Changes in gene expression profiles as they relate to the adult plant leaf rust resistance in the wheat cv. Toropi

Alice Casassola [a, *], Sandra P. Brammer [b], Márcia S. Chaves [b], José A. Martinelli [c], Francesca Stefanato [d], Lesley A. Boyd [d, e]

[a] Agronomy Post-Graduate Program, University of Passo Fundo, BR285, Passo Fundo, Rio Grande do Sul 99052900, Brazil
[b] Department of Biotechnology and Phytophatology, Brazilian Agricultural Research Corporation, BR285, Km294, Passo Fundo, Rio Grande do Sul 99001970, Brazil
[c] Department of Fitossanidade, Federal University of Rio Grande do Sul, Avenida Bento Gonçalves 7712, Porto Alegre, Rio Grande do Sul 91540000, Brazil
[d] Department of Genetics and Pre-Breeding, National Institute of Agricultural Botany, Huntington Road, Cambridge, Cambridgeshire CB3 0LE, UK
[e] Department of Life and Medical Sciences, University of Hertfordshire, Hatfield, Hertfordshire AL10 9AB, UK

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ABSTRACT

Leaf rust, caused by the foliar pathogen Puccinia triticina is a major disease of wheat in the southern region of Brazil and invariably impacts on production, being responsible for high yield losses. The Brazilian wheat cultivar Toropi has proven, durable adult plant resistance (APR) to leaf rust, which uniquely shows a pre-haustorial resistance phenotype. In this study we aimed to understand the interaction between P. triticina and the pre-haustorial APR in Toropi by quantitatively evaluating the temporal transcription profiles of selected genes known to be related to infection and defense in wheat. The expression profiles of 15 selected genes varied over time, grouping into six expression profile groups. The expression profiles indicated the induction of classical defence pathways in response to pathogen development, but also the potential modification of Toropi's cellular status for the benefit of the pathogen. Classical defence genes, including peroxidases, β-1,3-glucanases and an endochitinase were expressed both early (pre-haustorial) and late (post-haustorial) over the 72 h infection time course, while induction of transcription of other infection-related genes with a potential role in defence, although variable was maintained through-out. These genes directly or indirectly had a role in plant lignification, oxidative stress, the regulation of energy supply, water and lipid transport, and cell cycle regulation. The early induction of transcription of defence-related genes supports the pre-haustorial resistance phenotype in Toropi, providing a valuable source of genes controlling leaf rust resistance for wheat breeding.

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Introduction

In 2012/2013 Brazilian wheat production reached 5.5 million tons, of which 94.5% was produced in the southern region of the country [1]. Leaf rust is a major constraint to wheat production in this area, causing yield losses of up to 80% [2,3]. Chemical control of leaf rust in Brazil costs around US$ 30/ha and generally requires two sprays per crop [4]. In South America lost wheat production due to leaf rust is estimated to cost the industry 172 million dollars per year.

Breeding for wheat leaf rust resistance is complicated by the high level of genetic variation within the Brazilian Puccinia triticina population, exemplified by the pathogen's ability to rapidly overcome major resistance genes, resulting in new races [5].

Therefore, sources of durable APR, such as that found in the Brazilian cv. Toropi, have considerable value for Brazilian wheat breeding.

The bread wheat cv. Toropi (*Triticum aestivum* L.) was released as a commercial cultivar in 1965 with a partial level of APR to leaf rust (Fig. 1) which still remains effective despite 50 years of cultivation [6]. The APR in Toropi is a valuable "slow-rusting" type of resistance, being effective, while producing little or no selection pressure on the pathogen [7,8]. The APR in Toropi also displays a unique, pre-haustorial phenotype, restricting the formation of the primary infection structures; stomatal appressoria and sub-stomatal vesicles [9].

Although Toropi is derived from the cultivar Frontana there is no evidence that it contains the well characterized slow-rusting leaf rust APR gene *Lr34* [10]. Two recessive genes have been reported to be associated with the leaf rust APR in Toropi, temporary designated *Trp*-1 and *Trp*-2 [10]. *Trp*-1 and *Trp*-2 were located to chromosomes 1A and 4D, respectively [11]. In addition to leaf rust APR Toropi has a number of other agronomically important characteristics, including increased phosphorous absorption, translocation and distribution, tolerance to aluminum toxicity [12] and resistance to Fusarium head blight [13].

To identify the resistance mechanisms, genes and genetic pathways underlying the slow-rusting leaf rust APR in Toropi transcriptomics analyses were undertaken, looking at differential gene expression in Toropi flag leaf tissue at defined time points after *P. triticina* inoculation. Leaf tissue was sampled at eight time points after inoculation, including time points that represented the early stages of the pathogen's development and the pre-haustorial resistance in Toropi. The transcript profiles of 15 selected genes, previously identified as having a role in the cellular interactions between wheat, and both adapted and non-adapted isolates of the foliar pathogens causing leaf rust, powdery mildew and wheat blast (L. Boyd, unpublished data), were measured over the eight time points by quantitative PCR (qPCR). The putative function of these wheat infection-related genes in the colonization of wheat tissues by *P. triticina* and the pre-haustorial, leaf rust APR in Toropi is discussed.
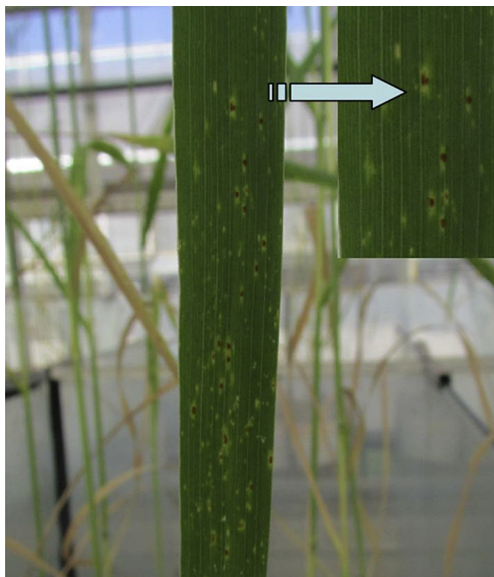

Fig. 1. Leaf rust phenotype on the wheat cv. Toropi. The adult plant leaf rust resistance in Toropi is characterized by a mixture of small, off-white to yellow flecks characteristic of necrotic and chlorotic plant reactions, and by the occasional leaf rust pustule. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Material and methods

Wheat-P. triticina inoculations and sampling

The wheat cv. Toropi was grown at 14 h light/10 h dark and 80% humidity until full expansion of the first flag leaf. Flag leaves were inoculated with urediniospores of *P. triticina*, race MDT-MR − Lr virulence: *Lr1, Lr3, Lr3ka, Lr10, Lr11, Lr14a, Lr14b, Lr17, Lr20, Lr23, Lr24, Lr26, Lr30* [14] − in a mineral oil suspension. Mock inoculations were carried out using the same mineral oil without urediniospores. After inoculation plants were maintained at 80% humidity, in total darkness for 24 h, before being returned to normal growing conditions. Leaf rust infection was verified 15 days post inoculation (Fig. 1).

Twelve *P. triticina* inoculated flag leaves and six mock-inoculated flag leaves were collected from individual plants at each of the following time points; 0, 1, 3, 6, 12, 24, 48 and 72 h after inoculation (hai). Total RNA was extracted from all flag leaf samples using RNeasy Plant Mini Kit (Qiagen) and DNA removed using TURBO DNA-free™ Kit (Ambion), according to the manufacturer's protocols.

Selection of candidate infection-related genes and quantitative PCR analysis

Wheat genes were selected for analysis from a global wheat transcriptomics study involving inoculation with adapted and nonadapted isolates of the fungal pathogens *Blumeria graminis*, *Magnaporthe grisea/oryzae*, and *P. triticina/hordei* (L Boyd; unpublished data). Differentially expressed probe sets were selected from the Agilent wheat microarray (http://www.genomics.agilent.com) that represented unique wheat unigenes. Ten wheat genes (Table 1) were selected that showed differential transcript profiles across 4 time points (12, 24, 36 and 48 hai) following inoculation with *P. triticina* and/or *P. hordei* (data not shown).

Primers were designed for qPCR using Primer3Plus. Toropi RNA samples from each time point were converted to cDNA using the SuperScript™ III First-Strand Synthesis System (Invitrogen™) according to the manufacturer's protocol. PCR amplification levels were normalized using geNorm (geNorm program v3.5) and three reference genes, ubiquitin [15], GAPDH and elongation factor-1α [16]. GeNorm calculates the normalization factor based on multiple control genes, resulting in more accurate and reliable normalization of gene expression data than is normally obtained using a single gene for normalization [17].

All qPCR were performed using SYBR® Green JumpStart™ Taq ReadyMix™ (Sigma−Aldrich) at 95 °C for 2 min; 40 cycles of 95 °C for 15 s, 60 °C for 1 min and 72 °C for 15 s; and then 95 °C for 15 s, 60 °C for 15 s and 95 °C for 15 s. The specificity of the reaction was verified by melt curve analysis and the efficiency of each primer was checked using the standard curve method [16,18]. Primers with slopes between −3.1 and −3.6, and reaction efficiencies between 90 and 110% were selected for the analysis (Table 1). Primers previously designed by Tufan et al. [18] for standard defence-related genes were also assessed (Table 2). The qPCR for each gene, on each of 3 biological replicates, was repeated 3 times.

Transcript levels of all 15 infection-related wheat genes in *P. triticina* inoculated flag leaf tissue were compared to the levels of transcript in the mock-inoculated control samples, at each time point, providing relative transcript levels for each gene. Three biological replicates were analyzed at each time point. The expression value for each biological replicate was an average of the normalized technical replicates. Relative expression values of the three inoculated biological replicates, at each time point were obtained by dividing each biological replicate by the average of the mock expression values at each time point.