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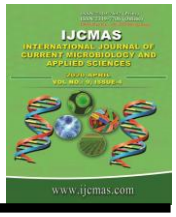

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Screening Elite Wheat Lines (*Triticum aestivum* L.) Possessing *Sr2* Gene for Variability in Expression of Pseudo Black Chaff

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A B S T R A C T

Stem rust is one of the most prevailing diseases of wheat crop severely affecting yield. The disease constrains wheat production and henceforth lags the demand. Identifying genes providing resistance from stem rust has been quite important in present scenario. One such gene providing resistance to stem rust is *Sr2* which has provided durable, broad-spectrum, adult plant resistance and has been used as a potential control measure against wheat stem rust in modern wheat breeding programs worldwide. PBC is morphological marker tightly linked to *Sr2* causes varying degrees of dark pigmentation on the stem internodes and glumes, is partially dominant and has been commonly used to select the presence of the gene in wheat germplasm. The present study has been designed for screening 30 elite wheat lines with *Sr2* gene for their variable expression with morphological marker PBC.

Introduction

Wheat (*Triticum aestivum* L. em Thell.) is the most important staple food crop in the world. It supplies about 25% of protein and 20% of the calories consumed by human beings from the daily diet. However, India is the second largest wheat producer in the world after China for the last 10 years (Braun *et al.*, 2010; Hawkesford *et al.*, 2013; ICAR-IIWBR, Annual Report-2015). Central Statistics Office (CSO), Government of India, has estimated 271.9 MT of the total food grain production in the year 2016-17. The national food demand is expected to increase drastically, similarly, the wheat demand is projected to increase 60% by 2050. Increasing production and productivity of any crop is not an easy task. There are several factors which directly or indirectly affect crop yield and comprehensively decrease wheat production by 29% globally (Rosegrant *et al.*, 1995;

Singh *et al.,* 2011). These yield-reducing factors can be broadly grouped into two groups- biotic and abiotic factors. Biotic factors include various types of diseases caused by fungi, bacteria, nematodes and insects while abiotic factors include various types of stresses like drought, heat, salinity, frost, nutrients excess or deficiency. Among the biotic factors, fungal diseases are most devastating in nature, contrastingly, wheat rusts are severely responsible to cause yield loss. Rusts are of three types; *viz.*, black rust (caused by *Puccinia graminis* f.sp. *tritici*), yellow rust (caused by *Puccinia striiformis*) and brown rust (*Puccinia recondita*). Among all rust diseases, the wheat stem rust or very often known as black rust is a significant disease that affects cereal crops including bread wheat, durum wheat, barley and triticale. Since pathogen evolves itself during the course of time with varied levels of virulence against prevailing resistance genes. About 58 stem rust resistance genes are catalogued till date (McIntosh *et al.,* 2005) in wheat. In recent studies, a total of five numerically designated wheat stem rust resistance genes; Sr2, Sr55 (Lr67/Yr46/ Pm46), Sr56, Sr57 (Lr34/Yr18/Pm38), and Sr58 (Lr46/Yr29/Pm39) along with several QTL associated with wheat stem rust resistance in diverse germplasm (Bhavani *et al.,* 2011; Njau *et al.,* 2013; Rouse *et al.,* 2014; Singh *et al.,* 2013a, b, c), has been reported. In the conventional wheat breeding program, selection for this gene has been performed so far through the use of the linked pseudo-black chaff (PBC) phenotype. Pseudo-black chaff or melanism or false black chaff is a partial dominant in nature and resulting from a deposition of melanoid pigments and is completely associated with the presence of the stem rust resistance gene *Sr2* (Sheen *et al.,* 1968; Hare and McIntosh, 1979; Kota *et al.,* 2006). Symptoms include brown to black discolouration of the glume extending from slight longitudinal marks to large black areas covering most of the glume surface. In the severe expression, the stem area below the last node may become distinctly brown to black discolouration. In addition, the trait appears post flowering with a peak at late milk soft dough stage. The symptom of pseudo-black chaff has a variable level of expression in different genetic backgrounds and environments (Hare and McIntosh, 1979). It appears frequently on glumes but also on peduncle and stem. High level of PBC expression (especially on glumes) also thought to be potent enough to reduce crop yield and makes it undesirable for farmers (Hare and McIntosh, 1979; Sheen *et al.,* 1968). During breeding program, presence of the *Sr2* gene can be confirmed by the PBC but it can also be checked at the molecular level. Several molecular markers linked with the *Sr2* gene have been identified. Microsatellite marker Xgwm533 was found to be tightly linked to the *Sr2* gene. The SSR marker, Xgwm533 largely used for the detection of the *Sr2* in the wheat germplasm. The expression of PBC can be manipulated on the molecular basis of Sr2 while maintaining adequate level expression of rust resistance (Kota *et al.,* 2006). Keeping these points in mind, the present investigation has been designed for screening elite wheat lines (*Triticum aestivum* L.) possessing *Sr2* gene for variability in expression of Pseudo Black Chaff.

Materials and Methods

The present was carried out at the Agricultural Research Farm, Institute of Agricultural Sciences (I. Ag. Sc.), B.H.U., Varanasi during Rabi season 2016-17. The experiment was conducted in a Randomized Complete Block Design with 30 elite lines of wheat in three replications on protected with fungicide (Tebuconazole formulation grade@1g/ liter) and non-protected conditions respectively.

Table.1 Comparison between the genotype having Sr2 gene in protected condition

Days to heading			Plant Height			Plot yield			Thousand grain weight		
	Ear	Ear + Peduncle +Lower internode		Ear + Peduncle	Ear + Peduncle +Lower internode		Ear + Peduncle	Ear + Peduncle +Lower internode		Ear	Ear + Peduncle +Lower internode
Mean	68.84	65.66	Mean	77.46	87.47	Mean	201.93	183.16	Mean	50.25	46.16
SD	3.86	2.73	SD	4.75	10.44	SD	34.12	35.76	SD	4.97	5.55
n	13	12	n	5	12	n	5	12	n	13	12
T Value	-1.8008		T Value	-2.0265		T Value	0.9978		T Value	1.9413	
P Value	0.0424		P Value	0.0304		P Value	0.1670		P Value	0.0322	
Result	Significant		Result	Significant		Result	Non-Significant		Result	Significant	

Table.2 Comparison between the genotype having Sr2 gene in non-protected condition

Days to heading			Plant Height			Plot yield			Thousand grain weight		
	Ear	Ear + Peduncle +Lower internode		Ear + Peduncle	Ear + Peduncle +Lower internode		Ear + Peduncle	Ear + Peduncle +Lower internode		Ear + Peduncle	Ear + Peduncle +Lower internode
Mean	67.53	70.52	Mean	79	87.47	Mean	178.33	220.88	Mean	52.54	46.58
SD	3.85	2.68	SD	4.64	12.53	SD	23.62	36.63	SD	7.37	4.40
N	13	12	n	5	12	n	5	12	n	5	12
T Value	-2.2315		T Value	-1.4469		T Value	-2.3751		T Value	2.0915	
P Value	0.0178		P Value	0.0824		P Value	0.0156		P Value	0.0269	
Result	Significant		Result	Non-Significant		Result	Significant		Result	Significant	

AUDPC		
	Ear	**Ear + Peduncle**
Mean	349.77	**324.36**
SD	136.80	**96.70**
N	13	**5**
T Value	**0.377**	
P Value	**0.355**	
Result	**Non-Significant**	

Observations were recorded for 6 characters, *viz.,* days to heading, plant height, plot yield, 1000-grain weight, area under disease progress curve (AUDPC) and pseudo-black chaff (PBC). Observations on 3 randomly selected plants from each genotype were collected and average data was taken into consideration. Molecular marker Xgwm533 was used to screen 30 lines for presence of *Sr2* gene.

Results and Discussion

In the present investigation, altogether total 30 genotypes (Appendix.1) were screened for the presence or absence of *Sr2* gene using *Xgwm533* marker in the experiment. Band pattern was visible at 120-bp. All the elite lines were showing the presence of this marker. Four traits *viz.,* DH, PH, PY, TGW were tested under both protected and non-protected condition (Table 1 and 2) and AUDPC was obtained under non-protected condition (Table 2). Pseudo-black chaff (PBC) was recorded in all the 30 genotypes possessing *Sr2* gene. There was a variable expression of PBC for all the genotypes. On the basis of phenotypic differences for PBC, all the 30 genotypes were distributed in 3 groups *viz.,* PBC on the ear only, on ear + peduncle and on the ear + peduncle + lower internode. All the 30 lines had PBC on the ear but only 13 genotypes out of it exhibited symptoms limited to ear only. Five genotypes showed PBC on both ear + peduncle whereas 12 genotypes could show PBC at all the 3 locations on the plant *i.e.,* ear + peduncle + lower internode. Impact of PBC was analyzed for 4 traits (days to heading, plant height, plot yield and 1000-grain weight), in protected condition by comparing T-test between means of traits of genotypes with ear, ear + peduncle and ear + peduncle + lower internode. Genotypes with PBC at all the 3 locations showed positive and significant impact for days to heading and plot yield. There was the positive and significant response for 1000-grain weight in those plants having PBC at only 2 places *i.e.,* on ear + peduncle. Positive but non-significant response for plant height was observed. AUDPC was reduced but non-significantly in genotypes that had symptoms only on ear + peduncle. McFadden, 1939; Hare and McIntosh, 1979; Kota *et al.,* 2006 supported the selection of desirable plants with help of presence of pseudo-black chaff.

Abbreviations: PBC- Pseudo Black Chaff; DH- Days to Heading; PT- Plant Height; PY- Plot Yield; TGW- Thousand Grain Weight; AUDPC-Area Under Disease Progress Curve.

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Appendix I.
Pedigree of 55 wheat genotype

1	**SONALIKA**
2	CROC_1/AE.SQUARROSA (205)//KAUZ/3/ENEIDA
3	ASTREB/OAX93.10.1//SOKOLL
4	CHIRYA.3
5	W15.92/4/PASTOR//HXL7573/2*BAU/3/WBLL1
6	SURUTU-CIAT
7	CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER /5/ PASTOR
8	MILAN/KAUZ/3/URES/JUN//KAUZ/4/CROC_1/AE.SQUARROSA (224)//OPATA
9	YAV_3/SCO//JO69/CRA/3/YAV79/4/AE.SQUARROSA (498)/5/2*OPATA
10	TILHI/SOKOLL
11	BCN/RIALTO
12	UP2338*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/MILAN/KAUZ//CHIL/ CHUM18/6/UP2338*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ
13	TILILA/TUKURU/4/SERI.1B*2/3/KAUZ*2/BOW//KAUZ
14	PBW 343/PASTOR
15	TILHI
16	NL 750
17	SW89-5124*2/FASAN
18	W462//VEE/KOEL/3/PEG//MRL/BUC
19	CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/ 2*KAUZ
20	JUPARE C 2001
21	ATTILA/3*BCN//BAV92/3/TILHI
22	ALTAR 84/AEGILOPS SQUARROSA (TAUS)//OPATA
23	ALTAR84/AE.SQUARROSA (219)//OPATA/3/WBLL1/FRET2//PASTOR
24	CIANO T 79
25	GAN/AE.SQUARROSA (897)//OPATA/3/BERKUT
26	ATTILA/3*BCN//BAV92/3/TILHI/4/SHA7/VEE#5//ARIV92
27	VORB/4/D67.2/PARANA 66.270//AE.SQUARROSA (320)/3/CUNNINGHAM
28	ASTREB/OAX93.10.1//SOKOLL
29	CMH79A.955/4/AGA/3/4*SN64/CNO67//INIA66/5/NAC/6/RIALTO
30	CROC_1/AE.SQUARROSA (205)//KAUZ/3/SASIA/4/TROST