Bangladesh J. Bot. **48**(4): 1199-1206, 2019 (December)

MOLECULAR ANALYSIS OF *YR* GENES FOR YELLOW RUST RESISTANCE IN BREAD WHEAT (*TRITICUM AESTIVUM* L. EM THELL)

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Keywords: Recombinant inbred lines, SSR markers, *Yr* genes, Grain yield

Abstract

Investigation was conducted to evaluate 210 recombinant inbred lines (RILs) of bread wheat to identify *Yr* genes using SSR markers and response to stripe rust reaction. Recombinant inbred lines were screened under epiphytotic conditions and data in terms of per cent leaf area infected were recorded using Modified Cobb's Scale. A total of seven *Yr* genes, namely Yr7, Yr18, Yr26, Yr29, Yr36, Yr47 and Yr53 were found to be linked to yellow rust resistance. Out of 40 Yr specific SSR markers, seven (Xgwm130 (Yr7), Xbarc 352 (Yr18), Xgwm 11 (Yr26), Xwmc 44 (Yr29), Xwmc 149 (Yr53), WKS1_I (Yr36) and Xcfb309 (Yr47) were found to be polymorphic in parental genotypes and RIL population indicating the presence or absence of Yr genes. Three RILs namely, 51, 52, 55 had largest number of resistant genes (4Yr genes) followed by six lines namely, RIL No. 20, 21, 23, 29, 39, 53 had 3Yr genes having disease score (0S). These RILs may be utilized for incorporation of specific Yr genes in well adapted genotypes for improvement of disease resistance in bread wheat.

Introduction

Wheat is one of the important leading cereals providing daily sustenance to the large proportion of world. Since the initiation of the Green Revolution in the mid 60s, India achieved remarkable increase in production and productivity of wheat. India is the second largest producer of wheat in the world and wheat production had touched a record of 97.44 mt from an area of 30.72 m ha during 2016-17 (Anon. 2017). It is one of the most significant food crops among cereals and serves as the staple food of about 36% of the entire world population (Talha *et al*. 2016). Increasing wheat yield potential in the developing world is a primary aim for food security concern (Duveiller *et al*. 2007). Both biotic and abiotic stresses are major hurdles for attaining this goal. Today, the most challenging task for wheat breeders is to increase grain yield as well as to improve the grain quality of crop (Goutam *et al*. 2013). Although wheat has a wide range of climatic adaptability, it is usually affected by many fungal diseases, the most devastating of which are the rust diseases.

All the three species of rusts, *viz.,* stem (black) rust (*Puccinia graminis* Pers. f. sp. *Tritici* Eriks. & E. Henn), leaf (brown) rust (*P. triticina* Eriks.) and stripe (yellow) rust *(P. striiformis* Westend f. sp. *tritici*) infect wheat crop. Rust epidemics caused yield losses of more than one million tons in 1950, 1964, 1990 and 2002 (Wan *et al*. 2004, 2007). Stripe (yellow) rust is a widespread disease across major wheat growing regions with diverse cropping systems, growing seasons and germplasm characteristics (Wellings 2011). Losses from stripe rust have been estimated to be at least 5.5 million tons per year at worldwide level (Beddow *et al*. 2015). The 78S84 and 46S119 are the most prevalent pathotypes of yellow rust in north-western plain zone of India and identified virulent against *Yr3*, *Yr9,* and *Yr27* genes (Prashar *et al*. 2008). In 2001, India faced a major epidemic due to breakdown of *Yr27* and up to 70% losses were estimated (Prashar *et al*. 2007). During 2010-11, stripe rust appeared in severe form in the plains of Jammu and

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Kashmir, foot hills of Punjab and Himachal Pradesh, parts of Haryana, and Tarai region of Uttarakhand (Sharma and Saharan 2011). Stripe rust continues to pose a threat to wheat cultivation worldwide (Sareen *et al*. 2012).

Stripe rust caused by the obligate biotroph fungus *Puccinia striiformis*, is a serious fungal disease for wheat, especially in cooler and moist environments (Chen *et al*. 2014). The temperature range for stripe rust infection is minimum of 0°C, optimal of 11°C and maximum of 23°C (Curtis *et al*. 2002). The wheat cultivars become susceptible to rusts due to their narrow genetic base and the rapid rate of evolution of the pathogen, making it necessary to search for new source(s) of resistance. Till now, nearly 76 major genes conferring resistance to stripe rust (*Yr1* to *Yr76*) have been identified (Dracatos *et al*. 2016, Xiang *et al*. 2016). Though remarkable progress has been made in breeding for stripe rust resistant varieties in India but the subsequent evolution of pathogenic races at much greater pace has challenged the breeding programmes (Khan and Saini 2009). Concerning co-evolution of plant and pathogen, the effectiveness of single gene to the resistance is limited and short-term. Use of diverse genes for providing resistance against stripe rust disease is the most economical and environmentally safe method. In view of these facts, **210** recombinant inbred lines of bread wheat were evaluated in this study for yellow rust resistance and molecular analysis of *Yr* genes. To identify genetically resistant and agronomically desirable genotypes for exploitation in a breeding programme aimed at improving grain yield potential of wheat.

Materials and Methods

The field experiment was conducted in the Research Area, Wheat and Barley Section, Department of Genetics and Plant Breeding, Chaudhary Charan Singh Haryana Agricultural University, Hisar, India located at 29°10'N latitude, 75°46'E longitude and altitude of 215.2 m above mean sea level. The present investigation was carried out on 210 RILs of bread wheat and two parents (WH542 as resistant parent and WH711 as susceptible parent) during Rabi season (November to April months) of 2015-16 and 2016-17. A mixture of stripe rust pathotypes 46S119, 47S103 and 78S84 was used to create epiphytotic conditions for screening parental genotypes and the RILs population obtained from Department of Plant Pathology, CCS Haryana Agricultural University, Hisar.

All the recombinant inbred lines of wheat including their parents were grown in 2 m paired rows, in 3 replications in randomized block design (RBD). Recommended package of practices were followed to raise the crop. The infector rows were grown after the interval of 10 entries as border rows of the experiment to ensure uniform infection. Data were recorded on the 5 plants/ replication/ RIL. Statistical analysis was done using OPSTAT software package (SPSS 1991).

Spray inoculums were done at tillering stage with urediospores of *Pst* (conc.10^6/ml). RILs were screened under epiphytotic conditions and data in terms of per cent leaf area infected were recorded using Modified Cobb's Scale (Peterson *et al*. 1948). Severity of disease was recorded in terms of per cent leaf area infection and pustule type was recorded as response.

A total of 40 *Yr* specific SSR molecular markers were used for studying molecular polymorphism and to detect *Yr* genes among RILs population. Genomic DNA was isolated from the young leaves of wheat plants by using cetyl trimethyl ammonium bromide (CTAB) extraction method (Murray and Thompson 1980, Saghai-Maroof *et al*. 1984 and Xu *et al*. 1994). PCR amplified DNA fragments for DNA markers were resolved by submerged horizontal electrophoresis in 2.5% (w/v) agarose gels. PCR amplified products were viewed under UV light fluorescence using photo UV transilluminator. The size (in nucleotides base pairs) of the amplified bands was determined based on its migration relative to standard DNA marker (100 bp DNA

ladder). PCR reactions were standardized using known markers for *Yr* genes. The presence of band run on agarose gel was taken as one and absence of band was read as zero in different lines and promising RILs were identified for *Yr* genes.

Results and Discussion

From the Table 1 it is apparent that resistant parent (WH 542) showed zero per cent infection while the susceptible parent (WH 711) showed 60% severity. Out of 210 RILs, 110 showed 0% infection, 46 RILs showed infection in traces, 10 RILs showed 0 - 5%, 6 showed 5.1 -10%, 9 showed 10.1 - 15%, 6 showed 15.1 - 20 %, 6 showed 20.1 - 30%, 14 showed 30.1 - 40% and 4 showed 60% severity. All recombinant inbred lines were categorized into highly resistant (HR), moderately resistant (MR) and susceptible (S) on the basis of disease score for yellow rust.

Table 1. Disease score data for yellow rust.

Disease score for yellow rust	Yellow rust reaction	RILs
0	HR	(110) WH542, 9,12, 13, 15, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 29, 31, 33, 35, 38, 39, 40, 43, 46, 51, 52, 53, 54, 55, 63, 64, 65, 66, 70, 72, 74, 75, 79, 80, 83, 90, 91, 92, 98, 100, 104, 108, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 122, 123, 124, 125, 126, 127, 130, 131, 132, 133, 134, 137, 138, 139, 140, 141, 142, 145, 146, 147, 148, 149, 150, 152, 153, 154, 163, 169, 170, 171, 172, 174, 177, 185, 188, 189, 190, 192, 193, 194, 195, 196, 197, 199, 200, 201, 202, 203, 204, 205, 206, 208, 209
TS	HR	(46) 3, 6, 7, 11, 16, 26, 28, 30, 32, 34, 36, 41, 47, 48, 50, 57, 62, 67, 69, 71, 73, 76, 77, 78, 81, 84, 85, 86, 87, 89, 93, 95, 97, 106, 107, 109, 128, 144, 159, 160, 162, 165, 166, 175, 176, 179
0-5S	HR	(10) 180, 184, 187, 191, 207, 10, 37, 135, 158, 198
5S-10S	MR	(6) 56, 88, 103, 136, 151, 181
10S- 15S	S	(9) 5, 44, 49, 59, 96, 102, 105, 161, 167
15S-20S	S	(6) 99, 168, 173, 182, 186, 210,
20-30S	S	(6) 4, 60, 94, 101, 129, 183
30-40S	S	(14) 1, 14, 42, 45, 58, 61, 68, 82, 121, 143, 156, 157, 164, 178
40-60S	S	(5) WH711, 2, 8, 110, 155

S - Susceptible, MS - Moderately susceptible, MR - Moderately resistance, R - Resistance.

Today, molecular markers are the best tools used to determine the level of genetic diversity among plants and can provide detailed characterization of genetic resources (Manifesto *et al*. 2001, Mir *et al*. 2012). The essential requirements for Marker Assisted Selection (MAS) in a plant breeding program is that markers should co-segregate with the desired trait, means to screen large populations and it should be available with high reproducibility across laboratories. Molecular marker aided selection methods resulted in significant improvement in breeding efficiency by reducing trial and error aspect of breeding process and also save time and cost. In the present study, 40 SSRs (*Yr* specific) were used for detecting polymorphism and to identify *Yr* genes in 210 recombinant inbred lines of bread wheat. Out of these, 7 *Yr* specific markers were polymorphic on parental genotypes. These were further screened in RIL population distinguishable on agarose gel electrophoresis. Seven *Yr* specific markers present on these parental genotypes include *Xgwm130 (Yr7), Xbarc 352 (Yr18), Xgwm 11 (Yr26), Xwmc 44 (Yr29), Xwmc 149 (Yr53), WKS1_I (Yr36), Xcfb309*

(Yr47). Yr7 gene linked to *Xgwm 130* primer was observed in 26 RILs in present material (Table 2). Similarly, Yao *et al*. (2006) identified a microsatellite marker *Xgwm 526* on the chromosome arm 2BL, linked closely with *Yr7* locus resistant to wheat stripe rust. *Yr18* gene linked to *Xbarc 352* primer was present in 16 RILs at molecular level, similar results were reported by Haque *et al*. (2014) that *Xgwm 130* is linked to both of *Lr34/Yr18* leaf and stripe rust resistance genes. *Yr26* gene linked to *Xgwm 11* primer was found to be present in 13 RILs, *Yr29* gene was present

Table 2. *Yr* genes present among recombinant inbred lines of bread wheat.

Yr gene	RILs (210)	Recombinant inbred lines
Xgwm130 (Yr7)	26 RILs	3, 7, 9, 18, 19, 20, 21, 23, 29, 30, 31, 35, 39, 46, 51, 52, 66, 70, 76, 79, 82, 117, 121, 170, 171, 190
Xbarc 352 (Yr18)	16 RILs	21, 22, 23, 24, 25, 32, 33, 67, 73, 103, 107, 110, 112, 113, 120, 123
Xgwm 11 (Yr26)	13 RILs	3, 6, 12, 15, 23, 24, 39, 40, 43, 51, 55, 93, 128
Xwmc44 (Yr29)	17 RILs	12, 13, 15, 18, 19, 20, 45, 63, 64, 85, 115, 124, 126, 133, 134, 135, 140
Xwmc 149 (Yr53)	17 RILs	51, 52, 53, 55, 62, 63, 65, 75, 180, 183, 191, 195, 196, 198, 201, 207, 208
WKS1_I (Yr36)	19 RILs	25, 27, 29, 39, 40, 51, 52, 53, 55, 56, 64, 67, 72, 73, 74, 114, 118, 119, 141
Xcfb309 (Yr47)	32 RILs	7, 8, 10, 17, 20, 21, 26, 27, 28, 29, 38, 46, 48, 52, 53, 54, 55, 66, 71, 74, 82, 89, 91, 92, 93, 94, 115, 117, 121, 124, 133, 135

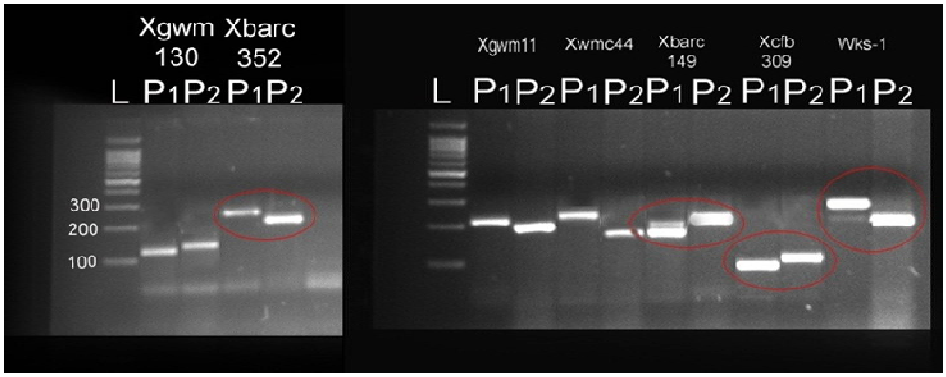
in 17 RILs at molecular level linked to primer *Xwmc 44*. This indicated the presence of stripe rust resistance against *Yr26 and Yr29* genes in the present set of material. Parveen *et al*. (2014) showed the presence of *Yr29* using *Xwmc 44* PCR based DNA markers as reported by Suenaga *et al*. (2001) and *Yr26/Yr15 using Xgwm11* reported by Ali *et al*. (2010) in the advanced wheat lines. *Yr53* gene was present in 17 RILs at molecular level linked to primer *Xwmc 149, Yr36* gene linked to *WKS1_I* primer was found to be located in 19 RILs. Fu *et al*. (2009) also confirmed the identity between *WKS1* and *Yr36* and transformed the susceptible wheat variety 'Bobwhite' with a 12.2-kb genomic fragment that includes the complete *WKS1* coding and flanking regions. Thirty two recombinant inbred lines showed the presence of *Yr47* linked to *Xcfb309* primer. Similarly, Bansal *et al*. (2011) also characterized the combination of flanking markers *gwm234* and X*cfb309* to ascertain the presence of *Lr52* and *Yr47* in segregating populations and characterized a valuable source of dual leaf rust and stripe rust resistance for deployment in new wheat cultivars AUS28183 and AUS28187. Xu *et al*. (2012) determined the location of *Yr53* on 2BL, using *Xwmc149* as one of the SSR marker along with *Xbarc349, Xwmc501, Xwmc441* indicating that the resistance gene is on the chromosome near the centromere of 2BL in durum wheat accession PI 480148.

Among these RILs, the RIL No. 4 was susceptible to yellow rust (30S), while the RIL No. 9 and 135 showed resistant reactions (0 - Ts) and confirmed by *Yr7, Yr29* and *Yr47* genes. This was followed by another group of 7 lines, namely RIL No. 5, 10, 12, 28, 32, 70, and 123 performed significantly better for grain yield. These lines also indicated resistant reaction to yellow rust (0 - 5S) as validated by the presence of *Yr7,Yr18, Yr26, Yr29,Yr47* genes. Another group of ten RILs mainly, RIL No. 1, 2, 3, 35, 36, 71, 93, 120, 127, and 139 also performed significantly better than their corresponding mean values for grain yield. Out of these lines, RIL No. 2 showed susceptible reaction (60S) score to yellow rust while other lines showed resistant reaction (0 - Ts) for yellow rust as indicated by the presence of *Yr7, Yr18, Yr26, Yr47* genes. With regard to disease resistance, three RILs namely, 51, 52, 55 had largest number of resistant genes (4*Yr* genes) with resistance score (0S) followed by six lines namely, RIL No. 20, 21, 23, 29, 39, 53 had 3*Yr* genes with resistance score (0S) (Table 3). These RILs may be utilized for incorporation of **present Yr** genes in well adapted

genotypes for improvement of disease resistance in bread wheat. Ali *et al*. (2010) screened 35 SSR primer pairs on the parents and on F_2 population, the result indicated that most of the resistant plants amplified same band as resistant parent while susceptible plants amplified same as susceptible parents. Ullah *et al*. (2016) reported that, out of 99 experimental lines, S19M93 and S23M41 markers revealed the presence of *Yr5* gene in 86 and 70 genotypes, respectively. While, *Xpsp3000* suggested presence of *Yr10* gene in 66 genotypes in spring bread wheat lines. Mukhtar *et al*. (2015) reported *Xpsp3000* has band size of 260 bp and characterized wheat germplasm for stripe rust resistance. This will facilitate gene pyramiding approaches against stripe rust and may be useful in future wheat improvement programs. Xu *et al*. (2014) validated 8 SSR and 6 STS molecular markers closely linked to the wheat stripe rust resistance gene *YrC591*, markers tightly linked to *YrC591* proved useful for tracing gene in wheat MAS breeding programs. Therefore, more resistance genes and QTL should be identified for agricultural use. It is important to develop diagnostic markers for marker-assisted selection (MAS) in order to ensure resistance diversity.

Table 3. Recombinant inbred lines of bread wheat identified with *Yr* genes.

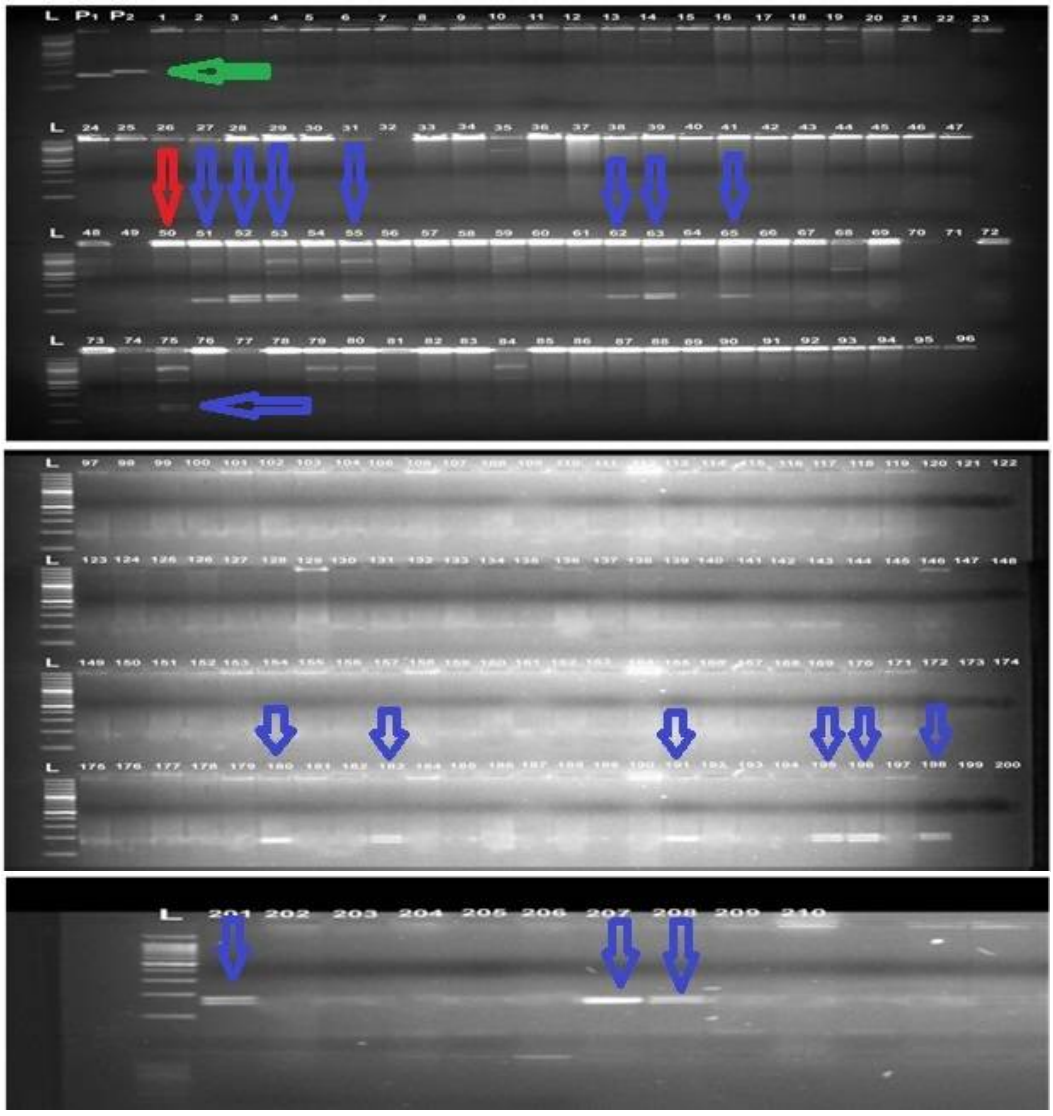
Total number of RILs	210
With 1 *Yr* gene	**60** (6, 8, 9, 10, 13, 17, 22, 26, 28, 30, 31, 32, 33, 35, 38, 43, 45, 48, 54, 56, 62, 65, 70, 71, 72, 75, 76, 79, 85, 89, 91, 92, 94, 103, 107, 110, 112, 113, 114, 118, 119, 120, 123, 126, 128, 134, 140, 141, 170, 171, 180, 183, 190, 191, 195, 196, 198, 201, 207, 208)
With 2 *Yr* gene	**25**(3, 7, 12, 15, 18, 19, 24, 25, 27, 40, 46, 63, 64, 66, 67, 73, 74, 82, 93, 115, 117, 121, 124, 133, 135)
With 3 *Yr* gene	**6**(20, 21, 23, 29, 39, 53)
With 4 *Yr* gene	**3**(51, 52, 55)



L - 100bp ladder, P_1 - Parent WH542, P_2 - Parent WH 711
Fig. 1. Parental polymorphism for seven *Yr* specific SSR markers.

A total of seven *Yr* genes, namely*Yr7, Yr18, Yr26, Yr29, Yr36, Yr47* and *Yr53* were found linked to yellow rust resistance. *Yr26* and *Yr36* were found effective with disease resistance (0 - Ts) in the present set of material. Three RILs namely, 51, 52, 55 had largest number of resistant genes (4*Yr* genes) followed by six lines namely, RIL No. 20, 21, 23, 29, 39, 53 which had 3*Yr* genes having disease score (0S). These RILs may be utilized for incorporation of present Yr genes in well adapted genotypes for improvement of disease resistance in bread wheat. The present study identified the lines that may be further utilized for improvement of yellow rust resistance in bread

wheat and further will help the researchers to uncover the critical areas of how to tackle this damaging disease instead of using fungicides that is contaminating the food chain.



L - 100bp ladder, P_1 - Parent WH542, P_2 - parent WH 711. Red arrow - absence of band in line no. 50, blue - presence of band in line no. 51, green- band at different bp.

Fig. 2. Allelic polymorphism among WH542, WH711 and RILs for *Xwmc149 (Yr53).*

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(Manuscript received on 29 October, 2018; revised on 12 February, 2019)