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# Marker assisted screening of breeding population of wheat segregating for stripe rust resistance using SSR markers

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**Abstract**
Screening of $F_2$ population from cross WH711 (susceptible parent) and PBW698 (resistant parent) against stripe rust was done on the basis of field reaction of yellow rust and 12 morphlogical traits. The genotyping of parents was done by using 80 SSR markers out of which 11 were found polymorphic. These 11 markers were used to screen 250 individuals of $F_2$ population. Mendelian inheritance was followed by five markers *viz.* Xgwm631, Cfa2040, Xsps3000, Barc76 and Xgwm 130 which segregated according to expected ratio of 1:2:1 with chisquare values 5.78, 4.23, 2.75, 9.2 and 7.6 respectively. Single marker analysis showed markers Xgwm631, Cfa2040 (Yr2, Yr6), Xsps3000 (Yr10), Xgwm130 (Yr7), Xwmc407 (Lr17), Barc46, Xgwm413 (Yr15), Barc187 (Yr27) and Xgwm273 (YrH52) were linked with yellow rust locus at 0.01% of significance hence these genes may be present in the breeding population. Cfa2040 was found linked with flag leaf area, number of spikelets and Barc181 with spike weight locus. Markers on chromosomes 7A and 1B showed LOD values 6.64 and 6.86 conferring tight linkage with yellow rust locus thus can be used for MAS.

**Keywords:** Marker segregation, single marker analysis, linkage, LOD

## Introduction

Wheat (*Triticum aestivum* L.) is one of the first domesticated food crops and has been the basic staple food of the major civilizations of Europe, Asia and North Africa. It is one of the most important cereal crops particularly in a country like India where it meets the food requirements of more than half of the human population. It occupies a vital position among food grain crops not only in terms of acreage and production but also because of its adaptation to a wide range of agro-climatic condition. The demand of wheat is increasing day by day because of ever increasing population; hence there is need for effective and quick selection of wheat strains which possess desired traits like high yielding, abiotic, biotic resistance etc. In India presently wheat occupies area of 30.60 million hectares with production of 99.70 million ton and productivity 3.37 ton per hectare (Anonymous 2018) [1].

Yellow rust caused by *Puccinia striiformis* (PST) Westend. f. sp. *tritici Eriks* is considered a major foliar disease which causes losses up to 75% in wheat production worldwide (Ma *et al*., 2001; Smith *et al*., 2002; Lin and Chen, 2007) [8, 16, 7]. Therefore, it is the major concern and problem for breeders and farmers (Marsalis and Goldberg, 2006) [9]. In 2001, India faced a major epidemic due to breakdown of Yr27 and up to 70% losses were estimated in PBW343 variety (Prashar *et al*., 2007) [13]. Pathotypes prevalent in north-western plain zone of India are 78S84 and 46S119. The arrival of new virulent pathogens is the main challenge in disease resistance program and to meet this, plant breeders have to put continuous efforts to develop resistant varieties. So far, more than 70 stripe rust resistance genes (Yr) have been reported in wheat with official and provisional designations (McIntosh *et al*., 2012) [10]. Closely linked SSR markers provide a powerful tool for pyramiding yellow rust resistance genes and marker assisted selection in breeding programs (Roders *et al*., 1998; Karakousis *et al*., 2003; Somers *et al*., 2004) [14, 5, 17]. DNA markers can be used for genetic improvement through selection of desirable traits such as disease resistance. Marker assisted selection (MAS), is expected to increase genetic response as they are least affected by environment thus increasing

efficiency and accuracy of selection.

## Material and Methods
### Plant material
The materials consisted of $F_2$ population of cross WH711 and PBW698. The population was planted in 4m rows along with parents and infector on borders in the fields of Department of Genetics and Plant Breeding, CCS-HAU, Hisar. Artificial inoculation of Pst 78S84 and 46S119 was done by spraying and disease severity was recorded using modified cobb scale (0-9) devised by Peterson *et al.* in 1948. Twelve Morpho-

physiological characters were also recorded. SSR markers were used for genotyping of parents and polymorphic markers were used for MAS in breeding population. Single marker analysis was done with the help of Win-QTL Cartographer.

### DNA isolation
DNA was isolated from young leaves of wheat plant using CTAB method modified by Sanghai-Maroof *et al.* 1984 [15]. Genotyping of the population was done using 80 SSR markers including Yr specific (Table 1).

**Table 1:** Band size and segregation ratio with chi square values of 11 polymorphic primers

| Marker | Bp | Segregation ratio | Gene action | Chi square value |
|---|---|---|---|---|
| Barc181 | 180-210 | - | - | 190.5 |
| Xgwm631 | 100-450 | 1:2:1 | Dominant | 5.78* |
| Cfa2040 | 255-270 | 1:2:1 | Dominant | 4.23* |
| Xsps3000 | 250-270 | 1:2:1 | Dominant | 2.75* |
| Barc187 | 110-130 | - | - | 185.5 |
| Barc76 | 200-220 | 1:2:1 | Dominant | 9.2** |
| Xgwm130 | 110-130 | 1:2:1 | Dominant | 7.6** |
| Xwmc407 | 120-150 | 15:1 | Duplicate | 4.57* |
| Xgwm273 | 150-200 | 13:3 | Inhibitory | 5.14** |
| Barc46 | 140-450 | 13:3 | Inhibitory | 1.92* |
| Xgwm413 | 100-120 | 13:3 | Inhibitory | 5.90** |

*Significant at 5% and **significant at 1%

**Table 2:** Linkage to yellow rust, leaf area and number of spikelets per spike of 11 Polymorphic primers on cross (WH711 X PBW698)

| Marker | Gene linked | Map Distance | Linkage group | $R^2$ Value | F Value |
|---|---|---|---|---|---|
| **Yellow rust** | | | | | |
| Xwmc407 | Lr 17 | 127.60 | 2A | 0.6247 | 0.000000000 8***** |
| Xgwm130 | Yr 7 | 000.74 | 7A | 0.6955 | 0.000000000 **** |
| Xgwm631 | | 153.36 | 7A | 0.3257 | 0.000000000 **** |
| Barc46 | | 0 00.00 | 1B | 0.2948 | 0.000000000 **** |
| Xgwm413 | Yr15 | 000.25 | 1B | 0.0793 | 0.000000001 **** |
| Barc181 | Yr 26 | 084.20 | 1B | 0.0033 | 0.837149181 |
| Barc187 | Yr 27 | 109.90 | 1B | 0.6059 | 0.000000000 **** |
| Xgwm273 | YrH52 | 36.30 | 6B | 0.6511 | 0.000000000 **** |
| Cfa2040 | Yr 2 or Yr 6 | 164.80 | 7B | 0.0994 | 0.000004156 **** |
| Xsps3000 | Yr10 | 223.1 | 1D | 0.0869 | 0.000081726 **** |
| Barc76 | Yr 18 | 235.1 | 7D | 0.0249 | 0.013609505 * |
| Leaf area | | | | | |
| Cfa2040 | Yr 2 or Yr 6 | 164.80 | 7B | 0.0994 | 0.045074152 * |
| Spike Weight | | | | | |
| Barc181 | Yr26 | 084.20 | 1B | 0.0033 | 0.015820085 * |
| Number of spikelet / spike | | | | | |
| Cfa2040 | | 164.80 | 7B | 0.0994 | 0.000927420 *** |

Significance at 5%, 1%, 0.1% and 0.01% levels are indicated by *, **, *** and ****, respectively

## PCR conditions
Genomic DNA along with SSR primers were amplified in applied biosystem thermocycler. The Optimization of PCR reaction was done by varying the concentrations of template DNA, dNTPs mix, primers and Taq DNA Polymerase in a reaction volume of 10 µl. The PCR was run with 50ng (1 µl in volume) DNA concentration and 3 units of Taq. The other constituents were 0.30 µl MgCl$_2$ 50 mM, 0.25 µl dNTPs mix (10µM), 0.20 µl of Forward primer and Reverse primer each, 7 µl Sterile distilled water making a total volume of 10 µl. The amplified products were stored at -20ºC till further use. Amplified products were resolved on 2.5% agarose gel. ABH scoring was followed and single marker analysis was done using Win-QTL cartographer V 2.5.

## Results and Discussion
### Inheritance of stripe rust
WH 711 was released in 2002 and it is famous variety among the farmers of Haryana state. It has very good plant characters like short height (100-105 cm), more no. of tillers (up to 15) and yield potential 45-50 q/ha. But with time it developed susceptibility against yellow rust. In this study we constructed a segregating population of 250 $F_2$ plants from a cross between WH711 and PBW 698, where PBW 698 acted as donor parent known to have Yr10 gene and showed a good resistance towards *Puccinia striiformis f. sp. tritici*.
In the phenotypic data analysis against severity of yellow rust under artificial inoculum (pst 78S84) pressure, the $F_2$ population segregated in 7:9 ratio for resistant and susceptible

plants, which was tested for goodness of fit with chi square value 4.15. Thus exhibits recessive duplicate type gene interaction or complimentary gene action. Here recessive allele at either of the two loci can mask the effect of dominant counterpart *i.e* the resistance will be shown only when dominant allele exits at both the loci if at any loci recessive allel is present it will show susceptible reaction. The findings were in agreement with Yang *et al.* (2016) [20], they also showed 7:9 (R:S) segregation against yellow rust while working on $F_2$ population (CH45 x CH42) of wheat. Whereas Wang *et al.* (2015) [19] found 90 resistant: 26 susceptible $F_2$ segregation ratios which were consistent with the expected 3:1, indicated single dominant gene is responsible for resistance in SE5756.

Genotyping of the two parents (WH 711 and PBW 698) was done using 80 simple sequence repeat (including Yr specific) satellite, out of which 11 were found polymorphic between the parent's genome and these were used to screen $F_2$ population.

On molecular basis primer Xgwm631, Cfa2040, Xsps3000, Barc76 and Xgwm 130 segregated in 1:2:1 with chi square values 5.78, 4.23, 2.75, 9.2 and 7.6 respectively, these markers showed single gene action and followed Mendelian inheritance according to which one fourth of the population is showing dominant allel, other one fourth have recessive allel and almost half of the population shows heterozygous condition. These markers do not have any epistatic effect due to other genes at different locus, thus they can be directly selected under MAS. Segregating ratio of 13:3 with chi square value 5.14, 1.92 and 5.90 was displayed by Xgwm273, Xgwm413 and Barc46 thus showing inhibitory epistasis type of gene interaction. This ratio shows interaction between two genes where one gene inhibits the effect of other gene in its presence. The gene can only express when inhibitory gene is in recessive form or absent. Xwmc407 showed segregation in 15:1 with chi square value 4.57 which shows duplicate gene action, whereas Barc181 and Barc187 did not fit in goodness of fit test (Table 4 and Plate 1, Plate 2). In Xwmc407 both genes exhibits same effect or govern the same trait, when recessive allel is present at both loci only then it shows susceptible reaction to yellow rust.

**Single marker analysis**
It is referred as single point analysis and it is the simplest method for detecting QTL associated with single marker. SMA is based on the idea that if there is an association between a marker genotype and trait value, it is likely that QTL is close to that marker locus. The $F_2$ population (mapping population) was screened using 11 Yr specific primers (polymorphic on parents). The analysis of molecular data combined with yellow rust reaction in field and other morpho-physiological traits showed that 9 Markers *viz.* Xgwm631, Cfa2040, Xsps3000, Xgwm130, Xwmc407, Barc46, Xgwm413, Barc187, Xgwm273 were linked with

yellow rust locus at 0.01% of significance. These markers are linked with various Yr genes (Table 5) and from the significant F value it may be inferred that these genes *i.e* Yr2, Yr6, Yr10, Yr7, Lr17, Yr15, Yr27 and YrH52 are present in the breeding population. Which is an efficient gene pyramid against the disease whereas earlier only Yr10 gene was known in parent PBW698. These markers can be efficiently utilized for identification of resistant plants at early stage and MAS can be done to develop resistant cultivars in short period of time. Cabuk *et al.* (2011) [3] screened $F_2$ progeny of wheat using SSR markers for yellow rust resistance genes (Yr7, Yr9, Yr15, Yr18, Yr26, and YrH52), which are present on chromosomes 1B, 2B and 7D. The molecular markers Xgwm526 (Yr7) and Xgwm273 (YrH52) were found to have the most conserved regions and Yr15 (Xgwm413) the least conserved regions among the cultivars. Asad *et al.* (2012) [2] evaluated $F_1$, $F_2$ and $F_3$ populations from cross Shaannong 104/Mingxian 169 with 104 SSR markers. Shaannong 104 carried a single dominant gene, which was closely linked with 6 SSR markers (Xgwm18, Xgwm273, and Xbarc187, Xgwm11, Xbarc137, Xbarc240) on 1B chromosome. Cheng *et al.* (2014) crossed Durum lines PI 331260 and PI 480016 (male) with Avocet S (female) and discovered that five markers Xgwm413, Xgwm498, Xgdm33, Xgwm11 and Xgwm18 were closely linked to Yr64 and Yr65.

Also, Cfa2040 showed linkage with flag leaf area and number of spikelets per spike which means that marker locus is somewhere located near to the locus of these two traits and Barc181 showed linkage with spike weight (Table 5). Hence direct selection for these linked traits may help for indirect selection for yellow rust resistance. In previous studies also specific genes were found linked to other agronomical trait locus for example Kumari *et al.* (2012) [6] also found that staygreen trait was linked with abiotic and biotic stresses, and identified two QTLs. Prasad *et al.* (1999) [12] analysed molecular marker (wmc41) and protein content data through single-marker linear regression approach and suggested linkage between Wmc41 and a QTL (QGpc.ccsu-2D.) for protein content.

Markers on chromosome 7A (Xgwm130 and Xgwm631) and 1B (Barc46, Xgwm413, Barc181 and Barc187) showed very strong linkage with LOD score 6.64 and 6.86 (Fig 1). The default LOD threshold value is 3.0, score greater than threshold favors linkage. Hence further supports the presence of Yr7, Yr15, Yr26 and Yr27 in the population, making this experiment a success towards developing yellow rust resistant varieties in future. The linked markers obtained from the present studies suggest a promising application of marker assisted techniques for crop improvement using flag leaf area, number of spikelets per spike and spike weight traits as measure for resistance against stripe rust. These identified markers can be used as strong tools for developing stripe rust resistant varieties in future, which is an emerging problem of global concern.
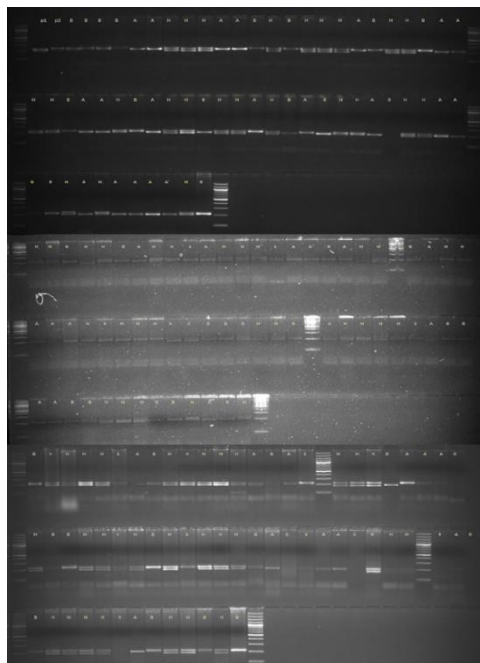
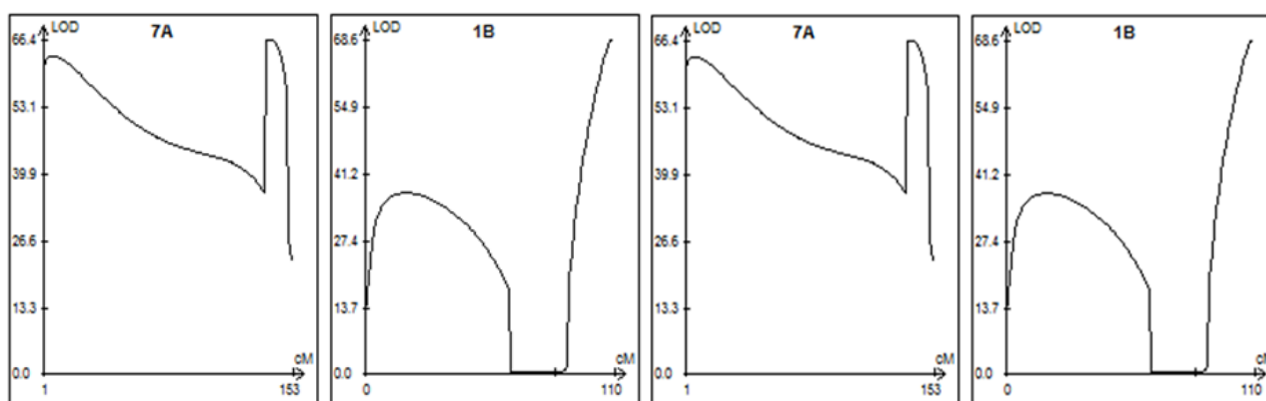**P1 and P2:** Segregation of Barc76 in F₂ population- A is like WH711, B is like PBW698 and H stands for heterozygote



**Fig 1:** LOD score on 7A and 1B chromosome of wheat

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