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 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 *Triticaceae* (= *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).
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 MgCl₂, 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 1: List of primers with their references used for confirmation of wheat leaf rust resistance gene Lr34

| Sl. No. | Primer  | Sequence                                      | Chromosome | Tₘ (°C) | Reference        |
|---------|---------|-----------------------------------------------|------------|---------|-----------------|
| 1       | wmc313  | F-GCAGCTCAATTATCTGCTGCGG<br>R-GGGTCCCTTGCTACTCATGTCT | 4A         | 59      | Sharma et al. 2015 |
| 2       | wmc468  | F-AGCTGGGTGTTAAACAGAGGAT<br>R-CATAAATGCTCCGACTCCCTTTTC | 4A         | 57      | Liu et al. 2010  |
| 3       | gwm610  | F-CTGGCTCTCCATGGTTTG<br>R-AATGGCAAGGTTATGAGG | 4A         | 61      | Kumar et al. 2013 |
| 4       | wmc707  | F-GCTAGCTGACACCTTTTCTT<br>R-TCAGTTTCCACTACCTTTT | 4A         | 58      | Liu et al. 2010  |
| 5       | gwm60   | F-TGTCCTACACGGACCACGT<br>R-GCATGAGATGCAAGAGG | 7A         | 61      | Wei et al. 2005  |
| 6       | wmc525  | F-GTTTACGATGTTGCTC<br>R-CTACGGATAATGCTTGGCT | 7A         | 60      | Kadam et al. 2012 |
| 7       | barc137 | F-GGCCAATCCCCACTTTCCA<br>R-CAGGCCCCTCTACACATT | 1B         | 60      | Shi et al. 2001  |
| 8       | wmc419  | F-GTTTGGATAAAACCCGGAGTC<br>R-CTACTTTCTGGTTATCAC | 1B         | 63      | Kadam et al. 2012 |
| 9       | gwm389  | F-ATCATGTCAGATTCCTTGAC<br>R-TGCCATGCAATACAGAT | 3B         | 55      | Malik et al. 2013 |
| 10      | barc62  | F-TGTCCTAGCATACATACAC<br>R-GCCAGACAAGAATGAGTGT | 1D         | 59      | Zhou et al. 2002 |
| 11      | wms130  | F-AGCTCTGTCTACGGAGAG<br>R-CTCTCTATATATCGGCTCC | 7D         | 58      | Suenaga et al. 2003 |
| 12      | KUDS    | F-ACGTTCAGATCAACCTGAA<br>R-GAATCTTGCAATCACGAG | 7D         | 58      | Lagudah et al. 2006 |
| 13      | cssfr1  | i) L34DINT9F<br>ii) L34PLUSR | 7D         | 58      | Lagudah et al. 2009 |
| 14      | cssfr2  | i) L34DINT9F<br>ii) L34MINUSR | 7D         | 58      | Lagudah et al. 2009 |
| 15      | barc352 | F-CCCTCTTCCGCTCCATCC<br>R-CTGTTTCCGCCAAATCTCGGT | 7D         | 60      | Sehgal et al. 2012 |
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 |          |
| 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 |          |
| 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 |          |
| 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 |          |
| 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        | 1 min|          |
| 58°C        | 1 min|          |
| 72°C        | 2 min|          |
| 94°C        | 30 sec|         |
| 58°C        | 30 sec|         |
| 72°C        | 30 sec|         |
| 94°C        | 50 sec|         |
| 58°C        | 5 min|          |
| 72°C        |      |          |
| 4°C         |      |          |
with UV transilluminator in Gel Documentation System (Flor chem.™ Alpha innotech, USA).

**Confirmation of F1 and detection of Lr34 gene in back cross generation by using molecular markers**

Simple sequence repeats (SSR) markers were used for confirmation of F1. The confirmed F1 (carrying leaf rust resistance gene Lr34) was used for crossing with NI5643 (lacking Lr34) and BC1 plants were developed. BC1 plants were screened by using molecular markers for presence of Lr34 and heterozygous plants having presence of Lr34 were recommended for developing BC2 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 BC1 generation containing leaf rust resistance alleles using molecular markers. Results thus obtained are presented under following headings.

**Development of F1**

F1 was developed by crossing NI5643 (lacking Lr34) and NIAW34 (carrying Lr34). Only one F1 plant survived.

**Molecular Marker analysis**

In this study Simple Sequence Repeat (SSR) markers were used to identify desirable wheat genotypes at BC1 generation containing leaf rust resistance alleles.

**Confirmation of F1**

The F1 plant obtained from cross NI5643 (lacking Lr34) × NIAW34 (carrying Lr34) was confirmed by using (SSR) marker gwm389. The F1 showed heterozygous amplification pattern (Plate 1).

**Development of BC1 plants**

The confirmed F1 was crossed with the recurrent parent NI5643 and BC1 plants were produced. Total 25 seeds were developed. These seeds were sown and 25 BC1 plants were raised (Plate 2).

**Screening of BC1 plants**

25 BC1 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 BC1 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 BC1 plants 13 were found positive for Lr34 (Plate 3).

**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.

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**Table 8:** Details of products size (bp) using SSR markers observed in BC1 plants during foreground selection

| Sl. No. | wms130       |
|---------|--------------|
| P1      | 110/130/170  |
| P2      | 110/135/175  |
| 1       | 110/135/175  |
| 2       | 110/130/170  |

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**Plate 3:** Screening of BC1 by wms130

**Plate 4:** Screening of BC1 by gwm389 and wmc313
Marker gwm389 amplified two alleles of 120bp and 140bp. Out of 25 BC₁ 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 BC₁ 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 | Genotypes |
|-----------------------------|------------|
| Lane No.                    | Genotypes  |
| P₁                          | NI5643     |
| P₂                          | NIAW34     |
| 1-25                        | BC₁ plants |

**Plate 5:** Screening of BC₁ wmc468 and wmc707

Marker wmc468 amplified two alleles of 140bp and 160bp. Out of 25 BC₁ 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 BC₁ 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 | Genotypes |
|-----------------------------|------------|
| Lane No.                    | Genotypes  |
| P₁                          | NI5643     |
| P₂                          | NIAW34     |
| 1-25                        | BC₁ plants |

**Plate 6:** Screening of BC₁ by gwm60 and wmc525

Marker gwm60 amplified three alleles of 180bp, 200bp and 220bp. Out of 25 BC₁ 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 BC₁ 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 | Genotypes |
|-----------------------------|------------|
| Lane No.                    | Genotypes  |
| P₁                          | NI5643     |
| P₂                          | NIAW34     |
| 1-25                        | BC₁ plants |

**Plate 7:** Screening of BC₁ by barc137 and barc62

Marker barc137 amplified two alleles of 250bp and 270bp. Out of 25 BC₁ 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 BC₁ 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).

BC₁ 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 BC₂.

**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 F₁ and production of BC₁ plants**

The F₁ generated from cross NI5643 (lacking Lr34)
× NIAW34 (carrying Lr34) was confirmed by using simple sequence repeats (SSR) marker gwm389. The F₁ showed heterozygous amplification pattern. This F₁ was used for crossing with the recurrent parent NI5643 (lacking Lr34) and BC₁ plants were produced.

**Table 9:** Details of products size (bp) using SSR markers observed in BC₁ 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     |
| 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 | 210/240 | 250     | 140     |
| 8       | 140     | 180/220 | 140     | 180     | 210/240 | 250     | 270     | 130     |
| 9       | 140     | 180/220 | 140/160 | 180/200 | 210/240 | 250     | 270     | 130     |
| 10      | 120/140 | 220     | 140     | 180/200 | 180/220 | 210/240 | 250/270 | 130     |
| 11      | 120/140 | 180     | 140     | 180     | 180/220 | 210/240 | 250/270 | 130     |
| 12      | 120/140 | 180     | 140     | 180     | 180/220 | 210/240 | 250/270 | 130     |
| 13      | 120/140 | 180     | 140     | 180     | 210     | 250/270 | 130/140 |
| 14      | 120/140 | 180     | 140     | 180     | 180/220 | 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     | 140     | 180     | 180/220 | 210/240 | 250/270 | 130     |
| 17      | 120/140 | 180     | 140     | 180     | 180/220 | 210/240 | 250/270 | 130     |
| 18      | 120/140 | 180     | 140     | 180     | 180/220 | 210/240 | 250/270 | 130     |
| 19      | 120     | 180     | 140/160 | 180/200 | 180/200/220 | 210/240 | 250/270 | 130     |
| 20      | 120/140 | 180     | 140     | 180     | 180/220 | 210/240 | 250/270 | 130     |
| 21      | 120     | 180     | 140     | 180     | 180/220 | 210/240 | 250/270 | 130     |
| 22      | 120     | 180     | 140     | 180     | 180/220 | 210/240 | 250/270 | 130/140 |
| 23      | 120/140 | 180     | 140     | 180     | 180/220 | 210/240 | 250/270 | 130/140 |
| 24      | 120     | 180     | 140     | 180     | 180/220 | 210/240 | 250/270 | 130     |
| 25      | 120/140 | 180     | 140     | 180     | 180/220 | 210/240 | 250/270 | 140     |

**Table 10:** BC₁ 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 |

Screening of BC₁ 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\textsubscript{1} plants were screened for the presence of Lr34, using SSR markers. Out of 25 BC\textsubscript{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 cssfr1, 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\textsubscript{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\textsubscript{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\textsubscript{1} plants and the heterozygous BC\textsubscript{1} plants containing Lr34 were identified. BC\textsubscript{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\textsubscript{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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