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## Emergence of a new race of leaf rust with combined virulence to *Lr14a* and *Lr72* genes on durum wheat

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### Abstract

Leaf rust is a foliar disease caused by the fungus *Puccinia triticina* that may severely reduce durum wheat yield. Resistance to this pathogen is common in modern durum germplasm but is frequently based on *Lr72* and *Lr14a*. After accounts of races with virulence to *Lr14a* gene in France in 2000, the present study reports the detection in 2013 for the first time of a new race with virulence to *Lr14a* and *Lr72*. The aim of this work was to characterize the virulence pattern of four Spanish isolates with virulence to *Lr14a*, and to discuss the consequences of this presence. Rusted leaves from cultivars 'Don Jaime' (*Lr14a*) and 'Gallareta' (*Lr72*) were collected in 2013 in the field at two Spanish sites, one in the south (near Cadiz) and another in the north (near Girona). Spores from single pustule for each cultivar and site were multiplied on susceptible cultivar 'Don Rafael'. Then, the four isolates were inoculated on a set of 19 isogenic lines Thatcher to characterize their virulence spectrum. All isolates presented the same virulence pattern. They were virulent on both *Lr14a* and *Lr72* and the race was named DBB/BS. This race was very similar to those reported in 2009-11, but with added virulence to *Lr14a*. The resistance based on *Lr14a* has therefore been overcome in Spain, by a new race that has likely emerged via stepwise mutation from the local predominating races. This information is important to guide breeders in their breeding programmes and gene deployment strategies.

**Additional key words:** resistance; *Puccinia triticina*; SSR marker.

**Authors' contributions.** Conceived and designed the experiments: IS and CR. Performed the experiments: NHS, DV, FA, JS. Analyzed the data: NHS and FMM. Contributed materials: IS, MHS and JCS. Wrote the paper: NHS, KA and FMM.

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### Introduction

Durum wheat (*Triticum turgidum* subsp. *durum*) is an important crop in the Mediterranean basin. One of the most important biotic constraints affecting this crop is leaf rust, caused by the fungus *Puccinia triticina*.

For more than three decades, the leaf rust resistance gene *Lr14a* has been effective in protecting durum wheat against leaf rust in Spain. The presence of this commonly occurring gene in durum wheat germplasm was first reported by Herrera-Foessel *et al.* (2008), who also found two closely linked SSR (Single Se-

quence Repeats) markers. The *Lr14a* gene is located on chromosome arm 7BL and was originally transferred from the emmer wheat 'Yaroslav' to common wheat, and from there to durum wheat. Many cultivars resistant to leaf rust are known to carry *Lr14a*, such as 'Creso', an Italian cultivar first released in 1974, its descendant 'Colosseo' (Maccaferri *et al.*, 2010), and some CIMMYT derived cultivars like 'Don Jaime' (Soleiman *et al.*, 2014a). All have been resistant to the prevailing leaf rust races in Spain until 2011 (Soleiman, 2013).

*Lr72*, previously known as *Lr<sub>Altar</sub>* (Herrera-Foessel *et al.*, 2014), was first described in the Mexican culti-

var ‘Altar C84’, released in Spain as ‘Gallareta’. This gene effectively protected the majority of durum wheat cultivars against the Mexican leaf rust races for nearly two decades, but the resistance it provided was overcome by the race BBG/BN in 2001 in Mexico, and later in other parts of the world (Singh *et al.*, 2004). Currently, *Lr72* gene is ineffective against most leaf rust races from durum worldwide, but it is still capable of protecting durum wheat from the races that are adapted to common hexaploid wheat (Herrera-Foessel *et al.*, 2014). In a survey on durum leaf rust in Spain in 2009–11 all races were avirulent to durum cultivars containing *Lr14a* and avirulent on ‘Altar’ (Soleiman, 2013). However, in France Goyeau *et al.* (2012) reported the existence of ‘durum’ races virulent on *Lr14a* but avirulent on ‘Altar’ (*Lr72*).

During the spring of 2013, uredinia with susceptible infection type were observed in field trials on durum wheat ‘Colosseo’ and ‘Don Jaime’ in the north and the south of Spain, confirming the virulence to *Lr14a*. The aim of this work was to characterize the virulence pattern of four Spanish isolates with virulence to *Lr14a*, and provide critical information to breeders to guide them in their gene deployment and use strategies.

## Material and methods

Leaves with uredinia of high infection type were collected in the field on ‘Don Jaime’ and ‘Gallareta’ durum wheat cultivars in April 2013 at two Spanish sites, Conil, near the city of Cadiz (36.27690° N, -6.08718° W) in Southern Spain, and La Tallada d’Empordà, near the city of Girona (42.08051° N, 3.05563° W) in Catalonia (northeast of Spain). Overall, four samples (one per cultivar and location) were analyzed. Spores from single uredinia from moderately infected leaves were inoculated on seedlings of the widely susceptible durum ‘Don Rafael’ in order to multiply each isolate separately. Inoculated plants were placed in a dark dew chamber for 14 h at 20 °C and 100% relative humidity.

A set of 19 near isogenic lines of the obsolete cultivar ‘Thatcher’ that have single gene for leaf rust resistance was used to determine the virulence spectrum of each isolate. ‘Thatcher’ was included in all tests as susceptible check. Nomenclature of races follows Soleiman *et al.* (2014b). The five sets of differential lines were: (1) *Lr1*, *Lr2a*, *Lr2c*, *Lr3*; (2) *Lr9*, *Lr16*, *Lr24*, *Lr26*; (3) *Lr3ka*, *Lr11*, *Lr17*, *Lr30*; (4) *Lr3bg*, *Lr13*, *Lr15*, *Lr18*; and (5) *Lr10*, *Lr14a*, *Lr23* and ‘Jupare’ (*Lr27+Lr31*, *Lr72*). Durum genotypes with known resistance genes were also included in the experiment:

breeding line Somateria (*Lr14a+*), ‘Colosseo’ (*Lr14a+*), ‘Don Jaime’ (*Lr14a+*), ‘Gallareta’ (*Lr72*), ‘Guayacán INIA’ (*Lr61*), and breeding lines Storlom (*Lr3+Lr72*) and Camayo (*Lr<sub>cam</sub>*) (Herrera-Foessel *et al.*, 2007). SSR markers *Xgwm344* and *Xgwm146* were used to detect the presence of the *Lr14a* gene. These markers are dominant and linked in coupling with that gene (Herrera-Foessel *et al.*, 2008).

Plants were grown in soil trays (60×40×10 cm), each one containing ten genotypes with four plants per genotype. When plants were at DC 16 (Zadoks *et al.*, 1974), the unfolded 5<sup>th</sup> leaf of each plant was inoculated with 6 mg of urediniospores mixed with talcum powder (1:50, v/v) per tray. Plants were incubated as described above and transferred the next day to a greenhouse compartment at 18–25 °C. Twelve days after inoculation the infection type (IT) was assessed according to a 0–4 scale (Stakman *et al.*, 1962). Virulence spectrum of the four new isolates was compared to that of the two races that were predominant in Spain from 2009 to 2011 (Soleiman, 2013).

## Results and discussion

The four isolates collected in 2013 had the same virulence spectrum, virulent on both *Lr14a* and *Lr72*, and the pathotype was designated as DBB/BS (Table 1). In a Spanish virulence survey in the period 2009–2011 on durum wheat leaf rust, seven races were identified among 75 isolates. The prevailing races were very closely related, with virulence on *Lr72* and avirulence on *Lr14a* (Soleiman, 2013). The four isolates examined in the present study were very similar to the races identified during the 2009–2011 period, but with added virulence on *Lr14a*, and all durum cultivars carrying this gene (Table 1). One plausible origin of the new race is that it evolved from the commonly found race DBB/BN through stepwise mutation acquiring virulence for the *Lr14a* resistance. The simultaneous detection of DBB/BS in both south and north of Spain supports this hypothesis. Mutations of avirulence loci in urediniospores, which are produced in massive quantities in wheat fields, are an important mechanism to generate new races (Huerta-Espino *et al.*, 2011).

The new race was also virulent on the wheat lines with *Lr72* as observed in field trials in Conil (I. Solis, unpublished data) and Gerona (D. Villegas, unpublished data) and in our study. This indicates that this pathotype is different from the French races, many of them with virulence on lines with *Lr14a* and avirulent on Altar C84 (*Lr72*) as described by Goyeau *et al.* (2012). This report of races with virulence on both *Lr14a* and *Lr72*

**Table 1.** Infection types (IT)<sup>[1]</sup> at 5<sup>th</sup> leaf stage on 20 near-isogenic Thatcher lines and eight genotypes with known *R*-genes inoculated with two leaf rust races collected on durum wheat in 2009-11 (DBB/BN and DBB/CN), and the new race collected in 2013 in Spain (DBB/BS).

|  | Isolates 2009-11 (old) |        | Isolates 2013 (new)            |                                    |                                 |                                  |
|--|------------------------|--------|--------------------------------|------------------------------------|---------------------------------|----------------------------------|
|  | DBB/BN <sup>[2]</sup>  | DBB/CN | Conil Altar<br>( <i>Lr72</i> ) | Conil D. Jaime<br>( <i>Lr14a</i> ) | Gerona Altar<br>( <i>Lr72</i> ) | Gerona<br>line with <i>Lr14a</i> |
|  |                        |        | DBB/BS                         |                                    |                                 |                                  |
| Thatcher                                   | 3                      | 3      | 4                              | 4                                  | 4                               | 4                                |
| <i>TcLr1</i>                               | ;                      | ;      | ;                              | ;                                  | ;                               | ;                                |
| <i>TcLr2a</i>                              | 1                      | 2      | 2                              | 2                                  | 1                               | 2                                |
| <i>TcLr2c</i>                              | 3                      | 3      | 3                              | 3                                  | 4                               | 3                                |
| <i>TcLr3</i>                               | 1                      | 1      | ;                              | 1                                  | 1                               | 1                                |
| <i>TcLr3bg</i>                             | 1                      | 1      | 1                              | 1                                  | 1                               | 1                                |
| <i>TcLr3ka</i>                             | ;                      | ;      | 1                              | 1                                  | 1                               | 1                                |
| <i>TcLr9</i>                               | 0                      | ;      | 0                              | ;                                  | 0                               | 0                                |
| <i>TcLr10</i>                              | 3                      | 3      | 4                              | 3                                  | 4                               | 4                                |
| <i>TcLr11</i>                              | 1                      | 2      | 1                              | 1                                  | 1                               | 1                                |
| <i>TcLr13</i>                              | 1                      | 2      | 1                              | 1                                  | 1                               | 1                                |
| <i>TcLr14a</i>                             | 2                      | 2      | 3                              | 4                                  | 4                               | 4                                |
| <i>TcLr15</i>                              | 2                      | 2      | 2                              | 2                                  | 1                               | 1                                |
| <i>TcLr16</i>                              | 1                      | 2      | 2                              | 2                                  | 2                               | 2                                |
| <i>TcLr17</i>                              | 1                      | ;      | 1                              | 1                                  | 1                               | 1                                |
| <i>TcLr18</i>                              | 1                      | 3      | 1                              | 2                                  | 2                               | 2                                |
| <i>TcLr23</i>                              | 3                      | 3      | 3                              | 4                                  | 4                               | 3                                |
| <i>TcLr24</i>                              | ;                      | ;      | ;                              | ;                                  | ;                               | ;                                |
| <i>TcLr26</i>                              | ;                      | ;      | 1                              | 1                                  | 1                               | 1                                |
| <i>TcLr30</i>                              | 1                      | 2      | 2                              | 2                                  | 2                               | 2                                |
| Jupare ( <i>Lr27+31,Lr72</i> )             | 0                      | ;      | ;                              | 1                                  | ;                               | ;                                |
| Gallareta ( <i>Lr72</i> )                  | 4                      | 4      | 4                              | 4                                  | 4                               | 4                                |
| Somateria ( <i>Lr14a+</i> ) <sup>[3]</sup> | 2                      | 2      | 3                              | 4                                  | 4                               | 4                                |
| Colosseo ( <i>Lr14a+</i> ) <sup>[3]</sup>  | 2                      | 2      | 3                              | 4                                  | 4                               | 4                                |
| Don Jaime ( <i>Lr14a+</i> ) <sup>[3]</sup> | ;                      | ;      | 4                              | 4                                  | 4                               | 4                                |
| Storlom ( <i>Lr3,Lr72</i> )                | ;                      | ;      | ;                              | ;                                  | ;                               | ;                                |
| Guayacán ( <i>Lr61</i> )                   | ;                      | ;      | ;                              | ;                                  | ;                               | ;                                |
| Camayo ( <i>LrCam</i> )                    | 1                      | 2      | 1                              | ;                                  | ;                               | ;                                |

<sup>[1]</sup> IT according to a 0-4 scale as described by Stakman *et al.* (1962) with some modifications. IT 0 to 2 (including ; that indicates the presence of only necrotic flecks on infected leaves) indicate an incompatible reaction (avirulent race), whereas IT 3 to 4 indicate a compatible response (virulent race). <sup>[2]</sup> Nomenclature of races follows Soleiman *et al.* (2014b). <sup>[3]</sup> Tested positive for the presence of *Lr14a* with the SSR markers *Xgwm344* and *Xgwm146*.

is of critical relevance for durum wheat breeders as breeding programs and gene deployment strategies will need to be modified. For this reason, an additional test on flag leaves would clearly confirm the virulence on both genes. While *Lr14a* gene still provides effective protection in many areas worldwide, including the Americas, Central India, East Africa and the Middle East (K. Ammar, unpublished data), its use in new crossing programs should be avoided, even in the presence of *Lr72*, based on the appearance of races with *Lr14a* virulence in France, Spain and Tunisia (Gharbi *et al.*, 2013), and likely in other areas of the Mediterranean Basin. Sources of resistance in addition to *Lr14a* gene (e.g. *Lr3*, *LrCam*, *Lr27+Lr31*, *Lr61*) will need to be used in order to maintain effective leaf rust resistance in durum wheat.

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