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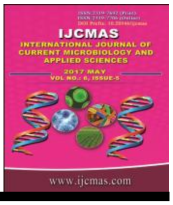

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Effect of Stem Rust Resistance Gene *Sr2+* on Spot Blotch (*Bipolaris sorokiniana*) of Wheat and Triticale

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ABSTRACT

Keywords

Stem rust,
Resistant gene,
Sr2+, *Puccinia graminis* f. sp. tritici,
Spot blotch,
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Spot blotch caused by *Bipolaris sorokiniana* is major disease problem in warm and humid areas of South Asian and other countries. Host resistance is an effective method to manage it and varieties with resistance to spot blotch and rusts are preferred. The stem rust resistant gene *Sr2* along with other *Sr* genes is widely used in wheat and Triticale breeding programme to provide resistance against stem rust pathotypes including new Ug99 (TTKS). The presence of *Sr2+* is also associated with morphological marker, pseudo black chaff (PBC) character, a dark pigmentation on the glumes, peduncle and below stem internodes of wheat. A total of 136 numbers of diverse genotypes of wheat and triticale having *Sr2+* gene in sole and in combination with other *Sr* genes (*Sr5+*, *Sr5+*, *Sr7b+*, *Sr8b+*, *Sr9b+*, *Sr9e+*, *Sr11+*, *Sr13+*, *Sr24+*, *Sr30+*, *Sr31+*) were evaluated against spot blotch under artificially inoculated and epiphytotics at ten hot spot locations in northern plains agro ecological zones in India during 2000-01 to 2011-12 crop seasons. Based on scoring of spot blotch severity on top two leaves at mild milk stage, the genotypes were categorization of in to R, MR, MS and S category. A total of 69.9% of genotypes possessing *Sr2+* genes were susceptible to spot blotch. Twenty genotypes possessing gene combinations of *Sr2+5+*, *Sr2+5+24+*, *Sr2+5+3*, *Sr2+8b+11+*, *Sr2+9e+11+*, and *Sr2+24+* were all susceptible to spot blotch. The resistant and susceptible genotypes with *Sr2+* gene were 30.1 and 69.9%, respectively. The results suggests that *Sr2+* and other stem rust resistant genes may be used cautiously in varieties meant for warm and humid regions where spot blotch and leaf rust are major diseases.

Introduction

The spot blotch caused by *Bipolaris sorokiniana* (Sacc.) Shoemaker in wheat and Triticale is a major disease problem in warmer and humid regions of India, Bangladesh and other South Asian countries. It causes losses up to 50% in grain yield and deteriorates seed quality. The host resistance is most effective and easily adopted way to manage the losses caused by this disease in grain and seed crop besides use of fungicides as pre sowing seed treatment and foliar sprays. Stem rust caused *Puccinia graminis* Pers.: Pers. f. sp. *tritici* Eriks. & E. Henn. is also one of the most devastating diseases affecting wheat yields globally. *Sr2* is a stem rust resistance gene that has been used in breeding for around 60 years as a source of durable and broad-spectrum adult plant resistance, which includes resistance to Ug99 and its related isolates. *Sr2* is located on the short arm of chromosome 3B and confers partial resistance only in the homozygous state (recessive resistance gene). It was originally transferred from Yaroslav emmer wheat into hexaploid wheat. Stem rust had been effectively managed using different resistant genes in wheat cultivars including *Sr2*. The new race of stem rust, Ug99 (TTKS), has been found infecting wheat having *Sr31* and *Sr38* genes (Pretorius *et al.*, 2000). The use of *Sr2* along with other resistant genes has been advocated to breed wheat varieties resistant to Ug99 (Singh *et al.*, 2011). The stem rust resistant gene, *Sr2* is recessive in nature and therefore difficult to incorporate. One partially dominant morphological marker linked to *Sr2* is the pseudo-black chaff (PBC) character. The PBC is a dark pigmentation that occurs on the glumes, peduncle and below stem internodes, but its levels of expression vary with genetic backgrounds and environments. A typical example of this is old popular variety 'Sonalika' in India. However, Sonalika has been found highly susceptible to spot blotch. The studies were therefore conducted to assess the performance of genotypes of wheat and Triticale having *Sr2* gene and its combinations with other *Sr* genes against spot blotch under artificially inoculated conditions at hot spot locations over years.

Materials and Methods

A total of 136 wheat (*Triticum aestivum* L. emend Fiori. & Paol., *T. durum* Desf., *T. dicoccum* Schrank *ex* Schübl.) genotypes and varieties including three genotypes of Triticale known to posses *Sr2* resistant gene in single or in combinations with other *Sr* genes, *Sr5+*, *Sr5+*, *Sr7b+*, *Sr8b+*, *Sr9b+*, *Sr9e+*, *Sr11+*, *Sr13+*, *Sr24+*, *Sr30+*, and *Sr31+* were planted in a meter line each and were grown as per recommended agronomic practices at hot spot multi locations of spot blotch at Karnal, Ludhiana, and Kaul, in North Western Plains zone, Faizabad, Varanasi, Sabour, Pusa, Shillongani, Coochbehar, and Kalyani in North Eastern Plains Zone during 2000-01 till 2011-12 crop seasons. A highly susceptible check genotype Raj 4015 was also repeat planted after every 20 test genotypes. The disease epiphytotics of spot blotch were created by artificially inoculating the spore suspension of *Bipolaris sorokiniana* isolates BS1, BS2, and BS9) initially at tillering and continued till boot leaf stage at 20 days intervals till disease appeared properly on check genotype. The spot blotch score was recorded in 0-9 double digit scale at mild milk stage according to method given by Singh and Kumar (2005). The categorization of resistance against spot blotch was done after taking the average and highest score over locations each year and later a mean of average and highest scores. The average was calculation by taking the both digits separately and value >0.5 was counted as one score. In case of average and highest score for categorization, the maximum score in each was used to categorize the resistance as per method of Singh *et al.*, (2016).

Spot blotch score		Category
Average score	Highest score	
00-13	Up to 35	Highly resistant (HR)
14-35	Up to 57	Resistant (R)
36-57	Up to 68	Moderately resistant (MR)
58-69	up to 79	Moderately susceptible (MS)
>69	>79	Susceptible (S)

Total numbers and percentage of resistant (R), moderately resistant (MR), moderately susceptible (MS) and susceptible (S) genotypes to spot blotch are calculated in case of each gene combination of stem rust genes.

Results and Discussion

The genotypes found R, MR, MS and S to spot blotch were 16, 25, 32 and 63, respectively.

Table.1 Presence of *Sr2* and its combination of genes in wheat and Triticale genotypes and their level of resistance to spot blotch

Rust resistant genes	**Total genotypes**	**Genotypes having different levels of resistance to spot blotch**											
		R		**MR**		**R+MR**		**MS**		**S**		**MS+S**	
		Nos.	**%**	**Nos.**	**%**	**Nos.**	**%**	**Nos.**	**%**	**Nos.**	**%**	**Nos.**	**%**
Sr2+	56	5	8.9	12	21.4	17	30.3	11	19.7	28	50.0	39	69.7
Sr2+5+	1	0	0.0	0	0.0	0	0.0	0	0.0	1	100.0	1	100.0
Sr2+5+24+	1	0	0.0	0	0.0	0	0.0	0	0.0	1	100.0	1	100.0
Sr2+5+31+	8	0	0.0	0	0.0	0	0.0	6	75.0	2	25.0	8	100.0
Sr2+7b+	2	1	50.0	0	0.0	1	50.0	0	0.0	1	50.0	1	50.0
Sr2+8b+	1	0	0.0	1	100.0	1	100.0	0	0.0	0	0.0	0	0.0
Sr2+11+	9	1	11.1	2	22.2	3	33.3	0	0.0	6	66.7	6	66.7
Sr2+13+	2	2	100.0	0	0.0	2	100.0	0	0.0	0	0.0	0	0.0
Sr2+8b+11+	1	0	0.0	0	0.0	0	0.0	0	0.0	1	100.0	1	100.0
Sr2+8b+9b+11+	5	1	20.0	0	0.0	1	20.0	1	20.0	3	60.0	4	80.0
Sr2+9b+	1	0	0.0	1	100.0	1	100.0	0	0.0	0	0.0	0	0.0
Sr2+9e+	8	1	12.5	1	12.5	2	25.0	1	12.5	5	62.5	6	75.0
Sr2+9e+11+	3	0	0.0	0	0.0	0	0.0	0	0.0	3	100.0	3	100.0
Sr2+24+	6	0	0.0	0	0.0	0	0.0	2	33.3	4	66.7	6	100.0
Sr2+30+	1	0	0.0	1	100.0	1	100.0	0	0.0	0	0.0	0	0.0
Sr2+31+	31	5	16.1	7	22.6	12	38.7	11	35.5	8	25.8	19	61.3
Total	136	16		25		41		32		63		95	
%		11.7		18.4		30.1		23.6		46.3		69.9	

R-Resistant, MR-Moderately Resistant, MS-Moderately Susceptible, S-Susceptible

Table.2 Leaf blight score in double digit scale (0-9) of wheat genotypes and released varieties possessing *Sr2+* gene and its combination of genes

S. No.	**Genotype/Variety**	**Spot blotch score**		**Catego-ry**	**S. No.**	**Genotype/ Variety**	**Spot blotch score**		**Catego-ry**
		HS	**Av.**				**HS**	**Av.**	
Sr2+ gene									
1	AKDW 2997-16 (d)	99	67	S	24	RAJ 4005	89	46	S
2	COW (W) 1	99	45	S	25	RAJ 4037	89	67	S
3	DDK 1022 (dico)	89	46	S	26	UP 2565	89	68	S
4	GW 337	99	67	S	27	VL 824	89	46	S
5	GW 349	99	78	S	28	VL 875	99	57	S
6	HD 2833 ©	99	68	S	29	DBW 14 ©	78	46	MS
7	HD 2864 ©	99	78	S	30	DDK 1029 (dico) ©	79	46	MS
8	HD 2874	99	79	S	31	GW 366 ©	79	46	MS
9	HD 2891	89	46	S	32	HD 2868	79	68	MS
10	HD 3093	89	35	S	33	HI 1535	79	57	MS
11	HD 4672	89	46	S	34	HI 8498 (d) ©	78	46	MS
12	HI 1514	99	46	S	35	HS 436	78	57	MS
13	HI 1563 ©	99	46	S	36	HW 5001	79	57	MS
14	HW 2044 ©	89	56	S	37	HPW 155	79	47	MS
15	HW 3070 ©	89	56	S	38	HUW 468	78	57	MS
16	HW 3080	99	68	S	39	HI 1544	79	57	MS
17	HUW 652	89	45	S	40	DT 46 (T) ©	68	36	MR
18	HUW 510	99	67	S	41	HD 2781 ©	68	46	MR
19	MP 4010 ©	99	57	S	42	HD 3086	67	35	MR
20	NIAW 1773	89	35	S	43	HI 1531	68	45	MR
21	PBW 175 ©	89	57	S	44	HI 1571 ©	58	36	MR
22	PBW 575	89	56	S	45	HS 431	68	46	MR
23	RAJ 3765 ©	89	67	S	46	HUW 533	68	46	MR
47	K 0307 ©	68	35	MR	70	K 0906	89	46	S
48	K 9644	67	45	MR	*Sr2+8b+9b+11+*				
49	PBW 589	67	35	MR	71	HD 2329 ©	99	79	S
50	TL 2934 (T)	68	57	MR	72	VL 832	89	35	S
51	TL 2942 © (T)	68	24	MR	73	VL 852	99	68	S
52	DBW 16 ©	47	24	R	74	HD 2865 ©	79	57	MS
53	HD 3075	57	35	R	75	K 1016	56	35	R
54	HD 3098	46	24	R	*Sr2+9b+* genes				
55	LOK 62	47	35	R	76	HS 490 ©	67	34	MR
56	UAS 320 (d) ©	57	34	R	*Sr2+9e+*				
Sr2+5+ genes					77	HI 8638	78	57	S
57	K 9107 ©	79	46	S	78	MACS 2846 ©	89	57	S

Sr2+5+ 24+ genes					79	PDW 233 (d) ©	89	57	S
58	DL 788-2 ©	99	67	S	80	RAJ 1555 ©	89	57	S
Sr2+5+31+ genes					81	NIDW 295 (d) ©	89	56	S
59	HS 445	99	68	S	82	WH 896 (d) ©	79	46	MS
60	PBW 509	99	57	S	83	WH 1080 ©	67	35	MR
61	PBW 519	99	68	S	84	HI 8703	56	35	R
62	VL 849	99	68	S	*Sr2+9e+11+* genes				
63	HS 375 ©	68	46	MR	85	GW 173 ©	99	57	S
64	PBW 343 ©	68	35	MR	86	GW 1189 (d)	89	68	S
65	PBW 373 ©	68	46	MR	87	HD 2285 ©'	99	78	S
66	PBW 502 ©	78	46	MS	*Sr2+11+* genes				
Sr2+7b+ genes					88	HI 8627 ©	99	79	S
67	WHD 948 (d)	89	45	S	89	K 8027	89	47	S
68	MACS 3828	57	34	R	90	LOK 1 ©	99	68	S
Sr2+8b+ genes					91	MP 3336	89	46	S
69	HS 295 ©	68	46	MR	92	PDW 291 (d) ©	89	68	S
Sr2+8b+11+					93	SONILAKA ©	89	68	S
94	GW 322 ©	68	46	MR	113	UP 2425 ©	89	57	S
95	HPW 266	68	46	MR	114	HD 2687 ©	79	46	MS
96	AKDW 4749 (d)	57	34	R	115	HS 240 ©	79	46	MS
Sr2+13+ genes					116	HW 5205	79	45	MS
97	PDW 329 (d)	47	24	R	117	HUW 549	79	35	MS
98	UPD 93 (d)	47	24	R	118	MACS 2496 ©	79	57	MS
Sr2+24+ genes					120	NIAW 917 ©	79	56	MS
99	LOK 45	99	67	S	121	PBW 396 ©	78	46	MS
100	PBW 524	99	68	S	122	PBW 533 ©	79	68	MS
101	RAJ 4125	79	46	S	123	PBW 573	79	46	MS
102	SKW 196 ©	99	78	S	124	UP 2822	79	35	MS
103	HI 1500 ©	78	35	MS	125	HW 5204	68	35	MR
104	HW 2004 ©	79	46	MS	126	HPW 251 ©	68	36	MR
Sr2+30+ genes					127	KRL 283	67	35	MR
105	HD 3076	68	24	MR	128	KRL 327	67	35	MR
Entries with *Sr2+31+* genes					129	PBW 547	68	45	MR
106	GW 344	89	58	S	130	PBW 581	67	45	MR
107	HS 365 ©	89	68	S	131	WH 1021 ©	68	46	MR
108	HS 459	99	46	S	132	HD 2733 ©	56	35	R
109	HW 1085	89	57	S	133	NIAW 1594	57	24	R
110	PBW 532	89	58	S	134	PBW 593	57	34	R
111	PBW 561	89	46	S	135	UP 2691	57	24	R
112	UAS 259	89	46	S	136	VL 738 ©	57	35	R

© -Released varieties

Fig.1 Per cent entries having stem rust resistant genes and resistant to spot blotch

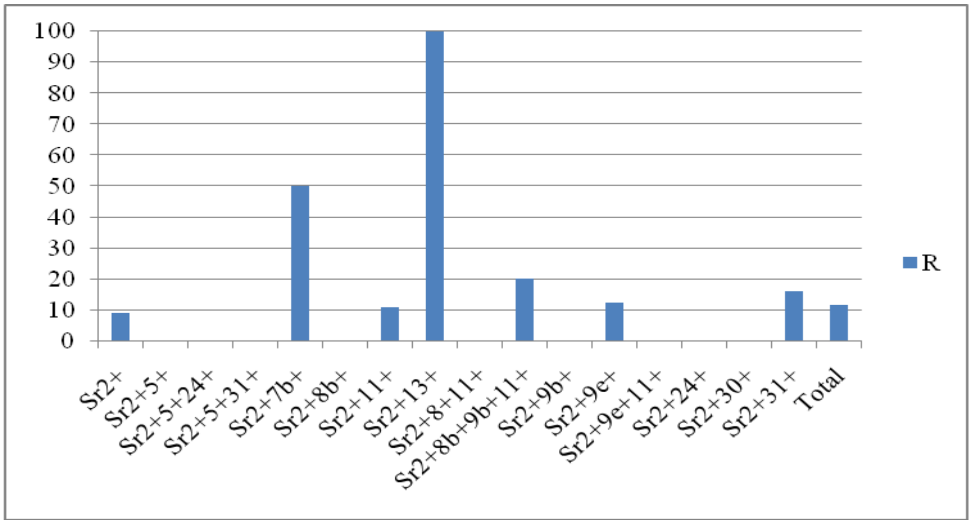


Fig.2 Per cent entries having stem rust resistant genes and moderately resistant to spot blotch

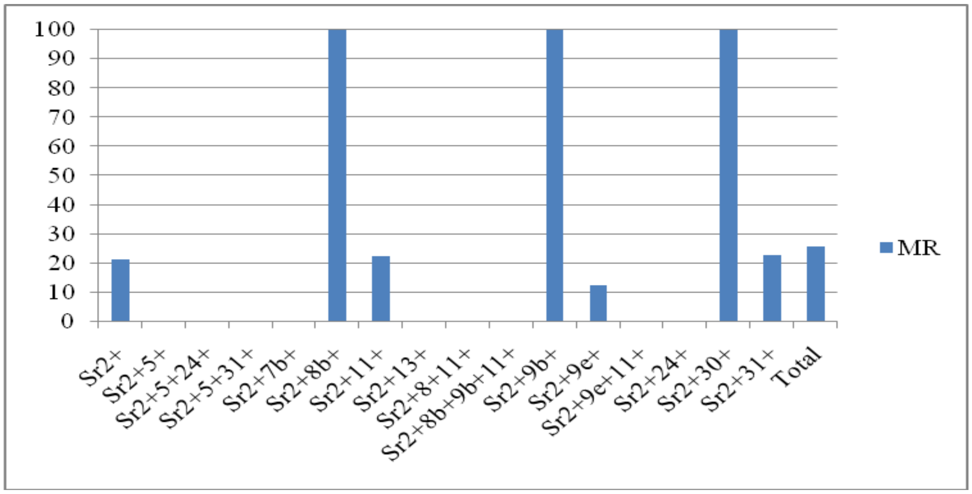


Fig.3 Per cent entries having stem rust resistant genes and moderate susceptibility to spot blotch

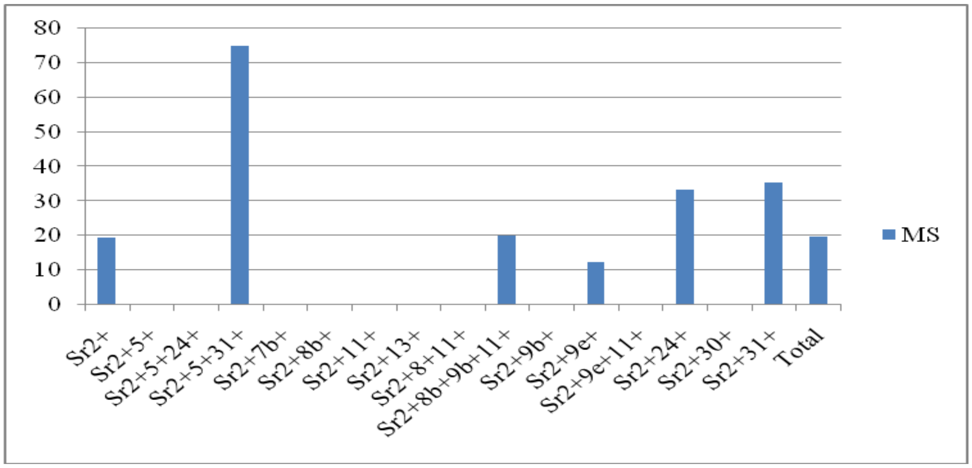


Fig.4 Per cent entries having stem rust resistant genes and susceptibility to spot blotch

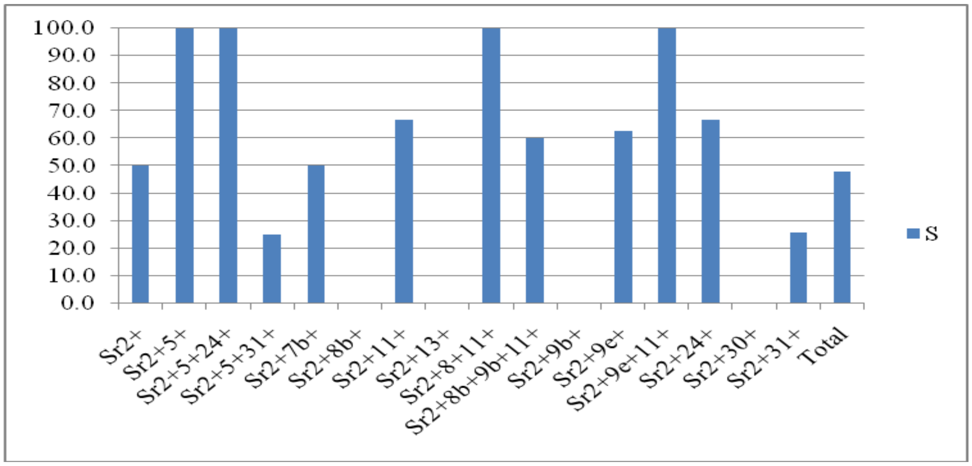


Fig.5 Per cent entries having stem rust resistant genes and R and MR to spot blotch

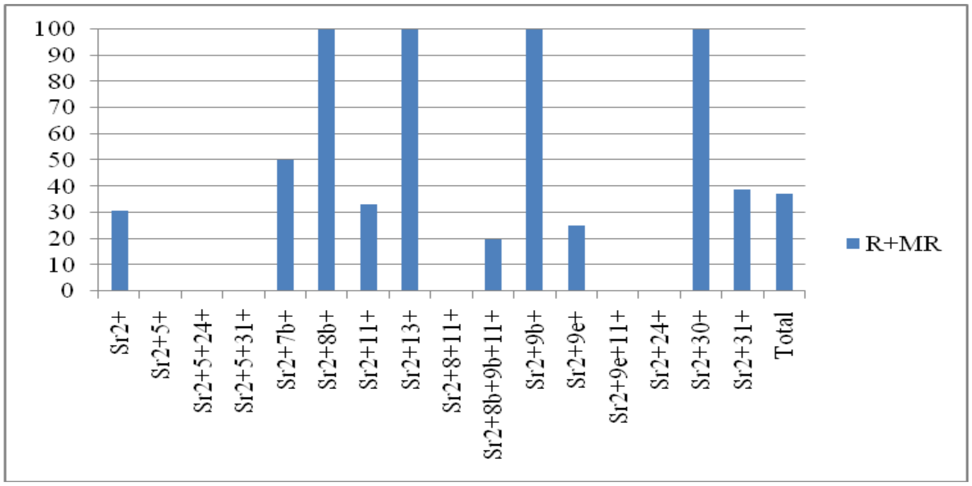


Fig.6 Per cent entries having stem rust resistant genes and MS and S to spot blotch

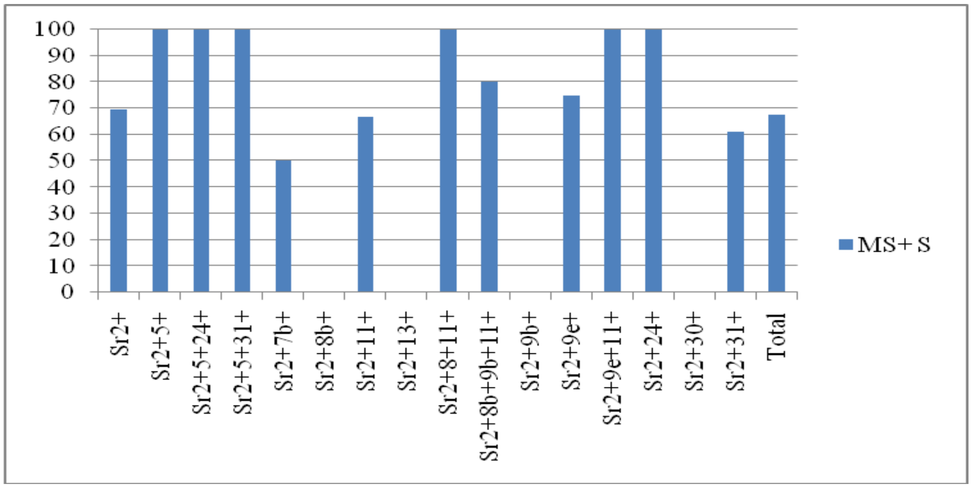
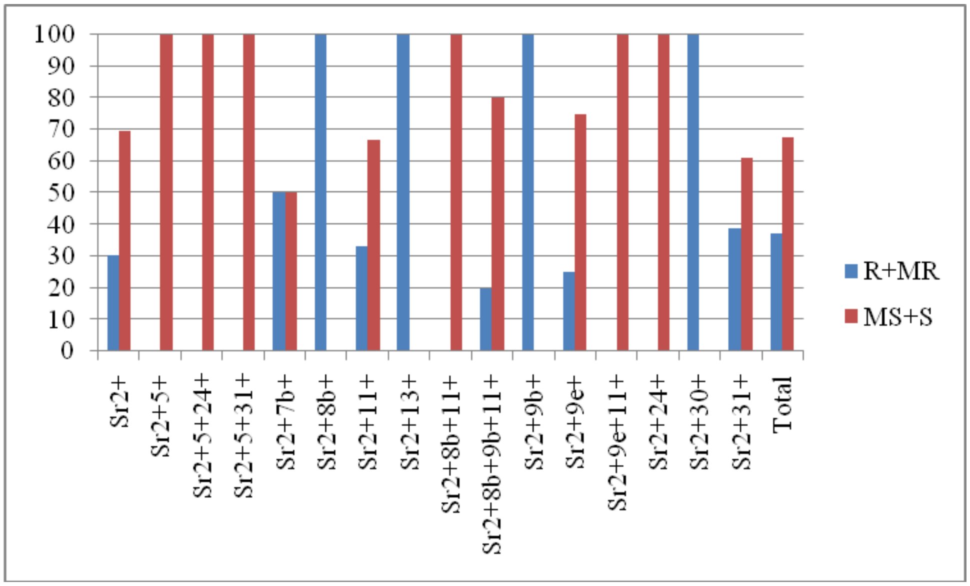


Fig.7 Degree of resistance to spot blotch in genotypes possessing different combinations of stem rust resistant genes



The resistant (R+MR) genotypes were 30.1% whereas susceptible (MS+S) were 69.9%. Twenty genotypes possessing gene combinations of *Sr2+5+*, *Sr2+5+24+*, *Sr2+5+3*, *Sr2+8b+11+*, *Sr2+9e+11+*, and *Sr2+24+* were all susceptible to spot blotch (Tables 1, 2, Figures 1-7). The genotypes with stem rust resistant gene combinations of *Sr2+8b+9b+11+*, *Sr2+9e+*, *Sr2+*, *Sr2+11+* and *Sr2+31+* were showing spot blotch susceptibility of 80.0, 75.0, 69.7, 66.7 and 61.7%, respectively.

Traditionally, Pseudo black chaff (PBC) has been used during rust breeding programmes morphological marker for the presence of *Sr2*. However, as its use is limited due to its partial dominance and variable level of expression in different genetic and environmental backgrounds (Bhowal and Norkhede, 1981). The *Sr2* gene is effective in the adult plant stage against all known pathotypes of stem rust including recently described Ug99 race and its variants in wheat (Singh *et al.*, 2011). Some gene specific and closely linked molecular markers for stem rust resistance genes including *Sr2* have been reported (Spielmyer *et al.*, 2003; Mago *et al.*, 2011). Bhardwaj *et al.*, (2011) reported gene matching of Indian wheat varieties for the presence of the *Sr2* gene under All India Coordinated Research Project on Wheat and Barley (AICRPWB). Out of 135 selected varieties, *Sr2* gene matching was in 71 varieties and *Sr2* has been found in 38 varieties released for North-west and central-southern regions of India. Among these 38 genotypes, presence of *Sr2* was confirmed in 35 genotypes with molecular markers CsSr2 and GWM533 (Bhardwaj *et al.*, 2011; Mago *et al.*, 2011). During host pathogen interaction study, scores of only 4 and 5 were found to be apparent observation for precise confirmation of *Sr2* during seedling chlorosis test (Brown, 1997).

The *Sr2* gene is located on short arm of wheat chromosome 3B which has provided broad-spectrum protection against stem rust since its introgression from Yaroslav (*Triticum turgidum* var. *dicoccum*) to Hope genotype. This gene has been widely deployed in Indian wheat breeding program along with *Sr9*, *Sr11* and *Sr31* genes to ensure durable resistance against stem rust, especially in varieties released for central and Southern regions of

India (Prashar *et al.,* 2008). The stem rust resistance genes (*Sr2*, *Sr24*, *Sr26*, *Sr38*, *Sr39*, *Sr31*, and *Sr1RSAmigo*) have been found resistant to Ug99 in few Iranian wheat cultivars (Mohammadi *et al.*, 2013).

Keeping in view of wide use of *Sr2* and other *Sr* genes, for management of stem rust, it is important to choose right combination of genes with *Sr2* to avoid high susceptibility of these to spot blotch. Further genetic and morphological studies may help to prove these observations.

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