Development of multiplex PCR to detect slow rust resistance genes Lr34 and Lr46 in wheat

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Abstract
Leaf rust caused by Puccinia triticina belongs to one of the most dangerous fungal diseases of wheat (Triticum aestivum L.) and is the cause of large yield losses every year. Here we report a multiplex polymerase chain reaction (PCR) assay, which was developed for detection of two important wheat slow rust resistance genes Lr34 and Lr46, using two molecular markers: csLV34 and Xwmc44, respectively. The presence of genes was analyzed in one winter wheat variety TX89D6435 and five spring wheat varieties: Pavon F76, Parula ‘S’, Rayon 89, Kern, Mochis 88. Both Lr34 and Lr46 genes were identified in variety TX89D6435, gene Lr34 was also identified in Parula ‘S’ and Kern varieties, and gene Lr46 occurs in Pavon F76 and Mochis 88 variety. None of the resistance genes tested was detected in the Rayon 89 variety. The use of the multiplex PCR method allowed to shorten the analysis time, reduce costs of analyses, and reduce the workload.

Keywords Leaf rust • Lr34 • Lr46 • Multiplex PCR • Wheat

Introduction
Leaf rust caused by the pathogen Puccinia triticina Erikss. & Henn. is one of the most destructive diseases of wheat (Kolmer et al. 2005). There are many fungicides that help control these fungal disease, but their use is expensive and can have a negative impact on the environment. The most efficient, economical, and environmentally sound method to mitigate the losses caused by pathogens is breeding for genetic resistance (Muthe et al. 2016). To date, more than 70 leaf rust resistance (Lr) genes have been cataloged (McIntosh et al. 2013), but many of these genes are race-specific and they have lost their effectiveness when new races of the pathogen are appearing (McCallum et al. 2007). Currently, breeding programs focus on producing cultivars with adult plant resistance (APR), also known as slow rusting or race non-specific genes. Slow rusting genes are a group of leaf rust resistance genes that give durable resistance only in adult plant (Bošković et al. 2008). APR is characterized by less and slower pathogen growth and reproduction despite a high infection type (Tiwari et al. 2009). An important advantage of genes is their pleiotropic effect on many pathogens, for example Blumeria graminis causing powdery mildew (Lillemo et al. 2008). Among all, the leaf rust resistance genes in wheat only four genes are known as slow rusting: Lr34 (Singh 1992), Lr46 (Singh et al. 1998), Lr67 (Dyck and Samboński 1979), and Lr68 (Herrera-Foessel et al. 2012).

The leaf rust resistance gene Lr34 (earlier LrT2) is the best known and most effective of slow rusting genes. The gene Lr34 was first described in the wheat line PI58548 and located on short arm of wheat chromosome 7D (Dyck 1987). Lagudah et al. (2006) developed PCR-based marker csLV34 that has been used extensively to identify the presence of Lr34 gene. The disadvantage is that the marker is not diagnostic in some genetic backgrounds, like Canadian wheat germplasm (Lagudah et al. 2009). Leaf tip necrosis (LTN) is one of the morphological markers associated with leaf rust resistance gene Lr34 and is also expressed in the absence of the pathogen (Lagudah et al. 2006). The Lr46 gene was first described in wheat cultivar Pavon F76 and was localized on chromosome 1BL (Singh et al. 1998). Lr46 shows a resistance phenotype in adult plants similar to Lr34, but the effects of Lr46 are not as pronounced as Lr34 (Martínez et al. 2001). Lillemo et al.
(2008) have shown that Lr46 has an additive effect on leaf rust resistance of Lr34. The gene Lr46 was mapped distal to Xwmc44 and proximal to Xgwm259 (Suenaga et al. 2003).

Material and methods

The aim of this study was to develop a multiplex PCR method for simultaneous identification of two most effective slow rust resistance genes: Lr34 and Lr46. Plant material consisted of six wheat cultivars Triticum aestivum L. derived from the National Small Grains Collection, the Agriculture Research Station in Aberdeen, USA: TX89D6435, Pavon F76, Parula ‘S’, Rayon 89, Kern and Mochis 88 (Table 1). The DNA was extracted from leaf tissue using the GeneMATRIX Plant & Fungi DNA Purification Kit (EURx Ltd., Poland). DNA quality and concentration was checked using the DeNovix spectrophotometer. In order to identify the Lr34 and Lr46 genes, two molecular markers were used: csLvs34 and Xwmc44. The STS marker csLV34 maps 0.4 cM from Lr34 and the sequence of primers (Merck) is as follows: csLV34F 5’- GTT GGT TAA GAC TGG TGA TGG -3’; csLV34R 5’- TGC TTG CTA TTG CTG AAT AGT -3’ (Lagudah et al. 2006). According to the literature, the size of the amplified product is a 150 bp (base pairs) band, indicative of the presence of the gene and a 229 bp band in susceptible genotypes. Locus of SSR marker Xwmc44 is located 0.4 cm from QTL for Lr46. A product of the microsatellite marker is 242 bp band for the presence of Lr46 gene, and the sequence of marker primers is as follows: WMC44F 5’- GGT CTT CTG GGC TTT GAT CCT G -3’, WMC44R 5’- GTT GCT AGG GAC CCG TAG TGG -3’ (Suenaga et al. 2003). The 25 μL mix composition of multiplex PCR volume consisted of the following: 12.5 μL 2× PCR TaqNovaHs PCR Master Mix (Blirt), which included 2× concentrated PCR reaction buffer, 4 mM MgCl2; 1.6 mM dNTPs mix (0.4 mM of each dNTP); 0.8 μL csLvs34 forward primer; 0.8 μL csLvs34 reverse primer; 1.2 μL Xwmc44 forward primer; 1.2 μL Xwmc44 reverse primer (the concentration for each primer was 100 μM); 2 μL DNA template (50 ng/μL) and 6.5 μL PCR grade water. PCR profile was modified with reference to standard protocol. The following annealing temperatures were tested: 55 °C, 60 °C for 30 s; elongation, 72 °C for 1 min), followed by the final extension for 7 min at 72 °C and final step at 4 °C. The reaction was carried out using the Labcycler thermal cyclers (SensoQuest GmbH). The products of amplification were prepared by adding 0.5 Midori Green Direct (NIPPON Genetics EUROPE) to each tube and were separated using 2% agarose (SIGMA) gel in 1× TBE buffer (BioShop) at 100 V for two and a half hours. A Molecular Imager Gel Doc™ XR UV system was used with the Biorad Bio Image™ Software to visualize the PCR products.

Results and discussion

Molecular markers can be successfully used in the identification of leaf rust resistance genes in wheat resistance breeding programs (Vida et al. 2009). The results showed that the amplification of csLvs34 marker was observed in TX89D6435, Parula ‘S’, and Kern varieties, but the size of the resulting Lr34 gene linked product was approximately 145 bp, which is smaller than reported by Lagudah et al. (2006). Differences in the size of products may result from the size of the DNA ladder used. Lagudah et al. (2006) used a 100 bp ladder molecular size markers. In our experiment we have used more precise, 50 bp DNA ladder, which showed that the csLvs34 marker product is smaller than 150 bp. Considering other varieties (Pavon F76, Rayon 89, and Kern) the PCR reaction with csLvs34 marker showed a 229 bp product, indicating the lack of the Lr34 gene. The analyses with the Xwmc44 marker linked to the Lr46 gene resulted in the identification of a 242 bp specific product in TX89D6435, Pavon F76, and Mochis 88 varieties. The accumulation of both Lr34 and Lr46 resistance genes was demonstrated by the multiplex PCR in TX89D6435 variety, so this variety can be a good source of non-race specific resistance to leaf rust (Table 1, Fig. 1).

Table 1

| No. | Cultivar     | Origin   | Plant ID | Presence of Lr34 | Presence of Lr46 |
|-----|--------------|----------|----------|------------------|------------------|
| 1.  | TX89D6435    | US, Texas| PI 584759| +                | +                |
| 2.  | Pavon F76    | Mexico   | PI 520003| –                | +                |
| 3.  | Parula ‘S’   | Mexico   | PI 520340| +                | –                |
| 4.  | Rayon 89     | Mexico   | PI 591784| –                | –                |
| 5.  | Kern         | US       | PI 672001| +                | –                |
| 6.  | Mochis 88    | Mexico   | PI 591791| –                | +                |
Authors declaration that they have no conflict of interests.

Conflict of interest The authors declare that they have no conflict of interests.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

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