# Molecular marker analysis of *Lr34* in Canada Western Red Spring wheat cultivars

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## ABSTRACT

The leaf rust resistance gene *Lr34* is an important component of the leaf rust resistance in Canadian wheat cultivars. It has provided effective resistance since it was incorporated into the cultivar Glenlea, registered in 1972. The Canada Western Red Spring (CWRS) wheat class is the predominant wheat class in Canada. The objective of this study was to analyze the molecular marker profiles of an historic collection of CWRS cultivars for microsatellite markers closely linked to *Lr34*. Cultivars released from 1900 to 2004 were included in the collection. These results were compared with genetic analyses of the leaf rust resistance in selected cultivars and with field leaf rust severity for these cultivars. Laura, registered in 1986, was the first major CWRS cultivar to carry *Lr34*, but since that time it has been incorporated into many of the leading CWRS cultivars. The cultivars Katepwa, AC Barrie, and Superb which were the most popular cultivars in the 1980s, 1990s and 2000s, respectively, all had the susceptible allele at the *Lr34* locus. Even though these cultivars had other genes for leaf rust resistance, they were overcome by the *Puccinia triticina* population and they suffered significant losses due to leaf rust. If *Lr34* had been present in these cultivars the losses would have been reduced. Determining the presence of *Lr34* in CWRS cultivars will help ensure that this important resistance gene is incorporated into future CWRS cultivars.

## INTRODUCTION

The western Canadian provinces of Alberta, Saskatchewan and Manitoba produce approximately 96% of the wheat in Canada (Statistics Canada). The Canada Western Red Spring (CWRS) cultivars are grown on approximately 70% of the area seeded to wheat in western Canada. Thatcher, the most popular CWRS cultivar from the 1940s to the 1970s (1) was completely susceptible to leaf rust, caused by *Puccinia triticina* Eriks. Breeding and research efforts resulted in the incorporation of resistance into subsequent cultivars. Commonly used resistance genes in this class include *Lr10*, *Lr13*, *Lr14a*, *Lr16*, *Lr21*, *Lr22a* and *Lr34* (2). Glenlea, registered in 1972, was the first major cultivar in western Canada to carry *Lr34* (3), although it did not have the end-use quality to be a CWRS cultivar (1). Since the 1970s Glenlea and subsequent cultivars with *Lr34* have maintained a moderate level of resistance. Combinations of resistance genes involving *Lr34* have often been very effective, partially due to the ability of *Lr34* to enhance the expression of other resistance genes (4). Laura, registered in 1986, was the first CWRS cultivar with *Lr34* (1,5) and the gene has been subsequently incorporated into more recent CWRS cultivars. Determining the presence of *Lr34* in current cultivars should help to predict the field resistance and durability of these cultivars and to aid decisions on selecting parents for future cultivars.

It is possible to postulate the presence or absence of *Lr34* in cultivars based on adult plant resistance. However, this is complicated by the presence of other resistance genes and the race non-specific nature of *Lr34*. Diagnostic molecular markers, tightly linked to *Lr34*, can help postulate the presence of this important gene. The csLV34 marker was reported to be tightly linked to *Lr34* (6) and diagnostic for the presence of the gene. This marker was recently used, in conjunction with phenotypic analysis, to postulate the presence of *Lr34* in 84 Australian wheat cultivars (7). The objective of our study was to characterize the molecular marker profiles of a large number of CWRS cultivars and breeding lines to postulate the presence or absence of *Lr34* in these lines.

## MATERIALS AND METHODS

DNA was extracted from 257 wheat lines including Canadian cultivars and breeding lines. They were analysed for the following markers linked to *Lr34*: gwm1220 (6), csLVMS1 (8), cam1, swm10 (9) and csLV34 (6). Simple sequence repeat (SSR) marker cam1 was developed by us from a Glenlea BAC clone that spanned swm10. The high number of alleles associated with cam1 (see below) is likely the results of the fact that it spanned three putative microsatellites. Marker csLV34, resolved on agarose gel, was determined as previously described (6). The four SSR markers were PCR amplified using a three primer labelling method modified from Schuelke (10) and resolved on an ABI 3130 Genetic Analyzer. Allele size was determined by fragment analysis using a modified version of Genographer.

## RESULTS

The SSR markers differed in the number of alleles observed in this diverse group of germplasm (Table 1). The csLV34 marker only had two alleles, while both csLVMS1 and swm10 had four alleles, gwm1220 had nine, and cam1 had ten. There have only been two alleles (csLV34a and csLV34b) reported to date for the csLV34 marker. The csLV34a allele (229bp) has been linked in repulsion with *Lr34*, whereas the csLV34b allele (150bp) was linked in coupling with *Lr34* in lines and cultivars known to contain *Lr34* (6). The check cultivar Thatcher had the expected csLV34a (229bp) allele while the near-isogenic line Thatcher-*Lr34* had the csLV34b (150bp) allele. There were eight different groups of lines based on their marker profiles for these five linked SSR markers. Group 1 had only two lines, Chinese Spring and the Canadian cultivar Glenlea. This group had the same pattern as the Thatcher-*Lr34* line except they had a 154bp allele for gwm1220 and the 361bp allele for cam1. Group 2 was the largest group postulated to have *Lr34*. This group included many of the Canadian CWRS cultivars previously reported to have *Lr34*, such as CDC Teal, Laura, and Pasqua. This group had the same pattern as Thatcher-*Lr34* except they had a 157bp allele for gwm1220 and the 361bp allele for cam1. Group 3 consisted of another group of Canadian CWRS cultivars postulated to have *Lr34* such as AC Cadillac, Peace, and Pacific (BW90). This group had the same marker pattern as Thatcher-*Lr34* except they had the 144bp allele for gwm1220, which was also found in Thatcher. Phenotypically many members of this group appear to have *Lr34*. Group 4 had the 229bp csLV34 allele, similar to Thatcher, but had the alleles for csLVMS1 and gwm1220 similar to Chinese Spring and Glenlea. This group was tentatively postulated to have *Lr34* since csLVMS1 and gwm1220 are known to flank *Lr34*. The alleles for swm10 and cam1 found in this group were not found in the other major groups. This group consisted largely of older cultivars including Canus (1935) and Lake (1954). The postulation of *Lr34* is uncertain for this group and further phenotypic analysis is needed to determine if *Lr34* is present. Group 5 consisted of 39 lines that had the same marker pattern as Thatcher except for csLV34 in which they had the 150bp allele typically associated with lines carrying *Lr34*, such as Thatcher-*Lr34*. Most of these lines were related and had pre-harvest sprouting resistance derived from RL4137, which was included in this group. AC Domain, previously postulated to have *Lr34* (11), was in this group. Based on the marker profile and inheritance of the csLV34b (150bp) allele in relation to field leaf rust evaluation of a segregating population generated from a cross of line within this group and Thatcher (unpublished data) we postulate that these lines do not have *Lr34*. It appears there has been a recombination between csLV34 and *Lr34* for this group of lines. Group 6 was the largest group of lines (117). This group had identical marker profiles as Thatcher. Thatcher, and its backcross derivatives, were used extensively in the development of CWRS cultivars (1) and so it is not surprising that this marker pattern was common in CWRS cultivars and breeding lines. Group 7 had the same marker pattern as group 6 and Thatcher, except they had the 141bp allele for gwm1220. Within this group were older cultivars such as Prelude (registered before 1923), Lee (1950) and Sinton (1975). Group 8 was composed of a diverse group of lines which had marker patterns different from the major groups. These lines had various alleles for gwm1220, swm10 and cam1. Lines in this group included older Canadian cultivars, such as Garnet (1925), and Regent (1939) along with more modern Canada Prairie Spring (CPS) cultivars, and foreign cultivars. These cultivars all had the 229bp allele for csLV34, similar to Thatcher.

**Table 1.** SSR marker profiles for 257 wheat cultivars and breeding lines

| Group | gwm 1220 | csLV MS1 | Xsw m10 | cam1 | csLV 34 | Total lines |
|---|---|---|---|---|---|---|
| Alleles | 9 | 4 | 4 | 10 | 2 | |
| **Checks** | | | | | | |
| Thatcher | 144 | 226 | 208 | 347 | 229 | 1 |
| Tc-*Lr34* | 161 | 224 | 206 | 357 | 150 | 1 |
| **Groups postulated to have *Lr34*** | | | | | | |
| 1 | 154 | 224 | 206 | 361 | 150 | 2 |
| 2 | 157 | 224 | 206 | 361 | 150 | 46 |
| 3 | 144 | 224 | 206 | 357 | 150 | 17 |
| 4 | 154 | 224 | 210 | 333 | 229 | 6 |
| **Groups postulated not to have *Lr34*** | | | | | | |
| 5 | 144 | 226 | 208 | 347 | 150 | 39 |
| 6 | 144 | 226 | 208 | 347 | 229 | 117 |
| 7 | 141 | 226 | 208 | 347 | 229 | 6 |
| 8 | Complex patterns/rare alleles | | | | | 24 |
| Total number of lines | | | | | | 257 |

## DISCUSSION

Determining the presence or absence of *Lr34* in wheat cultivars and breeding lines is important for leaf rust resistance but also important for stripe rust (*Yr18*), stem rust and powdery mildew resistance because resistance to these diseases is completely linked to *Lr34* (12). Previous studies have used leaf rust resistance, leaf tip necrosis, and csLV34 to postulate the presence of *Lr34* in wheat cultivars. There have been only two alleles detected to date for csLV34 and the csLV34b (150bp) allele is tightly linked in coupling to *Lr34* (6). There was also good agreement between the alleles for csLV34 and the phenotypic expression of *Lr34* in a group of 84 Australian cultivars (7). In our study the csLV34b (150bp) allele was generally present in lines known or postulated to have *Lr34* and the csLV34a (229bp) allele was found in lines without *Lr34*. The exceptions are groups 4 and 5. Group 4 had the same alleles for the flanking markers of *Lr34*, gwm1220 and csLVMS1, as group 1 (Chinese Spring and Glenlea) but had the csLV34a (229bp) allele. Group 5 contained a group of related lines derived from RL4137. The members of this group all had the csLV34b (150bp) allele but had alleles similar to Thatcher for all other SSR markers, which indicates that these lines do not have *Lr34* and the phenotypic evidence supports this hypothesis (data not shown). It is possible there was a recombination between the csLV34b allele and *Lr34* in the development of RL4137 that was subsequently inherited by the rest of these cultivars and lines. As a consequence, csLV34 is not thought to be diagnostic for *Lr34* in this group of CWRS derived material and must be used in combination with other *Lr34* associated markers There were two major groups (2+3) of CWRS cultivars that had the csLV34b allele and were known or postulated to have *Lr34*. These two major groups had the same marker patterns for csLV34, swm10 and csLVMS1 but differed for cam1 and gwm1220. These differences could reflect different sources of origin for *Lr34* within these CWRS lines. The largest group of lines, group 6, had the identical marker pattern to Thatcher and many members of this group are known or postulated to lack *Lr34*. This study helps to confirm that these lines do not carry *Lr34*. The information generated from this study should be useful in predicting the field leaf rust resistance of these cultivars and lines. It can also be used to make informed decisions on the choice of parents for future Canadian wheat cultivars.

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