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Inheritance Studies of Yellow Rust Resistance in Bread Wheat Genotypes for *Yr5* gene

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ABSTRACT: Yellow rust of wheat, caused by *Puccinia striiformis* Westend. f.sp. *tritici* poses a serious threat to quality and yield potential in cooler regions. The economic and environment friendly strategy to combat this disease is deployment of resistance genes. The aim of the study was to study the inheritance pattern of *Yr5* gene and test genetic linkage of marker *STS 7/STS 8* in F_2 segregating population derived from crosses between yellow rust susceptible parents HS 240 & DH 40 and resistant parent Avocet-*Yr5*. Two sets of F_2 segregating population derived from crosses HS 240 × Avocet-*Yr5* and DH 40 × Avocet-*Yr5* were evaluated phenotypically for their reaction to yellow rust disease under controlled conditions. The chi square analysis showed that resistance in segregating populations of two crosses was governed by single dominant gene and marker *STS 7/STS* can be utilized efficiently for selection of *Yr5* gene in the breeding material.

Keywords: Wheat, yellow rust, *Puccinia striiformis* Westend f.sp. *tritici*, *Yr5* gene, *STS7/STS8.*

INTRODUCTION

Wheat (*Triticum aestivum* L. em Thell) is an important cereal crop grown globally. Wheat production is a key component in sustaining global food security. Among various threats to wheat production, rust poses a serious problem to wheat cultivation worldwide. There are several evidences of increased yellow rust epidemics around the world which may be due to changing climatic conditions and increased adaptation of pathogen races. Yellow rust also known as stripe rust, caused by *Puccinia striiformis* Westend f.sp. *tritici* Eriks and Henn. (*Pst*), is an economically important foliar disease of wheat crop. In the past two decades, there has been global emergence of aggressive and genetically diverse pathogen populations which are adapted to warmer temperatures (Milus *et al*., 2009; Hubbard *et al*., 2015; Hovmøller *et al*., 2016). In India, yellow rust has become economically important in the recent past especially in cooler areas and is a threat in 10 mha area under Northern parts of India (Bhardwaj *et al*., 2019). Virulence on major seedling resistance genes including *Yr2*, *Yr6*, *Yr9*, *Yr11*, *Yr12*, *Yr17*, *Yr24* and *Yr27* has been reported (Wellings and McIntosh 1990; Nsabiyera *et al*., 2018; Gangwar *et al*., 2016). Only a few resistance genes are still effective against *Pst* races which urges the demand to develop durable resistant varieties. Deployment of resistant genes effectively and economically is important to reduce fungicide use and minimize crop losses. Yellow rust resistance genes have been identified progressively in wheat bringing the total number of catalogued genes to 70. Among all the R genes which are still effective against *Pst* races, *Yr5* is dominant seedling- expressed yellow rust R- gene originally identified in *T. aestivum* subsp. *spelta* var. *album* accession (Macer, 1963) and later to be shown in a number of spelta wheats (Kema, 1992). The gene is located on the long arm of chromosome 2B (Law, 1976). This gene can be used effectively in varieties grown in north western Himalayas in India where yellow rust poses havoc to wheat cultivation. In the present study, the inheritance pattern of *Yr5* gene was studied in the cross of Avocet-*Yr5* with the agronomically superior variety HS 240 and a doubled haploid genotype DH 40 which are suitable for cultivation in NWH zone but susceptible to yellow rust disease.

MATERIAL AND METHODS

The plant material for the study comprised of wheat genotypes HS 240 (spring wheat variety), DH 40 (a doubled haploid genotype developed by *Imperata cylindrica*- mediated doubled haploidy breeding technique (Chaudhary *et al.,* 2005), and Avocet-*Yr5* (resistant source for *Yr5* gene). DH 40 and HS240 were hybridized with Avocet-*Yr5* Two sets of F_2 population derived from crosses, HS 240 × Avocet-*Yr5* and DH 40 × Avocet-*Yr5* were tested for rust resistance and linkage with marker. The molecular marker used for the amplification was *STS7/STS8* (Chen *et al.*, 2003) (Table 1, Table 2).

Table 1: Molecular markers used for screening of targeted resistance genes.

Gene	Chromosome number	Marker	Type of marker	Primer sequence	Annealing temperature (°C)	Source
Yr 5	2B	*STS 7/STS8*	STS	F: GTACAATTCACCTAGAGT R: GCAAGTTTTCTCCCTATT	45	Chen *et al.* (2003)

Table 2: PCR conditions for the marker used for screening of targeted resistance genes.

Marker	Steps	Temperature and time		Cycles
STS 7/STS8	Initial denaturation	94 °C	4min	40
	Denaturation	94 °C	45sec	
	Annealing	45 °C	45sec	
	Extension	72 °C	60sec	
	Final extension	72 °C	10min	
	Storage	4 °C	∞	

A. Seedling resistance test

Seedling tests were conducted under controlled environment conditions. The parents and segregating generation were tested with pathotype110S119. Fully extended primary leaves were inoculated with the uredospore suspension. The seedlings were transferred to humid glass chamber for 48 hours. The inoculated seedlings were then transferred to glass house at about 15° C. The infection types were recorded 20 days after inoculation and were classified as resistant and susceptible according to Nayar *et al*. (1997). After phenotypic evaluation, F_2 populations were screened for analysis of marker gene association and inheritance of *Yr5* gene.

B. DNA isolation and PCR amplification

Genomic DNA was extracted from leaf samples as per CTAB method (Murray and Thompson 1980). The PCR reaction was performed in a total volume of 15µl, containing 100ng template DNA, 1× PCR Buffer, 2.5 mM $MgCl_2$, 0.2MM dNTP, 0.75U Taq DNA polymerase and 0.3µM of each primer. STS marker *STS7/STS8* was used for detection of *Yr5* gene (Chen *et al*., 2003). Amplification were performed in thermal Cycler at 94°C for 4 minutes followed by 40 cycles at 94°C for 45 seconds, 45°C for 45 seconds and 72°C for 60 seconds. A final elongation was performed at 72°C for 10 minutes. PCR products were analyzed by electrophoresis using 3% high resolution agarose gel melting in 1× TAE followed by staining with ethidium bromide and visualized with UV light.

C. Data Analysis

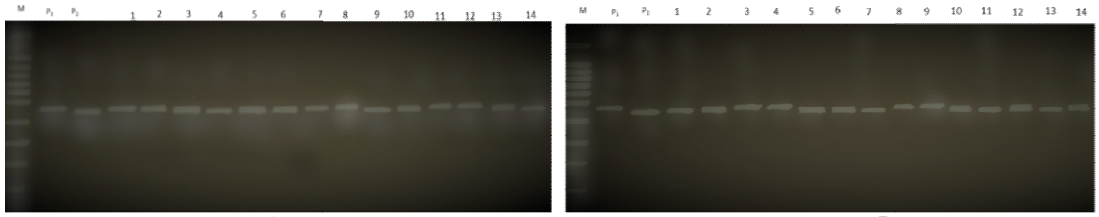
Chi square analysis was applied to check the validity of expected ratios to that of observed ratio in the segregating generation to test goodness of fit and investigate the inheritance of stripe rust resistance gene & molecular marker.

RESULTS AND DISCUSSION

In F_2 population derived from cross HS 240 × Avocet-*Yr5*, sixty three individuals exhibited resistant response and twenty four showed susceptible reaction. The segregation pattern in F_2 population developed from cross DH 40 × Avocet-*Yr5* revealed that sixty nine plants were resistant and twenty nine were susceptible to yellow rust (Table 3). The segregation ratio exhibited goodness of fit to 3:1 ratio in both the crosses. The segregation pattern was analogous to the ratio exhibited by single dominant gene. DNA samples from F_2 plants were analyzed to determine linkage between STS marker and resistant gene *Yr5*. STS marker *STS7/STS8* showed polymorphism in the parental genotypes. This marker was further used to analyze segregating ratio in F_2 individuals. The PCR amplification showed bands of 478bp in resistant homozygous individuals, 472bp in susceptible homozygous individuals and both the bands in heterozygous genotypes (Fig. 1). The resistant gene *Yr5* followed a segregation ratio of 1:3:1 with marker *STS7/STS8* in segregating F_2 population of crosses HS 240 × Avocet-*Yr5* and DH 40 × Avocet-*Yr5*. These results suggested that the yellow rust resistance to *Pst* strain is determined by a single dominant gene *Yr5*. There was no recombination between molecular marker and *Yr5* gene, indicating complete linkage between the two.

Table 3: Chi square analysis for segregation of resistance to stripe rust.

Cross	Number of Seedlings			Expected ratio	χ^2 value
HS 240 × Avocet-*Yr5*	**Resistant**	**Segregating**	**Susceptible**		
Phenotypic	63	-	24	3:1	0.62
Genotypic	27	36	24	1:2:1	0.25
DH 40 × Avocet-*Yr5*					
Phenotypic	69	-	29	3:1	0.29
Genotypic	24	45	29	1:2:1	0.56



A B

Fig. 1. (A) PCR Assay for stripe rust resistance gene *Yr5* in F_2 population of cross HS 240 × Avocet-*Yr5*. M- Marker, P_1- Avocet-*Yr5*, P_2- HS 240, 1 to 16= F_2 individuals of cross HS 240 × Avocet-*Yr5*. B: PCR Assay for stripe rust resistance gene *Yr5* in F_2 population of cross DH 40 × Avocet-*Yr5*. M- Marker, P_1- Avocet-*Yr5*, P_2- DH 40, 1 to 16= F_2 individuals of cross HS 240 × Avocet-*Yr5*.

CONCLUSION

R genes responsible for imparting genetic resistance to wheat yellow rust have proven to be ineffective after deployment. However, some genes like *Yr5* shows potential to combat the havoc caused by the *Pst* races. Inheritance pattern studies revealed that resistance in wheat genotypes genotypes is governed by the single resistance gene *i.e.Yr5*. STS marker *STS7/STS8* used in the study can identify individual gene and its co-segregation with the target gene indicate its possible use in recombining R genes as required.

FUTURE SCOPE

Yr5 is a race specific seedling resistance gene. It can be used in combination with other effective genes or with race non-specific adult-plant resistance genes which can be used to develop cultivars with durable resistance. Recent advances in molecular characterization of plant R- genes have underpinned the opportunities to develop diagnostic markers to combine major race-specific resistance with APR genes. The size of F_2 population used in the study was relatively small; therefore there is still scope to validate the results. The application of the marker will depend on the specificity of the actual marker allele associated with *Yr5* gene. Marker *STS7/STS8* can be used as ideal marker for marker assisted selection in future breeding endeavours.

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Conflicts of Interest. None.

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