderately resistant at both the locations and were found resistant anatomically. Cross K 1314 x SRRSN 6038 (5MR; 5R), SAWYT-HT-74 x SRRSN 6070 (5MR; 5R) and check variety Phule Samadhan (5MR; TR) recorded moderately resistant and resistant type infection at Niphad and Kolhapur, respectively. Similar, rust reaction was also recorded anatomically.

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