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GENETICS AND PLANT BREEDING

Marker Assisted Foreground Selection of Back Cross Genotypes for Leaf Rust Resistance in Wheat (*Triticum aestivum* L.)

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ABSTRACT

The investigation was undertaken to identify desirable wheat genotypes at BC_1 generation carrying leaf rust resistance alleles using molecular markers. The F_1 was generated from cross NI5643 (lacking *Lr34*) × NIAW34 (carrying *Lr34*). The F_1 was confirmed by using simple sequence repeats (SSR) marker gwm389. This F_1 was used for crossing with recurrent parent NI5643 (lacking *Lr34*) and BC_1 plants were produced. Twenty five BC_1 plants were screened for the presence of *Lr34*, by using SSR primers. For this purpose markers from chromosome 7D i.e. *cssfr1, cssfr2*, KUDS, *barc352* were used to screen parental genotypes. All these markers did not show polymorphism among parental genotypes. Therefore these were not used for foreground selection. Another marker from chromosome 7D, wms130 was used to screen BC_1 plants for the presence of *Lr34* (foreground selection). As per the SSR profile generated by primer wms130, the plants 1, 3, 4, 6, 10, 12, 13, 14, 15, 16, 18, 22 and 25 were found positive for *Lr34*. Another 10 markers i.e. gwm389, wmc313, wmc468, gwm610, wmc707, gwm60, wmc525, barc137, wmc419 and barc62 were used for background selection. The plants with heterozygous amplification pattern were selected for developing BC_2 plants. BC_1 plants that are heterozygous or positive for *Lr34* using all the markers are 1, 3, 4, 6, 10, 12, 13, 14, 15, 16, 18, 20, 22, 23 and 25. Based on foreground selection and background selection, a total of 15 plants were found carrying desirable alleles and were used for developing BC_2.

Highlights

- Present study was thus carried out with the objective to identify desirable wheat genotypes at BC_1 generation containing leaf rust resistance alleles using molecular markers.

Keywords: Wheat; Leaf rust; *Lr34* gene; foreground selection; background selection; SSR markers

Wheat (*Triticum aestivum* L.) is a major cereal crop in India and it is cultivated worldwide. It belongs to the tribe *Triticeae* (= *Hordeae*) in the grass family *Poaceae* (*Gramineae*) (Briggle and Reitz 1963). It is widely cultivated as cash crop because it produces a good yield per unit area.

Wheat production is subjected to many yield limiting biotic and abiotic stresses globally. Among biotic stresses, three rust diseases of wheat have been the most devastating throughout the world including Asia (Singh *et al.* 2004). According to Singh *et al.* (2004) stripe and leaf rust could adversely affect wheat production in Asia by 46% and 63%, respectively if susceptible wheat cultivars are grown.

Leaf (or brown) rust caused by *Puccinia triticina* Erikss, is an important and most widely distributed foliar disease of wheat world over. It has a potential of causing significant yield losses in India as it occurs in all the wheat growing areas. Depending upon severity and duration of infection, yield losses can reach up to 50% (McIntosh *et al.* 1995).

Rust can be managed most effectively and economically through cultivation of resistant

varieties. Breeding resistant varieties is the most successful and economic approach to combat leaf rust. The traditional approach of transferring *Lr* resistance genes from wheat related species or pyramiding genes in elite breeding lines is time consuming and very laborious.

Molecular markers are used for two purposes in resistance breeding (1) to monitor the incorporation of designated resistance genes or QTLs into elite wheat genotypes. (2) To identify resistance genes in varieties and lines where the genetic background is unknown. In hexaploid wheat, simple sequence repeats (SSR) are more informative and useful than any other marker system in molecular mapping because of their high polymorphism. Molecular markers were identified for most of the resistance genes against brown leaf rust (*Lr1, Lr3, Lr9, Lr10, Lr13, Lr14, Lr16, Lr20, Lr21, Lr22, Lr24, Lr25, Lr26, Lr28, Lr29, Lr32, Lr34, Lr35, Lr37, Lr39, Lr46, Lr47, Lr50, Lr51, Lr52, Lr57, Lr58* (Prins et al. 1997).

One of the markers associated with rust resistance gene pair *Lr34/Yr18* is leaf tip necrosis (*Ltn*). It shows complete linkage with rust resistance gene pair *Lr34/Yr18* (Singh 1992). *Ltn* plays major role in selecting genotypes with multipathogen resistance in wheat breeding programs. However, appearance of *Ltn* under field conditions is time consuming. For rapid identification of gene *Lr34,* use of quick screening methods like use of molecular markers is needed.

Variety NIAW34 was developed at Agricultural Research Station Niphad. Its yield potential is 40q/ha and average yield is 38-40q/ha. It is tolerant to aphids and resistant to black and brown rust. It is good for *chapati* and its protein content is high. It is recommended for late sown conditions. Variety NI5643 is also developed at Agricultural Research Station Niphad. Its yield potential is 25q/ha and average yield is 23-25q/ha. It is tolerant to aphids but susceptible to leaf rust. Though its yield potential is low, it is tolerant to aphids. For this purpose this variety was selected in present study for introgression of leaf rust resistant gene *Lr34,* from the variety NIAW34.

Present study was thus carried out with the objective to identify desirable wheat genotypes at BC_1 generation containing leaf rust resistance alleles using molecular markers.

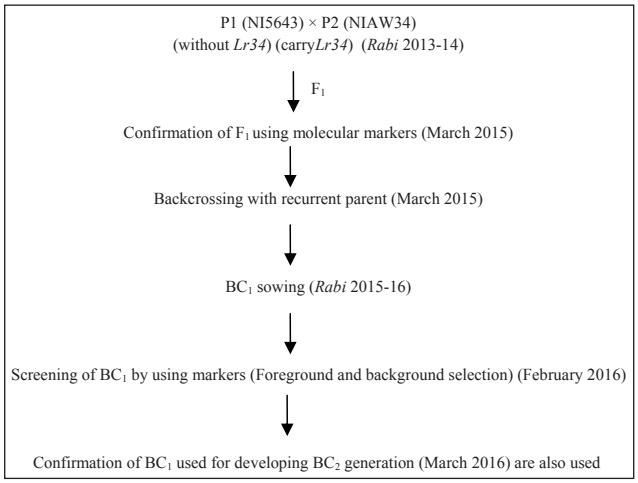
MATERIALS AND METHODS

The present research work entitled "Marker assisted foreground selection of back cross genotypes for leaf rust resistance in wheat (*Triticum aestivum* L.)" was carried out at State Level Biotechnology Center, Mahatma Phule Krishi Vidyapeeth, Rahuri (MS) during year 2014-15. The details of materials used and methods adopted in present study are mentioned under following subheadings.

Materials

Plant material

Leaf tissues from two parental genotypes (NIAW34 and NI5643), derived F_1 and 25 BC_1 plants were collected from Agricultural Research Station, Niphad, District Nashik (MS) and used for present research work. Details of population development is given in the following flowchart.



Molecular Markers

Simple Sequence Repeats (SSR) were used in the present investigation for identification of plants with presence of *Lr34* gene. Thirteen such markers were used to determine their efficiency in identifying *Lr34* gene in the wheat genotypes. In addition, two gene specific markers were also used.

METHODS

DNA Isolation

Genomic DNA was isolated from two parental genotypes, F_1 and 25 BC_1 plants by following CTAB (Cetyl Tri-methyl Ammonium Bromide) extraction method with some modifications as described by Helguera *et al.* (2005).

Table 1: List of primers with their references used for confirmation of wheat leaf rust resistance gene *Lr34*

Sl. No.	Primer	Sequence	Chromosome	T_a (^{0}C)	Reference
1	wmc313	F-GCAGTCTAATTATCTGCTGGCG R-GGGTCCTTGTCTACTCATGTCT	4A	59	Sharma *et al.* 2015
2	wmc468	F-AGCTGGGTTAATAACAGAGGAT R-CACATAACTGTCCACTCCTTTC	4A	57	Liu *et al.* 2010
3	gwm610	F-CTGCCTTCTCCATGGTTTGT R-AATGGCCAAAGGTTATGAAGG	4A	61	Kumar *et al.* 2013
4	wmc707	F-GCTAGCTGACACTTTTCCTTTG R-TCAGTTTCCCACTCACTTCTTT	4A	58	Liu *et al.* 2010
5	gwm60	F-TGTCCTACACGGACCACGT R-GCATTGACAGATGCACACG	7A	61	Wei *et al.* 2005
6	wmc525	F-GTTTGACGTGTTTGCTGCTTAC R-CTACGGATAATGATTGCTGGCT	7A	60	Kadam *et al.* 2012
7	barc137	F-GGCCCATTTCCCACTTTCCA R-CCAGCCCCTCTACACATTTT	1B	60	Shi *et al.* 2001
8	wmc419	F-GTTTCGGATAAAACCGGAGTGC R-ACTACTTGTGGGTTATCACCAGC	1B	63	Kadam *et al.* 2012
9	gwm389	F-ATCATGTCGATCTCCTTGACG R-TGCCATGCACATTAGCAGAT	3B	55	Malik *et al.* 2013
10	barc62	F-TTGCCTGAGACATACATACACCT R-GCCAGAACAGAATGAGTGCT	1D	59	Zhou *et al.* 2002
11	wms130	F-AGCTCTGCTTCACGAGGAAG R-CTCCTCTTTATATCGCGTCCC	7D	58	Suenaga *et al.* 2003
12	KUDS	F-ACGTTTCAGCATCAACCTGAA R-GAACTTGCAATCAAGTAGGAG	7D	58	Lagudah *et al.* 2006
13	*cssfr1* i) L34DINT9F ii) L34PLUSR	F-TTGATGAAACCAGTTTTTTTTCTA R-GCCATTTAACATAATCATGATGGA	7D	58	Lagudah *et al.* 2009
14	*cssfr2* i) L34DINT9F ii) L34MINUSR	F-TTGATGAAACCAGTTTTTTTTCTA R-TATGCCATTTAACATAATCATGAA	7D	58	Lagudah *et al.* 2009
15	barc352	F-CCCTTTCTCGCTCGCCTATCCC R-CTGTTTCGCCCAATCTCGGTGTG	7D	60	Sehgal *et al.* 2012

RNase treatment

Isolated DNA of wheat genotypes were purified by giving RNase treatment.

DNA amplification

Amplification reaction mixture was prepared in 0.2 ml thin walled flat capped PCR tubes, containing the following components. The total volume of each reaction mixture was 20 µl containing 2 µl of 1X Taq bufferB, 1.2 µl of 1mM $MgCl_2$, 1.6 µl of 3.2 mM dNTP mix, 1.6 µl of 0.32 picomole/ µl forward and reverse primers, 0.33 µl of 1 U taq-polymerase, 2 µl of template DNA and 9.67 µl of sterile distilled water. The DNA amplification was carried out in a Thermal Cycler (Eppendorf, Master Cycler Gradient, Germany). The temperature profiles set for PCR amplification of different primers are mentioned in Tables 2 to 7.

Agarose gel electrophoresis of amplified PCR products

PCR products were separated by Agarose gel (2%) electrophoresis. Agarose gels stained with Ethidium Bromide were used for DNA profiles visualization

Table 2: Temperature profile used for primer gwm389

Steps	Temperature	Time	Cycle(s)
Initial Denaturation	94°C	5 min	1
Denaturation	94°C	45 sec	
Annealing	55°C	45 sec.	40
Extension/Elongation	72°C	1 min.	
Final extension	72°C	10 min	1
Final hold	4°C	Till retrieval	-

Table 3: Temperature profile used for primer wms130

Steps	Temperature	Time	Cycle(s)
Initial Denaturation	94°C	5 min	1
Denaturation	94°C	20 sec	
Annealing	58 °C	20 sec.	45
Extension/Elongation	72°C	1 min.	
Final extension	72°C	7 min	1
Final hold	4°C	Till retrieval	—

Table 4: Temperature profile used for primer KUDS

Steps	Temperature	Time	Cycle(s)
Initial Denaturation	94°C	4 min	1
Denaturation	94°C	30 sec	
Annealing	58 °C	30 sec.	38
Extension/Elongation	72°C	1 min.	
Final extension	72°C	5 min	1
Final hold	4°C	Till retrieval	-

Table 5: Temperature profile used for primer barc352

Name of the steps followed	Temperature	Time	Cycle(s)
Initial Denaturation	94°C	4 min	1
Denaturation	94°C	30 sec	
Annealing	60 °C	30 sec.	38
Extension/Elongation	72°C	1 min.	
Final extension	72°C	5 min	1
Final hold	4°C	Till retrieval	—

Table 6: Temperature profile used for primers *cssfr1* and *cssfr2*

Temperature	Time	Cycle(s)
94°C	1min	
58°C	1min	5
72°C	2min	
94°C	30sec	
58°C	30sec	30
72°C	50sec	
94°C	30sec	
58°C	30sec	1
72°C	5min	
4°C	Till retrieval	-

Table 7: Temperature profile used for primers wmc313, barc62, gwm610, gwm60, barc137, barc525, wmc419, wmc468 and wmc707

Steps	Temperature	Time	Cycle(s)
Initial Denaturation	94°C	4 min	1
Denaturation	94°C	30 sec	
Annealing	*(varies according to primer as shown in table 3.1)	30 sec.	38
Extension/Elongation	72°C	1 min.	
Final extension	72°C	8 min	1
Final hold	4°C	Till retrieval	

with UV transilluminator in Gel Documentation System (Flor chem.™ Alpha innotech, USA).

Confirmation of F_1 and detection of *Lr34* gene in back cross generation by using molecular markers

Simple sequence repeats (SSR) markers were used for confirmation of F_1. The confirmed F_1 (carrying leaf rust resistance gene *Lr34*) was used for crossing with NI5643 (lacking *Lr34*) and BC_1 plants were developed. BC_1 plants were screened by using molecular markers for presence of *Lr34* and heterozygous plants having presence of *Lr34* were recommended for developing BC_2 plants.

RESULTS AND DISCUSSION

The long term goal of this investigation is to introgress leaf rust resistance gene *Lr34* from a donor genotype NIAW34 to a recipient genotype NI5643 through marker assisted selection. The specific objective of present investigation was to identify desirable wheat genotypes at BC_1 generation containing leaf rust resistance alleles using molecular markers. Results thus obtained are presented under following headings.

Development of F_1

F_1 was developed by crossing NI5643 (lacking *Lr34*) and NIAW34 (carrying *Lr34*). Only one F_1 plant survived.

Molecular Marker analysis

In this study Simple Sequence Repeat (SSR) markers were used to identify desirable wheat genotypes at BC_1 generation containing leaf rust resistance alleles.

Confirmation of F_1

The F_1 plant obtained from cross NI5643 (lacking *Lr34*) × NIAW34 (carrying *Lr34*) was confirmed by using (SSR) marker gwm389. The F_1 showed heterozygous amplification pattern (Plate 1).

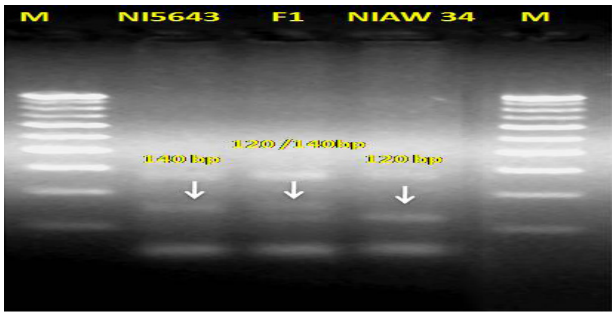


Plate 1: Confirmation of F_1 by primer gwm389

Development of BC_1 plants

The confirmed F_1 was crossed with the recurrent parent NI5643 and BC_1 plants were produced. Total 25 seeds were developed. These seeds were sown and 25 BC_1 plants were raised (Plate 2).

P_1 P_2 BC_1 BC_1

Plate 2: Field experiment conducted at ARS Niphad

Screening of BC_1 plants

25 BC_1 plants were screened for presence of *Lr34*

gene by using SSR markers. Screening was done by foreground and background selection.

Foreground selection

In foreground selection markers from chromosome 7D were used (as *Lr34* is present on 7D) to screen the parental genotypes. The markers cssfr1, cssfr2, KUDS and barc352 are tightly linked to *Lr34*. Therefore these were used to check polymorphism in parental genotypes. All these markers showed no polymorphism among parental genotypes. Therefore these markers were not used for foreground selection of desirable BC_1 plants. However another marker wms130 was found polymorphic and was used to confirm the heterozygotes (Table 8).

As per the SSR profile generated by primer wms130 the susceptible parent (NI5643) amplified alleles of size 110, 130 and 170bp and resistant parent (NIAW34) amplified alleles of size 110, 135 and 175bp. The plants 1, 3, 4, 6, 10, 11, 12, 13, 14, 15, 16, 18, 22, and 25 were found positive for *Lr34*. Out of 25 BC_1 13 plants were found positive for *Lr34* (Plate 3, Table 8).

Lane M- Marker 100 bp Ladder	
Lane No.	**Genotypes**
P_1	NI5643
P_2	NIAW34
1-25	BC_1 plants

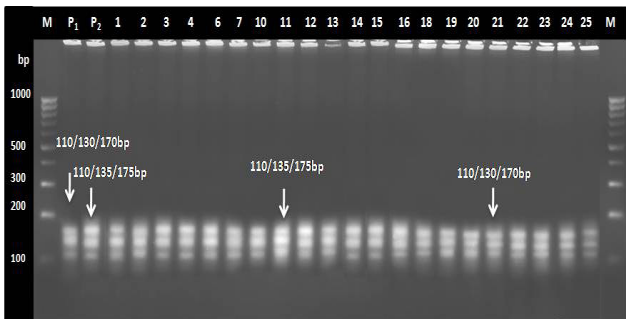


Plate 3: Screening of BC_1 by wms130

Table 8: Details of products size (bp) using SSR markers observed in BC_1 plants during foreground selection

Sl. No.	wms130
P_1	110/130/170
P_2	110/135/175
1	110/135/175
2	110/130/170
3	110/135/175
4	110/135/175
5	–
6	110/135/175
7	110/130/170
8	–
9	–
10	110/135/175
11	110/135/175
12	110/135/175
13	110/135/175
14	110/135/175
15	110/135/175
16	110/135/175
17	–
18	110/135/175
19	110/130/170
20	110/130/170
21	110/130/170
22	110/135/175
23	110/130/170
24	110/130/170
25	110/135/175

(- no amplification)

Background selection

Another 10 markers were used for background selection. The plants with heterozygous amplification pattern were selected. For some of the markers single bands were observed, therefore these markers (gwm610 and wmc419) were not considered for selection. The details of product size and banding patterns observed are given in Plates 4, Table 3.2.

Lane M- Marker 100 bp Ladder	
Lane No.	**Genotypes**
P_1	NI5643
P_2	NIAW34
1-25	BC_1 plants

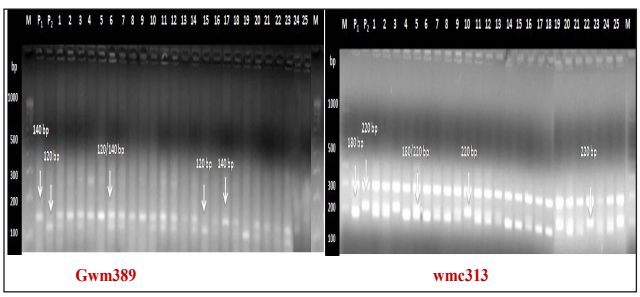


Plate 4: Screening of BC_1 by gwm389 and wmc313

Marker gwm389 amplified two alleles of 120bp and 140bp. Out of 25 BC_1 plants screened by primer gwm389, plants 1, 3, 6, 7, 10, 11, 12, 13, 14, 16, 18, 20, 21, 22, 23 and 25 were found heterozygous amplifying both the alleles. Marker wmc313 amplified two alleles of 180bp and 220bp. Out of 25 BC_1 plants screened by primer wmc313, plants 1, 2, 5, 7, 8, 9, 11, 12, 13, 16, 20, 21, 23 and 24 were found heterozygous amplifying both the alleles (Plate 4, Table 9).

Lane M- Marker 100 bp Ladder	
Lane No.	**Genotypes**
P_1	NI5643
P_2	NIAW34
1-25	BC_1 plants

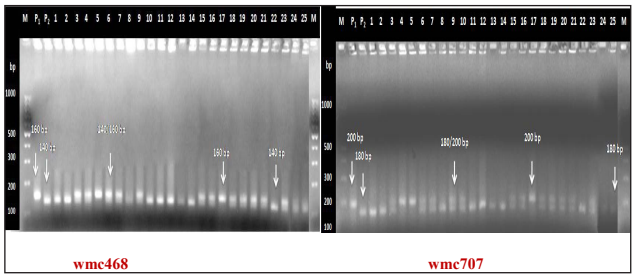


Plate 5: Screening of BC_1 wmc468 and wmc707

Marker wmc468 amplified two alleles of 140bp and 160bp. Out of 25 BC_1 plants screened by primer wmc468, plants 3, 4, 6, 7, 9, 15, 16, 18, 19, 20, 21 and 23 were found heterozygous amplifying both the alleles. Marker wmc707 amplified two alleles of 180bp and 200bp. Out of 25 BC_1 plants screened by primer wmc707, plants 3, 6, 7, 9, 10, 15, 16, 18, 19, 20, 21 and 23 were found heterozygous amplifying both the alleles (Plate 5, Table 9).

Lane M- Marker 100 bp Ladder	
Lane No.	**Genotypes**
P_1	NI5643
P_2	NIAW34
1-25	BC_1 plants

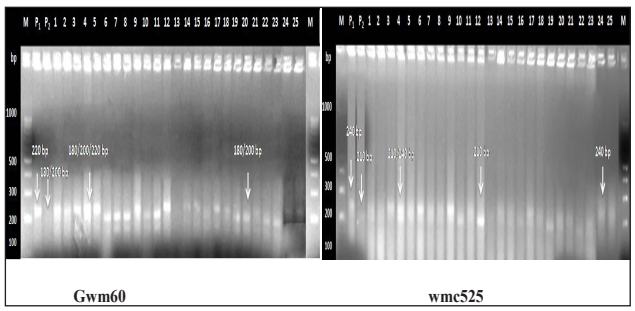


Plate 6: Screening of BC_1 by gwm60 and wmc525

Marker gwm60 amplified three alleles of 180bp, 200bp and 220bp. Out of 25 BC_1 plants screened by primer gwm60, the plants 1, 2, 4, 9, 12, 14, 15, 17, 18 and 21 were found heterozygous amplifying all the three alleles. Marker wmc525 amplified two alleles of 210bp and 240bp. Out of 25 BC_1 plants screened by primer wmc525, plants 1, 2, 4, 5, 6, 7, 8, 9, 11, 14, 15, 17, 18, 20, 23 and 25 were found heterozygous amplifying both the alleles (Plate 6, Table 9).

Lane M- Marker 100 bp Ladder	
Lane No.	**Genotypes**
P_1	NI5643
P_2	NIAW34
1-25	BC_1 plants

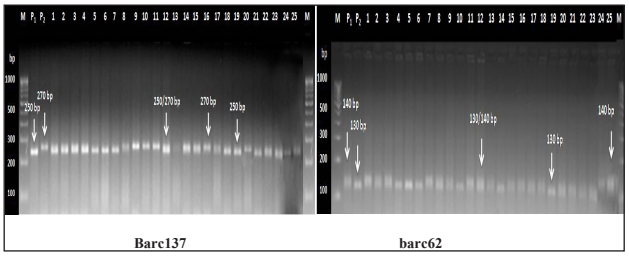


Plate 7: Screening of BC_1 by barc137 and barc62

Marker barc137 amplified two alleles of 250bp and 270bp. Out of 25 BC_1 plants screened by primer barc137, plants 1, 2, 3, 4, 12, 14, 15, 17, 20, 22, 23 and 25 were found heterozygous amplifying both the alleles. Marker barc62 amplified two alleles of 130bp and 140bp. Out of 25 BC_1 plants screened by primer barc62, plants 1, 2, 3, 8, 9, 12, 13, 15, 16, 17, 18, 20, 21, 22 and 23 were found heterozygous amplifying both the alleles (Plate 7, Table 9).

BC_1 plants that are heterozygous or positive for *Lr34* with all the markers used are- 1, 3, 4, 6, 10, 12, 13, 14, 15, 16, 18, 20, 22, 23 and 25. Based on foreground and background selection, a total of 15 plants were found carrying desirable alleles and were used for developing BC_2.

DISCUSSION

The results obtained in this research work have been described and explained in previous chapter. The discussion based on the results of experiment is mentioned in this chapter.

Confirmation of F_1 and production of BC_1 plants

The F_1 generated from cross NI5643 (lacking *Lr34*)

Table 9: Details of products size (bp) using SSR markers observed in BC_1 plants during background selection

Sl. No.	*gwm389*	*wmc313*	*wmc468*	*wmc707*	*gwm60*	*wmc525*	*barc137*	*barc62*
P1	140	180	160	200	220	240	250	140
P2	120	220	140	180	180/220	210	270	130
1	120/140	180/220	140	180	180/200/220	210/240	250/270	130/140
2	140	180/220	140	180	180/200/220	210/240	250/270	130/140
3	120/140	220	140/160	180/200	220	240	250/270	130/140
4	140	180	140/160	200	180/200/220	210/240	250/270	130
5	140	180/220	160	200	220	210/240	250	130
6	120/140	180	140/160	180/200	180/220	210/240	250	130
7	120/140	180/220	140/160	180/200	180/220	210/240	250	140
8	140	180/220	140	180	180/220	210/240	270	130/140
9	140	180/220	140/160	180/200	180/200/220	210/240	270	130/140
10	120/140	220	140	180/200	180/220	210	270	130
11	120/140	180/220	140	180	180/220	210/240	270	140
12	120/140	180/220	140	180	180/200/220	210	250/270	130/140
13	120/140	180/220	140	180	_	240	270	130/140
14	120/140	180	140	180	180/200/220	210/240	250/270	130
15	120	180	140/160	180/200	180/200/220	210/240	250/270	130/140
16	120/140	180/220	140/160	180/200	180/220	210	270	130/140
17	140	180	160	200	180/200/220	210/240	250/270	130/140
18	120/140	180	140/160	180/200	180/200/220	210/240	250	130/140
19	120	220	140/160	180/200	180/220	210	250	130
20	120/140	180/220	140/160	180/200	180/220	210/240	250/270	130/140
21	120/140	180/220	140/160	180/200	180/200/220	240	250	130/140
22	120/140	220	140	180	180/220	210	250/270	130/140
23	120/140	180/220	140/160	180/200	180/220	210/240	250/270	130/140
24	120	180/220	140	180	180/220	240	250	140
25	120/140	220	140	180	180/220	210/240	250/270	140
Total no. of heterozygous plants	**16**	**14**	**12**	**12**	**10**	**16**	**12**	**15**

Table 10: BC_1 plants heterozygous or positive for *Lr34* with all the markers used

Sl. No.	*gwm389*	*barc137*	*barc62*	*wmc313*	*gwm60*	*wmc525*	*wmc468*	*wmc707*
P1	140	250	140	180	220	240	160	200
P2	120	270	130	220	200/180	210	140	180
1	140/120	250/270	140/130	180/220	220/200/180	240/210	140	180
10	140/120	270	130	220	200/180	210	140	200/180
16	140/120	270	140/130	180/220	200/180	210	160/140	200/180
20	140/120	250/270	140/130	180/220	200/180	240/210	160/140	200/180
23	140/120	250/270	140/130	180/220	200/180	240/210	160/140	200/180

× NIAW34 (carrying *Lr34*) was confirmed by using simple sequence repeats (SSR) marker gwm389. The F_1 showed heterozygous amplification pattern. This F_1 was used for crossing with the recurrent parent NI5643 (lacking *Lr34*) and BC_1 plants were produced.

Screening of BC_1 plants

Three hundred elite wheat lines were earlier screened by Pawar *et al.* (2013) for identification of *Lr34* gene. Among them 60 lines showed presence of the *Lr34* gene. The percent of confirmed plant

was 20%. In the present investigation BC_1 plants were screened for the presence of *Lr34*, using SSR markers. Out of 25 BC_1 plants, a total of 15 plants carrying desirable alleles were selected. The percent of confirmed plants was 60% which is more than that of Pawar *et al.* (2013).

Foreground selection using markers from chromosome 7D

Thirty eight wheat genotypes comprising susceptible as well as resistant to leaf rust gene *Lr34* were earlier used by Muthe (2015) for validation of known markers linked with the gene *Lr34* conferring resistance to leaf rust in wheat. Amplification was carried out using STS marker csLV34, SSR markers (from chromosome 7D) wms130, barc352, gwm389, and KUDS and gene specific markers *cssfr1*, *cssfr2*, *cssfr5*. SSR primers wms130, barc352, gwm389 produced both the *Lr34+* and *Lr34-* alleles by indicating presence and absence of *Lr34* gene within selected genotypes.

Similarly in the present study the SSR marker wms130 was used for foreground selection of BC_1 plants. The susceptible parent (NI5643) amplified alleles of size 110, 130 and 170bp and resistant parent (NIAW34) amplified alleles of size 110, 135 and 175bp. Other markers i.e. cssfr1, cssfr2, KUDS, barc352 were used to check polymorphism in parental genotypes. These markers showed no polymorphism among parental genotypes. Therefore these were not used for foreground selection of desirable BC_1 plants. This means that utility of marker is dependent on genotypes used.

Background selection

Ten SSR markers i.e. gwm389, wmc313, wmc468, gwm610, wmc707, gwm60, wmc525, barc137, wmc419 and barc62 were used for background selection of desirable BC_1 plants and the heterozygous BC_1 plants containing *Lr34* were identified. BC_1 plants that are heterozygous or positive for *Lr34* using all the markers are - 1, 3, 4, 6, 10, 12, 13, 14, 15, 16, 18, 20, 22, 23 and 25. Based on foreground and background selection, a total of 15 plants were found carrying desirable alleles and were used for developing BC_2.

CONCLUSION

The SSR primer (wms130) tightly linked to Lr34, can be used in foreground selection of desirable wheat genotypes at BC1 generation carrying leaf rust resistant gene Lr34. SSR primer cssfr1, cssfr2, KUDS, barc352 failed to produce polymorphic amplification pattern in these genotypes hence, may not be used. SSR markers gwm389, wmc313, wmc468, gwm610, wmc707, gwm60, wmc525, barc137, wmc419 and barc62 can be used for background selection of desirable wheat genotypes at BC1 generation carrying leaf rust resistance gene Lr34. Based on foreground and background selection, a total of 15 plants carrying desirable alleles were selected.

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REFERENCES

Briggle, L.W. and Reitz, L.P. 1963. Classification of *Triticum* species and of wheat varities grown in United States. Technical bulletin No.1278.

Helguera, M., Vanzetti, L., Soria, M., Khan, I.A., Kolmer, J. and Dubcovsky, J. 2005. PCR markers for *Triticum speltoides* leaf rust resistance gene *Lr51* and their use to develop isogenic hard red spring wheat lines. *Crop Sci.*, **45**(2): 728-734.

Kadam, S., Singh, K., Shukla, S., Goel, S., Vikrm, P., Pawar, V., Gaikwad, K., Chopra, R.K. and Singh, N. 2012. Genomic associations for drought tolerance on the short arm of wheat chromosome 4B. Funct. Integr. Genomics. DOI: 10.1007/s10142-012-0276-1.

Kumar, S., Kumari, P., Kumar, U., Grover, M., Sigh, A.K., Singh, R. and Sengar, R.S. 2013. Molecular approaches for designing heat tolerant wheat. *J. Plant Biochem. Biotechnol.*, DOI: 10.1007/s13562-013-0229-3.

Lagudah, E.S., Krattinger, S.G., Herrera-Foessel, S., Singh, R.P., Huerta-Espino, J., Spielmeyer, W., Brown-Guedira, G., Selter, L.L. and Keller, B. 2009. Gene-specific markers for the wheat gene *Lr34/Yr18/Pm38* which confers resistance to multiple fungal pathogens. *Theor. Appl. Genet.*, **119**: 889–898.

Lagudah, E.S., McFadden, H., Singh, R.P., Huerta-Espino, J., Barina, H.S. and Spielmeyer, W. 2006. Molecular genetic characterization of the *Lr34/Yr18* slow rusting resistance gene region in wheat. *Theor. Appl. Genet.*, **114**: 21-30.

Liu, L., Wang, L., Yao, J., Zheng, Y. and Zhao, C. 2010. Association aping of six agronomic traits on chromosome 4A of wheat (*Trticum aestium* L.) *Mol. Plant Breed*, DOI: 10.5376/ mpb.2010.01.0005.

Malic, R., Tiwar, R., Arora, A., Kumar, P., Sheoran, S., Sharma, P., Singh, R., Tiwari, V. and Sharma, I. 2013. Genotypic characterization of elite Indian wheat genotypes using

molecular markers and their pedigree analysis Aust. *J. Crop Sci.,* **7**: 561-567.

McIntosh, R.A., Wellings, C.R. and Park, R.F. 1995. Wheat rusts: An atlas of resistance genes. CSIRO Publications, Melbourne, 208. DOI: 10.1007/978-94-011-0083-0.

Muthe, S.T. 2015. Validation of known markers linked with the gene *LR34* conferring resistance against leaf rust in wheat in wheat *(Triticum aestivum* L.) Master's thesis submitted to Mahatma Phule Krishi Vidyapeeth Rahuri (unpublished).

Pawar, S.L., Kumar, P., Duhan, J.S., Saharan, M.S., Bharadwaj, S.C., Tiwari, R. and Sharma, I. 2013. Characterization of adult plant leaf rust resistance gene *Lr34* in Indian wheat genotypes using an STS marker. *J. Wheat Res.,* **5**(1): 15-20.

Prins, R., Marais, G.F., Pretorius, Z.A., Janse, B.J.H. and Maris, A.S. 1997. A study of modified forms of the *Lr19* translocation of common wheat. *Theor. Appl. Genet.,* **95**: 424-430.

Sehgal, S.A., Tahir, R.A. and Nawaz, M. 2012. Molecular characterization of wheat genotypes using SSR markers. *Int. J. Bioautomation.,* **16**(2): 119-128 119.

Sharma, S., Ghimire, S.L., Niroula, R.K., Ojha, B.R. and Thapa, D.B. 2015. Marker assisted screening of Nepalese wheat genotypes and advanced lines for resistance to different races of wheat rust species. *Agri. Biol. J. North Amer.,* DOI: 10.5251/abjna. 2015. 6.4.108.117.

Shi, J., Ward, R. and Wang, D. 2001. Application of a high throughput, low cost, non denaturing polyacrylamide gel system for wheat microsatellite mapping. *National Fusarium Head Blight Forum Proc.* Dec. 8-10 (2001).

Singh, R.P., William, H.M., Huerta-Espino, J. and Rosewarne, G. 2004. Wheat rust in Asia: Meeting the challengers with old and new technologies Proc. 4th Int. Crop Sci. Cong., Brisbane, Australia 26 Sep-1 Oct.

Singh, R.P. 1992. Association between gene *Lr34* for leaf rust resistance and leaf tip necrosis in wheat. *Crop Sci.,* **32**: 874-878.

Suenaga, K., Singh, R.P., Huerta-Espino, J. and William, H.M. 2003. Microsatellite markers for genes *Lr34/Yr18* and other quantitative trait loci for leaf rust and stripe rust resistance in bread wheat. *Genet. Resistance,* **93**(7): 881-890.

Wei, Y.M., Hou, Y.C., Wu, W., Zhang, Z.O., Liu, D.C. and Zheng, Y.L. 2005. Microsatellite DNA polymorphism divergence in chinese wheat (*Triticum aestivum* L.) landraces highly resistant to fusarium head blight. *J. Appl. Genet.,* **46**(1): 3-9.

Zhou, W.C., Kolb, F.L., Bai, G.H., Shaner, G. and Domier, L.L. 2002. Genetic analysis of scab resistance QTL in wheat with microsatellite and AFLP markers. *Genome,* **45**: 719–727.