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Genetics of stem rust resistance in common wheat genotypes WR95 and Selection T3336

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Abstract

Genetic stocks WR95 and Sel.T3336 were screened against most prevalent races of stem rust *viz.*, 40A, 40-1 and 117-6 at seedling stage under controlled conditions. Both the genetic stocks, WR95 and Sel.T3336 were found resistant to all the three races. Genetic analysis revealed that WR95 carries a single recessive gene for rust resistance. An effort was made to locate the gene using monosomic series, however, the gene proved to be hemizygous ineffective in monosomic F_1s. WR95 was also screened with validated molecular markers of the stem rust resistance genes *Sr24, Sr25, Sr26, Sr31, Sr36, and Sr38*. None of these genes could be detected in WR95 showing that the resistance gene present in WR95 is diverse from these genes. Sel T3336 showed segregation for one stem rust resistance gene against race 40-1. However, screening with molecular markers showed the presence of stem rust resistance genes *Sr*24 and *Sr*26 in Sel.T3336. The resistance identified in WR95 may prove very useful in breeding.

Key words: Wheat, stem rust, inheritance, molecular marker

Introduction

Though wheat is infected by several diseases, fungal diseases particularly rusts (*Puccinia* spp.) cause substantial damage to wheat crop under severe epidemic conditions and inflict yield reductions world over including India. Stem rust caused by *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & Henn., is known to cause significant yield losses in susceptible cultivars under the circumstances of adequate inoculum and congenial environmental conditions.

Stem rust is a warm-temperature disease that develops at optimum level between 20 and 35^{0}C. In India, central and peninsular regions are particularly prone to stem rust where favourable environmental conditions exist. About 55 stem rust resistance genes have been documented [1] so far. A large number of designated genes having their origin in *Triticum aestivum* have become ineffective to Indian pathotypes of stem rust. However, the alien genes (*Sr*24, *Sr*25, *Sr26, Sr27, Sr31, Sr36* and *Sr38*) derived from related genera and species confer moderate to high degree of resistance to the prevalent Indian stem rust races [2]. Breeding for rust resistance is the most economical way of controlling the disease because large scale use of fungicides is not practically feasible and also not eco-friendly. Identification and characterization of rust resistance sources is important for proper utilization of these sources in breeding programme. In this communication, characterization of the stem rust resistance has been attempted in two genetic stocks of common wheat.

Materials and methods

Plant material

The present investigation comprised two resistant genetic stocks, WR95 and Selection (Sel.)T3336 exhibiting resistance to stem rust under field conditions over the years. The genetic stock WR95 has been developed from the complex cross Kalyansona/Gigas//HD1999/Sonalika*3/*T. carthlicum,* whereas Sel.T3336 is derived from the cross Lok Bharti x DARF/Kite. Agra Local, Lal Bahadur and NI5439 were used as susceptible parents in genetic analysis.

Pathogen

Three pathotypes of *Puccinia graminis triticii viz.*, 40A, 40-1 and 117-6 were used for screening of WR95 and Sel.T3336 for resistance. These are the most commonly

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prevalent pathotypes in different wheat growing regions of India. The initial inoculum of all the pathotypes was obtained from DWR Regional Station, Flowerdale, Shimla and was multiplied on susceptible cultivar Agra Local in isolation in glass house at IARI, New Delhi. Stem rust pathotypes 40A and 40-1 were used for inheritance studies. The two resistant genetic stocks, WR95 and Sel.T3336 were also screened with validated molecular markers for stem rust resistance genes to identify genes conferring resistance.

Seedling test

For seedling testing with stem rust pathotypes, ten days old seedlings grown in pan trays were hand inoculated with talcum mixed urediospores of individual pathotype using lanceolate needle. Inoculated seedlings were kept in humidity chamber for 36h and then shifted to glass house benches at 22^0C mean temperature. Observations were recorded following the scale proposed by Stakman *et al.* [3].

DNA extraction, PCR amplification and Electrophoresis

Genomic DNA from WR95 and Sel.T3336 was isolated using CTAB method of Saghai-Maroof *et.al.* [4] with minor modifications. DNA concentration was estimated by Hoefer DYNA Quant 200 Fluorimeter (Hoefer Scientific San Fransisco, USA) using Hoechst 33258 (Bisbenzimide) as the fluorescent dye and calf thymus DNA as the standard [5]. The flourimeter was calibrated with 100 µg/ml calf thymus DNA solution in 2 ml of assay solution. The concentration of the sample under study was then measured directly in ng/ml by adding 2 µl of DNA sample to 2 ml of the assay solution. DNA sample was diluted with appropriate amount of sterilized water to yield a working concentration of 20 ng/µl and stored at –20°C until used for PCR amplification.

The details of the markers that were used for screening of stem rust resistance genes are given in the (Table 1). PCR amplification was carried out with 20 ng of genomic DNA, 2.5 mM $MgCl_2$, 1U Taq DNA polymerase, 1x PCR buffer without $MgCl_2$, 0.25 µM of each of primers and 200 µM of dNTPs. The volume was made up to 25 µl with sterile distilled water. PCR tubes containing the above components were capped and centrifuged at 10,000 rpm for 1 minute to allow proper settling of reaction mixture. For each primer, positive control and negative control were taken along with genetic stocks and were subjected to PCR in separate reaction mixture. Thermocycling was carried out in a PE-Thermocycler. To each PCR tube 2.5 µl of 6x loading dye was added. Agarose gel of required concentration was prepared according to the product size (Table 1) in case of each primer, in 1x TAE buffer with 4 µl ethidium bromide (10mg/ml) per 100 ml of gel volume. Electrophoresis was carried out at 70 V for 3hours till the bromophenol blue dye travelled less than $2/3^{rd}$ the length of gel. A 100bp ladder was used along the PCR product for comparing the product size. The resolved amplification products were visualized under UV light on a UV-Transilluminator. The gel was photographed using a Gel Documentation System. Presence and absence of bands were recorded in positive control as well as in case of genetic stocks.

Results and discussion

Both genetic stocks WR95 and Sel.T3336 were screened for stem rust resistance at seedling stage against three pathotypes of stem rust pathogen *Puccinia graminis tritici viz.*, 40A, 40-1 and 117-6. WR95 showed infection type 1^- to 1 whereas Sel.T3336 produced infection type $1^=$N to 1^-, thus showing high degree of resistance against test pathotypes (Table 2). WR95 does not have any known effective stem rust resistance gene in its parentage, therefore, determining the identity of the gene conferring resistance was important.

Though WR95 showed resistance to all the three stem rust pathotypes used in the present study but for inheritance studies only two stem rust pathotypes, *viz.,* 40A and 40-1 were considered. All the plants in F_1 derived from crosses, WR95 x Agra Local, WR95 x Lal Bahadur and WR95 x NI5439 showed susceptible reaction when tested with stem rust pathotype 40A indicating that resistance in WR95 against this pathotype is recessive. The F_2 population derived from these crosses segregated into resistant and susceptible plants against pathotype 40A (Table 3). In the cross WR95 x Agra Local, out of 75 F_2 plants, 17 plants were resistant while 58 showed susceptible reaction. The observed frequency of F_2 plants fitted well in the expected ratio of 1 resistant: 3 susceptible plants as evident from the non-significant Chi-square values of 0.21 (P-value 0.75 - 0.50). Similarly the F_2 populations of cross WR95 x Lal Bahadur segregated into 18 resistant and 51 susceptible individuals out of 69 F_2 plants as in previous cross. The observed frequency of F_2 plants fitted well in the expected ratio of 1 resistant: 3 susceptible plants as evident from the non-significant Chi-square value of 0.04 (P-value 0.90-0.75). Similar results were observed in the F_2 population derived from cross WR95 x NI5439 which segregated into 15 resistant and 59 susceptible plants. The observed frequency of F_2 plants fitted well in the expected ratio of 1 resistant: 3 susceptible plants

Table 1. Molecular markers and PCR conditions used for screening stem rust resistance genes

Stem rust resistance gene	Primer	PCR Cycle	Dominant/ codominant	Base pairs	Gel conc.
Sr24/Lr24	SCS1326	94^0C-4min.; 40cycles (92^0C-1min.; 60^0C-1 min.;72^0C-2 min.); 72^0C-5 min.	Dominant	607	1.5% Agarose
Sr25/Lr19	GbF GbR	94^oC-5 min.; 30 cycles (94^oC-1.30 min.; 55^oC-2min.;72^oC-1.30 min.); 72^oC-5 min	Dominant	130	2% Agarose
Sr26	Sr26#43-F Sr26#43-R	94^0C-3 min.; 30 cycles (94^0C-30s; 56^0C-30s; 72^0C-40s);20^0C-1 min	Dominant	207	2% Agarose
Sr31	Lag95-F Lag95-R	95^0C-3 min.; 30 cycles (94^0C-30s, 57^0C-1 min, 72^0C-1 min); 72^0C-10 min	Codominant	1100	0.8% Agarose
Sr36	Stm773-F Stm773-R	95^0C-4 min., 40 cycles (94^0C-30s, 60^0C-30s, 72^0C-30s); 72^0C-7min	Codominant	192/ 162	3% Metaphore
Sr38/Lr37	VENTRIUP	94^0C-4min.;40cycles(92^0C-1 min.; 60^0C-1min.;72^0C-2 min.);72^0C-5 min	Dominant	252	2% Agarose

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Table2. Screening of WR95 and Sel.T3336 against different races of stem rust

Stem rust pathotype	Genetic stock		Susceptible parent
	WR95	Sel.T3336	Agra local
40A	1	1^-	4
40-1	1^-	$1^=$N	4
117-6	1^-	$1^=$	4

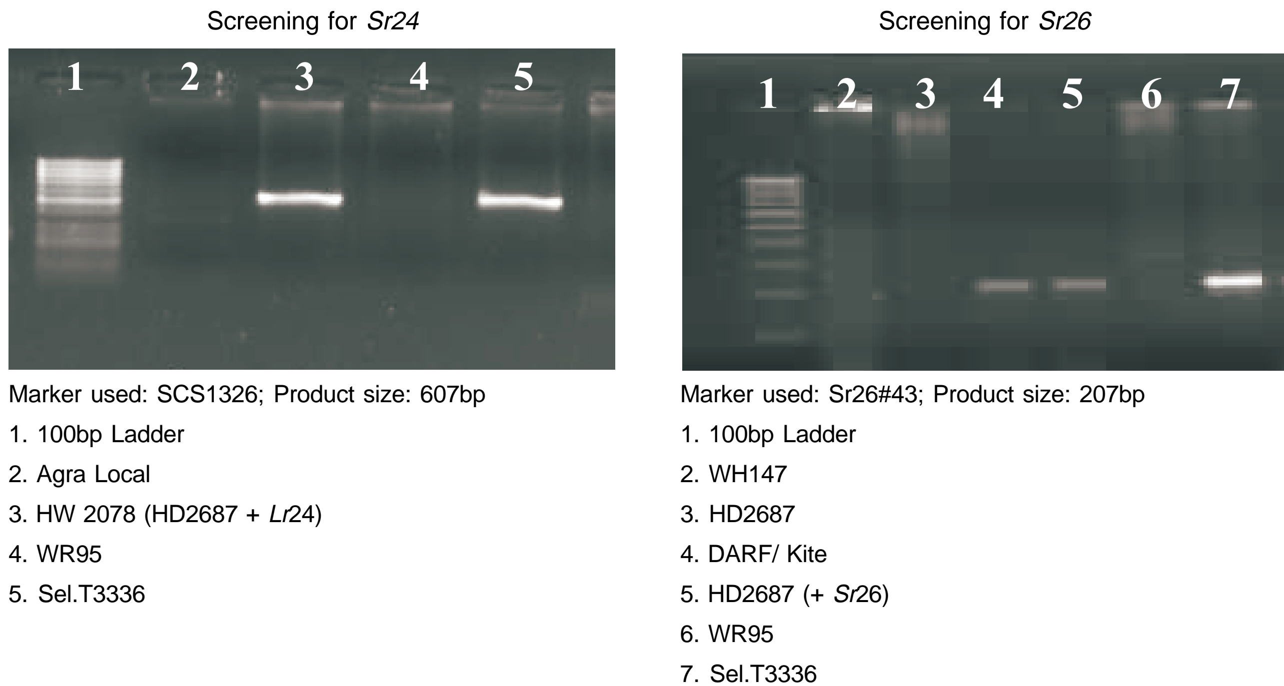
(P-value 0.50-0.30). Inheritance of stem rust resistance in WR95 was also studied for race 40-1 which is virulent on *Sr*24. A single recessive gene controlled stem rust resistance was observed against race 40-1 also (Table 3).

One of the parents involved in Sel.T3336 is DARF/ Kite carrying stem rust resistance genes, *Sr*24 and *Sr*26; hence, it is likely that Sel.T3336 carried these stem rust resistance genes. Since pathotype 40-1 is virulent on *Sr*24 but Sel.T3336 showed resistance to this pathotype, it can be presumed that Sel.T3336 carries stem rust resistance gene *Sr*26 conferring resistance to race 40-1.

For genetic analysis of stem rust resistance in Sel.T3336 only one pathotype 40-1 was used which can differentiate between genes *Sr*24 and *Sr*26 present in one of the parents of Sel.T3336. All the F_1 plants showed resistant reaction against race 40-1 indicating the dominant nature of the resistance gene. The F_2 generation segregated into resistant and susceptible plants. Out of 67 plants studied in F_2, 51 were resistant and 16 susceptible, which fits well in the expected ratio of 3 resistant: 1 susceptible with a non-significant Chi-square value of 0.04 (P-value 0.90-0.75).

Though test of allelism is commonly used to determine allelic relationship among different sources of rust resistance, however, with the availability of robust molecular markers for different rust resistance genes, it is possible to establish the identity of a gene by screening with molecular markers. Genetic analysis revealed that genetic stocks WR95 and Sel.T3336 carry genes for stem rust resistance. Screening of Sel T3336 with validated molecular markers specific for genes *Sr*24, *Sr*25, *Sr*26, *Sr*31, *Sr*36, and *Sr*38 showed the presence of two stem rust resistance genes i.e. *Sr*24 and *Sr*26 (Fig. 1.). This explains the resistance of Sel.T3336 against race 40-1, which is virulent on *Sr*24. Genetic analysis of stem rust resistance in Sel.T3336 showed segregation for a single dominant gene which is identified by molecular markers as *Sr*26. Thus, Sel. T3336 carrying *Sr*24 and *Sr*26 can be used as a donor for these *Agropyron* derived rust resistance genes. WR95 was also screened with the same set of molecular markers used for screening of Sel.T3336. However, none of the genes i.e. *Sr*24, *Sr*25, *Sr*26, *Sr*31, *Sr*36, *and Sr*38 could be detected with molecular markers specific for these genes.

Effort was made to locate the stem rust resistance gene in WR95 on specific chromosome using Chinese Spring monosomic series. All 21 F_1s of crosses between monosomics for individual chromosome and WR95 were susceptible. Therefore,it was concluded that the stem rust resistance gene in WR95 is possibly hemizygous ineffective.



Marker used: SCS1326; Product size: 607bp

1. 100bp Ladder
2. Agra Local
3. HW 2078 (HD2687 + *Lr*24)
4. WR95
5. Sel.T3336

Marker used: Sr26#43; Product size: 207bp

1. 100bp Ladder
2. WH147
3. HD2687
4. DARF/ Kite
5. HD2687 (+ *Sr*26)
6. WR95
7. Sel.T3336

Fig. 1. Screening of genetic stocks WR95 and Sel.T3336 with Sr24 and Sr26 specific molecular markers

Table 3. Segregation of crosses of WR95 and Sel.T3336 with susceptible parents in F_2 generation against stem rust pathotypes, 40A and 40-1

Parents/Cross	Stem rust race 40A					
	Generation	No of plants		Expected ratio	χ^2	P Value
		Resistance	Susceptible			
WR95 × Agra Local	F_1	0	12			
	F_2	17	58	1:3	0.21	0.75-0.50
WR95 × Lal Bahadur	F_1	0	10			
	F_2	18	51	1:3	0.04	0.90-0.75
WR95 × NI5439	F_1	0	10			
	F_2	15	59	1:3	0.77	0.50-0.30
Total of 3 crosses		50	168	1:3	0.50	0.50-0.30
			Heterogeneity χ^2		0.52	0.50-0.30
Stem rust race 40-1						
WR95 × Agra Local	F_1	0	10			
	F_2	25	51	1:3	2.52	0.20-0.10
WR95 × Lal Bahadur	F_1	0	8			
	F_2	19	44	1:3	0.89	0.50-0.30
WR95 × NI5439	F_1	0	5			
	F_2	19	60	1:3	0.04	0.90-0.75
Total of 3 crosses		63	155	1:3	1.77	0.20-010
			Heterogeneity χ^2		1.68	0.20-0.10
Sel.T3336 x NI5439	F_1	10	0			
	F_2	51	16	3:1	0.04	0.90-0.75

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Majority of already designated stem rust resistance genes are of dominant nature and under monogenic control [6]. Field resistance controlled by a single recessive gene was reported in cultivars like Kota [7], S227, S308 and E4853 [8]. The designated stem rust resistance gene *viz*., *Sr*30 [9] and *Sr*8b [10] were found to be recessive in nature. In a wheat-rye recombinant line, 'Selection 212', the resistance to stem rust pathotype 122 and 40A was governed by a single recessive gene [11]. This recessive stem rust resistance gene of WR95 may prove very useful in breeding programme.

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