A unique race of the wheat stem rust pathogen with virulence on *Sr31* identified in Spain and reaction of wheat and durum cultivars to this race

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ABSTRACT

Wheat stem rust, caused by *Puccinia graminis* f. sp. *tritici*, is a re-emerging disease, posing a threat to wheat production. In Spain, stem rust has been rarely detected since 1970s, but infection was observed in wheat fields in 2018. We analyzed six stem rust samples collected in Rota, Cádiz province and one from Monteagudo del Castillo, Teruel province. All the samples from Rota were typed as race TKTTF, whereas the sample from Monteagudo del Castillo, collected in a wheat field adjacent to barberry bushes, was typed as race TKHBK. This race has a unique and significant virulence combination that includes virulence to *Sr31*, *Sr33*, *Sr53* and *Sr59*, and is avirulent to Rusty, a durum line developed for universal susceptibility to the wheat stem rust pathogen. TKHBK is the first race outside the Ug99 race group with virulence to *Sr31* and the first known race with virulence to *Sr59*. Genotyping studies indicate that race TKHBK does not belong to the Ug99 or TKTTF race groups and constitutes a previously unknown lineage. Two hundred bread and durum wheat cultivars and breeding lines from Spain were evaluated against TKHBK and TKTTF and six additional races. Resistance was observed to all the races evaluated. Molecular markers confirmed the presence of *Sr38*, *Sr31*, *Sr24*, *Sr7a*, and *Sr57* in bread wheat, and *Sr13* in durum wheat. The re-emergence of wheat stem rust in Spain and the occurrence of unique virulences underscore the need to continue surveying and monitoring this disease.

1. INTRODUCTION

Wheat stem rust, caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*), is a devastating disease of bread (*Triticum aestivum* L.) and durum (*T. turgidum* ssp. *durum* (Desf.) Husn.) wheat. For several decades, wheat crops have been protected globally from this disease by the widespread use of effective resistance genes and the eradication of the alternate host, common barberry (*Berberis vulgaris* L.) in the United States and western Europe (Hermansen, 1968; Roelfs, 1985). Wheat stem rust is a re-emerging disease, exemplified by the occurrence, evolution into new virulent variants, and rapid spread of races in the Ug99 race group (Newcomb et al., 2016; Singh et al., 2015). This race group is of special concern because it possesses virulence on *Sr31,* a gene of rye origin that has been globally deployed mostly through germplasm developed by the CIMMYT wheat breeding program (Singh et al., 2006). *Sr31* remained effective for over six decades and likely contributed to the global decline of stem rust (Singh et al., 2006). In addition, severe epidemics and localized outbreaks caused by races outside of the Ug99 race group have been reported in recent years in east Africa (Olivera et al., 2015), Central Asia (Skolotneva et al., 2020), the Caucasus region of Eurasia (Olivera et al., 2019), and Europe (Berlin, 2017; Olivera Firpo et al., 2017). Many of these recent outbreaks have been associated with highly diverse pathogen populations and novel virulence combinations (Berlin, 2017; Olivera et al., 2019), indicating that the alternate host is likely playing an important role in pathogen variation and disease epidemiology.

In Spain, stem rust was an important disease of wheat, with several epidemics occurring in the period between the 1940s and 1960s (Martínez-Moreno & Solís, 2019; Zadoks, 1967). The rapid replacement of low productive and susceptible local varieties (‘Aragon 03’, ‘Pane 237’) by short-cycle and partially resistant cultivars from CIMMYT (‘Siete Cerros 66’, ‘Cajeme’,

68 'Yecora', and 'Anza') in the 1970's (Lupton, 1992) resulted in a drastic reduction of stem rust
69 infections in Spanish wheat crops (Martínez-Moreno & Solís, 2019). Although this disease has
70 been rarely detected for almost five decades, infections started being observed in wheat fields in
71 recent years (Martínez-Moreno & Solís, 2019).

72 As stem rust has not been a problem in Spain since the 1970s, limited effort was put towards
73 breeding for resistance to this disease, and information is currently unavailable for stem rust
74 resistance in the cultivars that have been grown in the country during the last 50 years. As this
75 disease is being detected more frequently in Spain and other European countries, it is crucial to
76 understand and improve stem rust resistance in the local bread and durum wheat varieties. The
77 objectives of this study were to: 1) analyze stem rust samples collected at two locations in Spain
78 in 2018, and 2) evaluate bread and durum wheat cultivars and breeding lines for resistance to stem
79 rust races identified in Spain and elsewhere that carry important virulence combinations.
80

81 **2. MATERIALS AND METHODS**

82 **2.1 Race typing of wheat stem rust samples**

83 Seven samples of stem rust infected tissue were collected from bread wheat at two
84 locations: six samples from a wheat nursery at the Rota Station in the province of Cádiz
85 (36.88ºN, 6.29ºW, altitude = 50 m) and one from a farmer's field adjacent to rust-infected
86 barberry (*B. garciae* Pau) bushes in Monteagudo del Castillo in the province of Teruel (40.46ºN,
87 0.81ºW, altitude = 1,420 m). Dried samples were mailed to the USDA-ARS Foreign Disease-
88 Weed Science Research Unit (FDWSRU) in Ft. Detrick, MD (USA) following the shipping and
89 receiving protocols according to the USDA APHIS PPQ permit conditions for handling
90 international *Pgt* cultures. Samples received at FDWSRU were increased on the susceptible

wheat ‘McNair 701’ (CItr 15288), collected in gelatin capsules (size 00), and stored in a −80°C freezer. After December 1st, cultures were shipped to Cereal Disease Laboratory (CDL) in St. Paul, MN (USA) and stored in a -80ºC freezer until processing.

Race identification was based on the North American stem rust differential set (Jin et al., 2008). All samples and isolates were further characterized on additional monogenic lines carrying the following resistance genes: *Sr7a*, *Sr13c*, *Sr22*, *Sr25*, *Sr26*, *Sr27*, *Sr32*, *Sr33*, *Sr35*, *Sr37*, *Sr39*, *Sr40*, *Sr43*, *Sr47*, *Sr50*, *Sr51*, *Sr52*, *Sr53*, *Sr59*, *Sr8155B1*, *SrSatu*, and *Sr1RS^{Amigo}* (Olivera Firpo et al., 2017). Durum cultivars Iumillo (*Sr9g,12,+*) and Leeds (*Sr9e,13b,+*), and barley line Q21861A (*Rpg1*, *rpg4*, *Rpg5*) were also included in the evaluation. One isolate (18SPA092-1) derived from the sample collected in Monteagudo del Castillo was evaluated on two additional wheat lines carrying *Sr31* (DK42-2 and Line E/Kavkaz-2) and a set of eight universal susceptible bread and durum wheat lines.

Each sample was first inoculated onto the differentials and the additional monogenic lines set and two single-pustule isolates were derived from each original sample. Experimental procedures for inoculation, incubation, and disease assessment were performed as described by Jin et al. (2007). Single-pustule–derived cultures were increased in isolation on susceptible wheat ‘McNair 701’. Each increased isolate was evaluated on the differential lines and the set of additional resistance lines. Race designation was based on the letter code proposed by Roelfs & Martens (1988). Urediniospores from all pure cultures were increased in isolation on ‘McNair 701’ and stored at -80ºC.

2.2 Genotyping of isolate 18SPA092-1

DNA was extracted from *Pgt*-infected leaf tissue following the protocol described by Olivera et al. (2015). Genotyping was performed using a custom Illumina single nucleotide polymorphism (SNP) array (PgtSNP 3.0k chip) and data was filtered as described by Olivera et al. (2019). After removing loci with monomorphic or missing data, a final data set containing 1,838 SNP loci was used for the phylogenetic analysis. Analysis was performed using R (version 3.4.3: R Core Team, Vienna, Austria) with the package Poppr version 2.6.1 (Kamvar et al., 2015). A distance matrix was calculated using Prevosti's distance (Prevosti et al., 1975) and Neighbor-joining analysis (Saitou & Nei, 1987) was used to construct a phylogenetic tree. Bootstrap values were calculated using 5,000 sample replicates and a 75% cutoff using the aboot function. A set of 25 reference isolates from previously defined clades I (Ug99 race group), II (race JRCQC), III (races TRTTF and TTRTF), IV (TKTTF race group), and V (isolates from Georgia and Germany 2013) (Olivera et al., 2015; 2019; Olivera Firpo et al., 2017) were included in the analysis (Supplementary Table S1).

2.3 Germplasm evaluation for stem rust response

A total of 120 bread wheat and 80 durum wheat cultivars and breeding lines were evaluated for stem rust response at the seeding stage. The 120 bread wheat genotypes included 59 varieties currently cultivated in Spain, 44 breeding lines from the Institute of Agrifood Research and Technology (IRTA) breeding program, and 17 old varieties from the IRTA program that are no longer deployed. The 80 durum wheat genotypes included 40 current varieties cultivated in Spain, 18 breeding lines from IRTA program, and 22 old varieties from different breeding programs including IRTA. These 200 genotypes were evaluated against two *Pgt* races recovered from the stem rust samples collected in Rota and Monteagudo del Castillo,

and six additional races. These additional *Pgt* races included: two variants in the Ug99 race group, TTKSK (carrying *Sr31 + Sr38* virulence) and TTKTT (carrying *Sr31 + Sr38 + Sr24 + SrTmp* virulence) (Newcomb et al., 2016); two races with combined virulence on *Sr9e* and *Sr13b* (JRCQC and TTRTF) (Olivera et al., 2019); TTTTF, the race with the broadest virulence spectrum in the Unites States and race QFCSC, the predominant race in the United States in the last decade. Five seedlings per entry were inoculated on fully expanded primary leaves 8 to 9 days after planting. Experimental procedures in inoculation and disease assessment were performed as described by Jin et al. (2007). Wheat cultivar McNair 701 was used as the susceptible control. Disease evaluation was repeated once. The presence of specific stem rust resistance genes was postulated based on race specificity and infection types at the seeding stage.

2.4 Molecular marker analysis

Genomic DNA from 117 bread wheat and 80 durum wheat genotypes was isolated from 8 to 9-day-old seedlings following a modified cetyltrimethylammonium bromide extraction method (Rouse et al., 2012). The amount and purity of DNA was determined using a NanoDrop ND-1000 (NanoDrop Products). For bread wheat genotypes, DNA markers specific to resistance genes *Sr24* (Mago et al., 2005), *Sr31* (Mohler et al., 2001), *Sr38* (Helguera et al., 2003), and *Sr57/Lr34/Yr18/Pm38* (Lagudah et al., 2009) were assessed to confirm their presence or absence in the evaluated germplasm. We developed two Kompetitive Allele-Specific Polymorphism (KASP) markers to predict the presence of *Sr7a*: *KASP-IWB12146* and *KASP-IWB47019*. Both markers were derived from molecular markers in a custom 90K SNP Illumina array (Wang et al., 2014) that were identified as linked to *Sr7a* in a genome-wide association study of United States spring wheat (Bajgain et al., 2015). The primer sequences for *KASP-IWB12146* are allele-

specific sequences 5'-GGAAGACGCCGATGGTGCCAA, 5'-GAAGACGCCGATGGTGCCAG, and common primer 5' CATTTCGGGTCCGTGAAGCTGAATT. The primer sequences for *KASP-IWB47019* are allele-specific sequences 5'- CACATCTGTTGAATCATTCACACTA, 5'-CACATCTGTTGAATCATTCACACTG, and common primer 5'-GGAAGACAATCCTTCGAGCGTACAT. The KASP markers were assayed as described for other KASP markers developed in our lab (Nirmala et al., 2017). For durum wheat genotypes we used KASP markers developed by S. Dreisigacker as described on the MASWheat webpage (https://maswheat.ucdavis.edu/protocols/Sr13) to discriminate the *Sr13* alleles (*a*, *b*, and *c*) based on the corresponding *Sr13* resistant haplotypes (Zhang et al., 2017).

3. RESULTS

Two races were recovered from the samples collected in Spain in 2018: TKTTF and TKHBK. Race TKTTF was recovered from the samples collected in Rota Station, whereas race TKHBK was identified from the sample collected in Monteagudo del Castillo. Race TKTTF produced high infection types on differentials carrying *Sr5*, *Sr6*, *Sr7b*, *Sr8a*, *Sr9a*, *Sr9b*, *Sr9d*, *Sr9e*, *Sr9g*, *Sr10*, *Sr17*, *Sr21*, *Sr30*, *Sr36*, *Sr38*, *SrTmp*, and *SrMcN* (Table 1). Isolates of race TKTTF were avirulent on all the additional stem rust resistance genes tested in this study (Table 2). Race TKHBK produced high infection types on *Sr5*, *Sr6*, *Sr7b*, *Sr8a*, *Sr9b*, *Sr9e*, *Sr9g*, *Sr17*, *Sr21*, *Sr31*, *Sr38*, and *SrMcN* (Table 1; Table 2). It has also exhibited virulence on lines carrying *Sr33*, *Sr53*, and *Sr59* (Table 2; Figure 1). In addition, the isolate of race TKHBK was avirulent on Rusty, a durum wheat line bred for stem rust susceptibility (Table 3).

Isolate 18SPA092-1 was genotyped with a custom PgtSNP 3.0k chip, and a phylogenetic analysis was performed with 25 reference isolates representing the previously defined clades. It had a unique multilocus genotype and formed a sister branch to clades I (Ug99 race group) and II (JRCQC race group) that was well supported (Figure 2).

Seedling resistance was observed in bread wheat cultivars and breeding lines to races TKHBK and TKTTF (Table 4). Fifty-one (67.1%) cultivars and 40 (90.9%) breeding lines were resistant to race TKHBK, whereas 29 (38.2%) and 20 (45.5%) cultivars and breeding lines exhibited a resistant response to race TKTTF, respectively. Resistance in bread wheat was also observed to the other six races evaluated. For bread wheat cultivars, the percentage of resistance ranged between 17.1% and 90.1% (Table 4). The lowest frequencies of resistance were to the two variants in the Ug99 race group; 17.1% and 18.4% for TTKSK and TTKTT, respectively (Table 4). Similar percentage of resistance was observed with races TTRTF (69.7%) and TTTTF (67.1%), whereas the greatest frequencies of resistance observed were to races JRCQC (88.2%) and QFCSC (90.1%). The percentage of resistance in bread wheat breeding to races TTKSK and TTKTT was 27.3% and 22.7%, respectively (Table 4). Twenty-two (50.0%) and 14 (33.3%) breeding lines were resistant to races TTRTF and TTTTF, respectively. The highest percentage of resistance in bread wheat breeding lines was to races QFCSC (100%) and JRCQC (97.7%). Nine (12.0%) cultivars and five (11.8%) breeding lines were resistant to all races evaluated (Table 4).

All the durum wheat cultivars and breeding lines were resistant to race TKHBK, whereas 39 (62.9%) cultivars and 10 (55.6%) breeding lines were resistant to race TKTTF (Table 5). Resistance was also observed to the other six races evaluated, and the percentage of resistance for each race was comparable between the cultivars and breeding lines. Thirty-eight (61.3%) and

37 (59.7%) durum cultivars exhibited a resistant response to races TTKSK and TTKTT, whereas nine (50%) breeding lines were resistant to both races (Table 5). The highest percentages of resistance were to races QFCSC (96.8% and 100% for cultivars and breeding lines, respectively), followed by race TTRTF (83.3% for cultivars and 75.8% for breeding lines). Resistance to races JRCQC and TTTTF was between 50% and 60% for both cultivars and breeding lines. Twenty-six (41.9%) durum wheat cultivars and six (33.3%) breeding lines were resistant to the eight races evaluated (Table 5).

Based on disease reaction to the eight races evaluated, we postulated three *Sr* genes alone or in combination in the 120 bread wheat cultivars and breeding lines evaluated. The most predominant gene was *Sr38*, postulated in 23 cultivars and 11 breeding lines (Table 6). *Sr31* was postulated to be present in 15 cultivars and 4 breeding lines, whereas *Sr24* was postulated in only one cultivar. The presence of additional resistance genes was postulated in 41 genotypes carrying either *Sr31* (17 entries) or *Sr38* (24 entries) (Table 6). Thirty-seven of these postulated genes, that were not able to be identified with the eight races used in this study, were effective against race TKHBK. Alleles of *Sr13* (*a/c* and *b*) were pustulated to be present in 11 durum cultivars and three breeding lines. *Sr13a/c* alleles were postulated in three cultivars, whereas *Sr13b* allele was postulated in five cultivars and three breeding lines (Table 7).

We used DNA markers to confirm the presence of *Sr38*, *Sr31*, *Sr24* and *Sr7a* genes in bread wheat genotypes. *Sr38* was confirmed in 33 (43.4%) cultivars and 17 (38.6%) breeding lines (Table 6; Figure 3). All the cultivars and lines that were postulated to carry *Sr38* based on seedling phenotypes were confirmed with the DNA marker. Seventeen cultivars and breeding lines that were not postulated to carry *Sr38*, tested positive for the DNA marker, suggesting the presence of additional stem rust resistance genes in these genotypes. *Sr31* was confirmed with

DNA markers in 12 (15.8%) bread wheat cultivars and four (9.1%) breeding lines (Table 6; Figure 3). Two cultivars ('Balsamina' and 'Variety 39'), postulated to carry *Sr31* based on seedling phenotypes, tested negative for the DNA marker (Table 6). The presence of *Sr24* in 'Variety 52' was confirmed with the DNA marker. *Sr7a* was detected in six bread wheat cultivars and five breeding lines (Table 6; Figure 3). One cultivar ('Variety 20') of the eleven that tested positive for the *Sr7a* marker, displayed a seedling phenotype that didn't match with the *Sr7a*-positive genotype. We also ran the DNA marker corresponding to adult plant resistance gene *Sr57* and tested positive in 10 (13.2%) and four (9.1%) cultivars and breeding lines, respectively (Table 6; Figure 3). The three effective alleles (*a, b,* and *c*) of *Sr13* gene were confirmed by DNA markers in both durum cultivars and breeding lines. *Sr13a, b,* and *c* were detected in five (8.1%), eight (12.9%), and 14 (22.6%) durum cultivars, respectively (Table 7; Figure 4). *Sr13a* and *c* alleles were detected in two (11.1%) breeding lines, whereas *Sr13b* was confirmed in five lines (27.8%). All the genotypes postulated to carry the *Sr13b* allele based on seedling phenotypes were confirmed with the DNA marker (Table 7). All but one genotype postulated to carry *Sr13a* or *c*, which cannot be distinguished with the *Pgt* races used in this study, were confirmed to carry one of these alleles. 'Variety 38', that was postulated to carry *Sr13a/c*, was confirmed by DNA marker to carry the *Sr13b* allele (Table 7). Eighteen cultivars and breeding lines resistant to all races evaluated tested positive for the *a, b* or *c* allele with the *Sr13* DNA marker, indicating the presence of additional effective resistance genes in these genotypes.

4. DISCUSSION

Wheat stem rust is a reemerging disease, causing significant impact in many bread and durum wheat-growing regions in the world. During the last 50 years, European countries have not experienced significant stem rust outbreaks as a result of the barberry eradication programs in western Europe (Hermansen, 1968) and the widespread use of broad-spectrum fungicides. However, recent outbreaks and epidemics reported in Germany (Olivera Firpo et al., 2017), Italy (Nocente et al., 2011), and Sweden (Berlin, 2017) are a strong indication that once again stem rust poses a threat to wheat production in Europe.

Pgt race TKTTF is widely distributed in the Middle East and East Africa (Singh et al., 2015) and has caused a devastating epidemic in Ethiopia in 2013-2014 (Olivera et al., 2015). In Europe, isolates of race TKTTF were first identified in Germany in 2013, where unusual stem rust infestations were observed in spring wheat nurseries (Olivera Firpo et al., 2017). Now, race TKTTF appears to be widely distributed in Europe (Hovmøller, 2021). It has also been detected in Morocco (Olivera & Jin, *unpublished*). It is not surprising that race TKTTF was identified in both Morocco and south-western Spain (Cádiz) as the southwesterly winds along the Atlantic coast from Morocco into Spain play a significant role in dispersing rust spores from north-west Africa into Europe (Zadoks, 1967).

Virulence to *Sr31* has been recognized as a distinct phenotypic feature of the Ug99 race group, as thus far, only races in this group have been reported to have virulence to this important gene (Newcomb et al., 2016; Singh et al., 2015). Although the variants of the Ug99 race group have been observed only in Africa and the Middle East, the occurrence of *Pgt* isolates in Spain with virulence on *Sr31*, raised concerns about the potential incursion of Ug99 into Europe. Isolate 18SPA092-1 with a novel race designation TKHBK, is the first race outside the Ug99 race group with virulence on *Sr31*. It is also the first *Pgt* race to be avirulent to ‘Rusty’, a durum

wheat line developed as universal susceptible to all known *Pgt* races (Klindworth et al., 2006). Furthermore, race TKHBK is not genotypically related to *Pgt* isolates that have been recently reported in Europe (TKTTF race group, clade IV; or TTRTF, clade III-B), and to other races belonging to previously described clades (JRCQC, clade II; races from sexual population from Georgia, clade V) (Olivera et al., 2105; 2019; Olivera Firpo et al., 2017). TKHBK is the first known race with virulence to *Sr59*, a recently identified gene of rye origin. These unique and highly unusual virulence/avirulence combinations and multilocus genotype strongly suggested that race TKHBK is likely a product of sexual origin from the alternate host. The impact of barberry in stem rust epidemiology has been documented previously in Spain; barberry (*Berberis* sp.) was recognized as a source of local stem rust inoculum and pathogen variability (Salazar & Brañas,1973). The identification of race TKHBK motivated an interest to investigate further the current role of barberry in stem rust epidemiology in several regions in Spain (Villegas et al., *unpublished*).

To assess crop vulnerability to wheat stem rust, we evaluated 120 bread wheat and 80 durum wheat cultivars and breeding lines from Spain for resistance to races TKTTF and TKHBK, and to six other races with significant virulence combinations. Results show that resistance is present in common and durum wheat. Stem rust resistance genes were postulated in the 120 bread wheat genotypes evaluated and confirmed with DNA markers. The most frequent gene was *Sr38*, present in ~40% of the cultivars and breeding lines. However, *Sr38* does not confer protection to the races responsible for the recent outbreaks in Europe, including TKTTF and TTRTF (Olivera et al., 2015; 2019). It is also ineffective against all variants in the Ug99 race group (Newcomb et al., 2016; Jin et al., 2007) and race TKHBK identified in this study. *Sr31* was detected in 12 bread wheat cultivars and four breeding lines and, may have been introduced into Spain when

CIMMYT cultivars ('Siete Cerros 66', 'Cajeme', 'Yecora', and 'Anza') were broadly adapted by local farmers in the 1970's (Lupton, 1992; Martínez-Moreno and Solís, 2019). *Sr7a* effective against some isolates of race TKTTF was detected at a low frequency in bread wheat cultivars and breeding lines. Adult plant resistance gene *Sr57* was detected in low frequency in both cultivars and breeding lines. As this set of cultivars and breeding lines were only tested at the seedling stage, field evaluations for stem rust response are necessary to confirm the marker results.

The frequency of resistance to all the races evaluated was comparable between both cultivars and breeding lines of bread wheat. These results confirm that stem rust has not been a priority in the national breeding programs, because of the low occurrence observed in the last decades (Martínez-Moreno & Solís, 2019). The knowledge generated in this study is a valuable tool for breeders to help designing crossing strategies to improve stem rust resistance in the future. A higher frequency of resistance was observed in Spanish durum wheat compared to bread wheat for all the races evaluated except for JRCQC. Race TKHBK was avirulent to all the durum wheat genotypes evaluated. Similarly, 13 durum cultivars susceptible to all the other six races evaluated, were resistant to race TKHBK. This avirulence, including that to Rusty, is highly unique and currently being investigated.

Three phenotypic alleles (*a, b* and *c*) of the *Sr13* gene were postulated to be present in this durum wheat collection and confirmed with DNA markers. *Sr13* is a major component of stem rust resistance in durum wheat worldwide (Klindworth et al., 2007; Singh et al., 2015). Therefore, it is not surprising that this gene was detected in about half of the cultivars and breeding lines evaluated. As with bread wheat, there was no significant increase in the level of stem rust resistance in the durum breeding lines compared to the cultivars. The frequencies of resistance to all races

evaluated were comparable between both improvement categories, and only for *Sr13b* there was a significant increase in its frequency in the breeding lines compared to the cultivars. An increase in the frequency of *Sr13b* represents a risk to durum production as this gene is ineffective against TTRTF (Olivera et al., 2019), a race responsible for the severe stem rust epidemic on durum wheat in southern Italy (Patpour et al., 2018).

Wheat stem rust is re-emerging in Spain, following the same trend observed across Europe. In addition to a virulent race (TKTTF) with broad distribution across Europe and other important wheat growing regions in the world, this study identified a unique race (TKHBK) with significant virulence combination. Barberry has historically played a significant role in stem rust epidemiology in Spain, and the detection of race TKHBK is likely an indication that the alternate host is active in generating new virulence combinations. Pathogen monitoring in the cereal and alternate hosts is critical to detect new races that may overcome resistance in wheat varieties. In addition, there is a need to better understand the basis of stem rust resistance in common and durum wheat cultivars in Spain. This study provided the first effort in characterizing common and durum wheat cultivars and breeding lines for resistance to races of the stem rust pathogen with significant virulence. Our results indicate that resistance is available in both species, and cultivars resistant to all the races evaluated can be a source of resistance genes to improve and diversify stem rust resistance.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

Bajgain, P., Rouse, M.N., Bulli, P., Bhavani, S., Gordon, T., Wanyera, R. et al. (2015) Association mapping of North American spring wheat breeding germplasm reveals loci conferring resistance to Ug99 and other African stem rust races. *BMC Plant Biology, 15*, 249.

Berlin, A. (2017) Stem rust attacks in Sweden heralds the return of a previously vanquished foe. Swedish University of Agricultural Sciences, SLU News. https://www.slu.se/en/ew-news/2017/11/stem-rust-attacks-in-sweden-heralds-the-return-of-a-previously-vanquished-foe/.

Helguera, M., Khan, I.A., Kolmer, J., Lijavetzky, D., Zhong-Qi, L. & Dubcovsky, J. (2003) PCR assays for the *Lr37-Yr17-Sr38* cluster of rust resistance genes and their use to develop isogenic hard red spring wheat lines. *Crop Science, 43*, 1839–1847.

Hermansen, J.E. (1968) Studies on the Survival and Spread of Cereal Rust and Mildew Diseases in Denmark. Copenhagen, Denmark*: Hertz Bogtrykkeri.*

Hovmøller, M.S., Patpour, M., Rodriguez-Algaba, J., Thach, T., Justesen, A. & Hansen, J.G. (2021) DGG report of yellow and stem rust genotyping and race analyses 2020. *Global Rust Reference Centre*. Available at: https://agro.au.dk/fileadmin/www.grcc.au.dk/International_Services/Pathotype_YR_results/GRRC_annual_report_2020.pdf Accessed on [10 May 2021]

Jin, Y., Singh, R.P., Ward, R.W., Wanyera, R., Kinyua, M., Njau, P. et al. (2007) Characterization of seedling infection types and adult plant infection responses of monogenic *Sr* gene lines to race TTKS of *Puccinia graminis* f. sp. *tritici*. *Plant Disease, 91*, 1096-1099.

Jin, Y., Szabo, L.J., Pretorius, Z.A., Singh, R.P., Ward, R. & Fetch Jr., T. (2008) Detection of virulence to resistance gene *Sr24* within race TTKS of *Puccinia graminis* f. sp. *tritici*. *Plant Disease, 92*, 923-926.

Kamvar, Z. N., Brooks, J. C., & Grünwald, N. J. (2015) Novel R tools for analysis of genome-wide population genetic data with emphasis on clonality. *Frontiers in Genetics*, *6*, 208.

Klindworth, D.L., Miller, J.D. Jin, Y. & Xu, S.S. (2007) Chromosomal locations of genes for stem rust resistance in monogenic lines derived from tetraploid wheat accession ST464. *Crop Science, 47*, 1441-1450.

Klindworth, D.L., Miller, J.D. & Xu, S.S. (2006) Registration of 'Rusty' durum wheat. *Crop Science, 46*, 1012–1013.

Lagudah, E.S., Krattinger, S.G., Herrera-Foessel, S., Singh, R.P., Huerta-Espino, J., Spielmeyer, W. et al. (2009) Gene-specific markers for the wheat gene *Lr34/Yr18/Pm38* which confers resistance to multiple fungal pathogens. *Theoretical and Applied Genetics, 119*, 889-898.

384 Lupton, F.G.H. (1992) Changes in varietal distribution of cereals in central and western Europe.
385 In: Lupton, F.G.H. (Ed.), *Agro-Ecological Atlas of Cereal Growing in Europe.* Volume IV.
386 Pudoc. Wageningen, pp. 161.
387 Mago, R., Bariana, H.S., Dundas, I.S., Spielmeyer, W., Lawrence, G.J., Pryor, A.J. et al. (2005)
388 Development of PCR markers for the selection of wheat stem rust resistance genes *Sr24* and
389 *Sr26* in diverse wheat germplasm. *Theoretical Applied Genetics, 111,* 496–504.
390 Martínez–Moreno, F. & Solís, I. (2019) Wheat rust evolution in Spain: an historical review.
391 *Phytopathologia Mediterranea, 58,* 3-16.
392 Mohler, V., Hsam, S.K.L., Zeller, F.J., & Wenzel, G. (2001) An STS marker distinguishing the
393 rye-derived powdery mildew resistance alleles *Pm8/Pm17* of common wheat. *Plant Breeding,*
394 *120*, 448–450.
395 Newcomb, M., Olivera, P.D., Rouse, M.N., Szabo, L.J., Johnson, J., Gale, S. et al. (2016)
396 Characterization of Kenyan isolates of *Puccinia graminis* f. sp. *tritici* from 2008 to 2014
397 reveals virulence to *SrTmp* in the Ug99 race group. *Phytopathology, 100*, 986-96.
398 Nirmala, J., Saini, J., Newcomb, M., Olivera, P., Gale, S., Klindworth, D. et al. (2017) Discovery
399 of a novel stem rust resistance allele in durum wheat that exhibits differential reactions to
400 Ug99 isolates. *G3: Genes Genomes Genetics, 7*, 3481-3490.
401 Nocente, F., Sereni, L., Matere, A. & Pasquini, M. (2011) Recent Occurrence of *Puccinia*
402 *graminis* f. sp. *tritici* in Italy: Pathogen virulence composition and seedling resistance of
403 durum and common wheat. *Cereal Research Communications, 39*, 77-87.
404 Olivera, P.D., Newcomb, M., Szabo, L.J., Rouse, M.N., Johnson, J., Gale S, et al. (2015)
405 Phenotypic and genotypic characterization of race TKTTF of *Puccinia graminis* f. sp. *tritici*
406 that caused a wheat stem rust epidemic in southern Ethiopia in 2013/14. *Phytopathology, 105*,

917-928.

Olivera, P.D., Sikharulidze, Z., Dumbadze, R., Szabo, L.J., Newcomb, M., Natsarishvili, K. et al. (2019) Presence of a sexual population of *Puccinia graminis* f. sp. *tritici* in Georgia provides a hotspot for genotypic and phenotypic diversity. *Phytopathology*, *119*, 2152-2160.

Olivera Firpo, P.D., Newcomb, M., Flath, K., Szabo, L.J., Carter, M., Luster, D.G. et al. (2017) Characterization of *Puccinia graminis* f. sp. *tritici* isolates derived from an unusual wheat stem rust outbreak in Germany in 2013. *Plant Pathology*, *66*, 1258-1266.

Patpour, M., Hovmøller, M.S., Hansen, J.G., Justesen, A.F., Thach, T., Rodriguez-Algaba, J. et al. (2018) Epidemics of yellow rust and stem rust in Southern Italy 2016-2017. In: BGRI 2018 Technical Workshop. Available at: https://www.globalrust.org/content/epidemics-yellow-and-stem-rust-southern-italy-2016-2017 Accessed [10 May 2021].

Prevosti, A., Ocaña, J., & Alonso, G. (1975) Distances between populations of *Drosophila suboscura* based on chromosome arrangement frequencies. *Theoretical and Applied Genetics*, *45*, 231-241.

Roelfs, A.P. (1985) Wheat and rye stem rust. In: Roelfs A.P. & Bushnell, W.R. (Eds.), *The Cereal Rusts Vol. II: Diseases, Distribution, Epidemiology and Control*. Academic Press, Orlando, FL, pp 4-39.

Roelfs, A.P. & Martens, J.W. (1988) An international system of nomenclature for *Puccinia graminis* f. sp. *tritici*. *Phytopathology*, *78*, 526-533.

Rouse, M.N., Nava, I.C., Chao, S., Anderson, J.A. & Jin, Y. (2012) Identification of markers linked to the race Ug99 effective stem rust resistance gene *Sr28* in wheat (*Triticum aestivum* L.). *Theoretical Applied Genetics*, *125*, 877–885.

Saitou, N. & Nei, M. (1987) The neighbor-joining method: A new method for reconstructing

phylogenetic trees. *Molecular Biology and Evolution*, *4*, 406-25

Salazar, J. & Brañas, M. (1973) Physiologic races of wheat black rust (*Puccinia graminis* Pers. var. *tritici* Eriks. et Henn.) detected in Spain in the years 1968–1971. *Cereal Rusts Bulletin, 1*, 21-23.

Singh, R.P., Hodson, D.P., Jin, Y., Huerta-Espino, J., Kinyua, M., Wanyera, R. et al. (2006) Current status, likely migration and strategies to mitigate the threat to wheat production from race Ug99 (TTKS) of stem rust pathogen. *CAB Reviews: Perspectives in Agriculture Veterinary Science Nutrition and Natural Resources*, *1*, 1-13.

Singh, R.P., Hodson, D.P., Jin, Y., Lagudah, E.S., Ayliffe, M.A., Bhavani, S. et al. (2015) Emergence and spread of new races of wheat stem rust fungus: Continued threat to food security and prospects of genetic control. *Phytopathology*, *105*, 872-884.

Skolotneva, E.S., Kosman, E., Patpour, M., Kelbin, V.N., Morgounov, A.I., Shamanin, V.P. et al. (2020) Virulence phenotypes of Siberian wheat stem rust population in 2017–2018. *Frontiers in Agronomy*, *2*, 6. https://doi.org/10.3389/fagro.2020.00006

Wang, S., Wong, D., Forrest, K., Allen, A., Chao, S., Huang, B.E., et al. (2014) Characterization of polyploid wheat genomic diversity using a high-density 90 000 single nucleotide polymorphism array. *Plant Biotechnology Journal*, *12*, 787–796.

Zadoks, T.C. (1967) Epidemiology of wheat rust in Europe. *International Journal of Pest Management B, 13:1*, 29-46.

Zhang, W., Chen, S., Abate, Z., Nirmala, J., Rouse, M.N. & Dubcovsky, J. (2017) Identification and characterization of *Sr13*, a tetraploid wheat gene that confers resistance to the Ug99 stem rust race group. *Proceedings of the National Academy of Sciences USA, 114*, E9483-9492.

SUPPORTING INFORAMTION LEGEND

Supplementary Table S1. SNP genotypic clades of 25 reference isolates of *Puccinia graminis* f. sp. *tritici.*

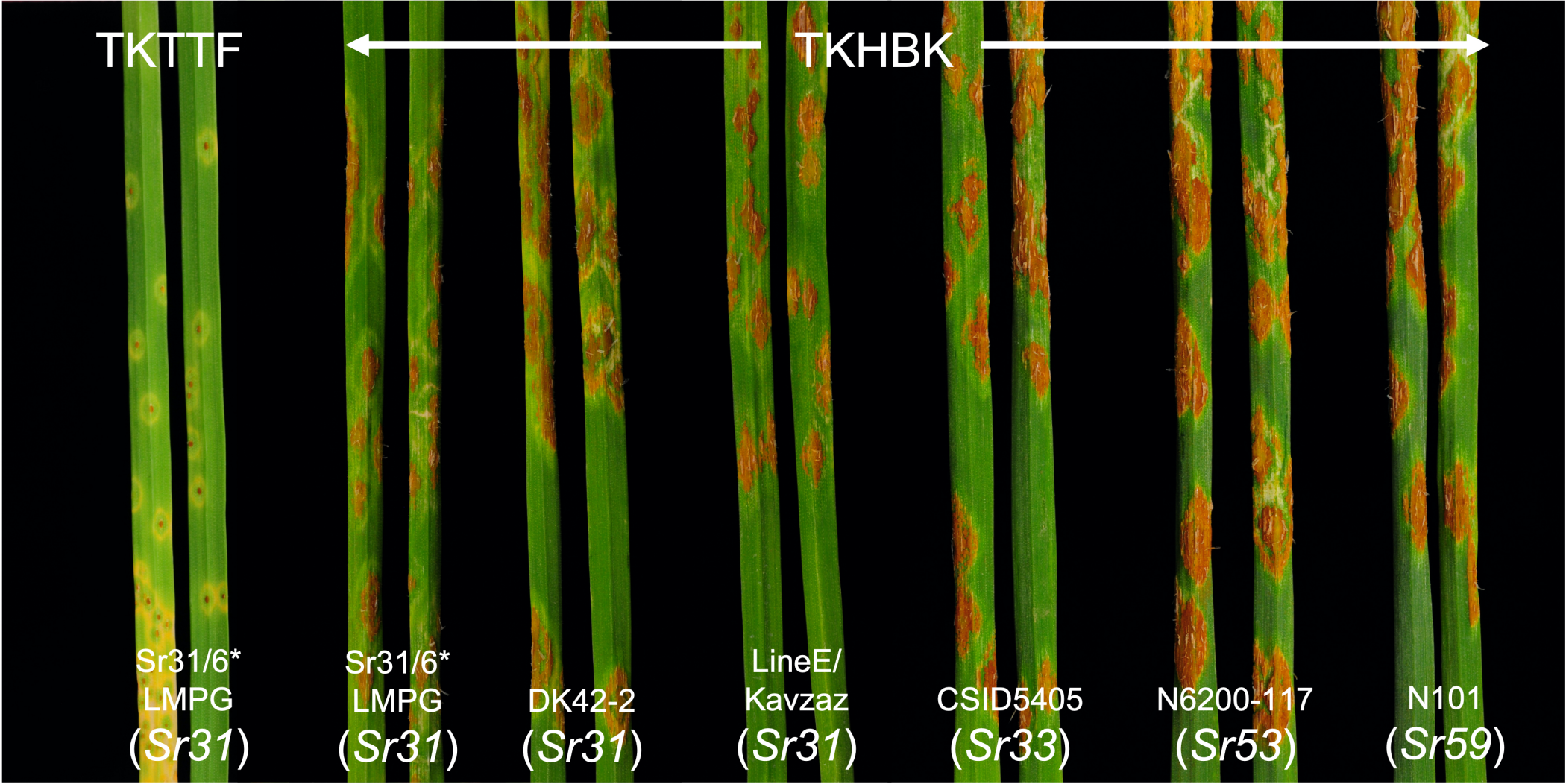
FIGURE LEGENDS

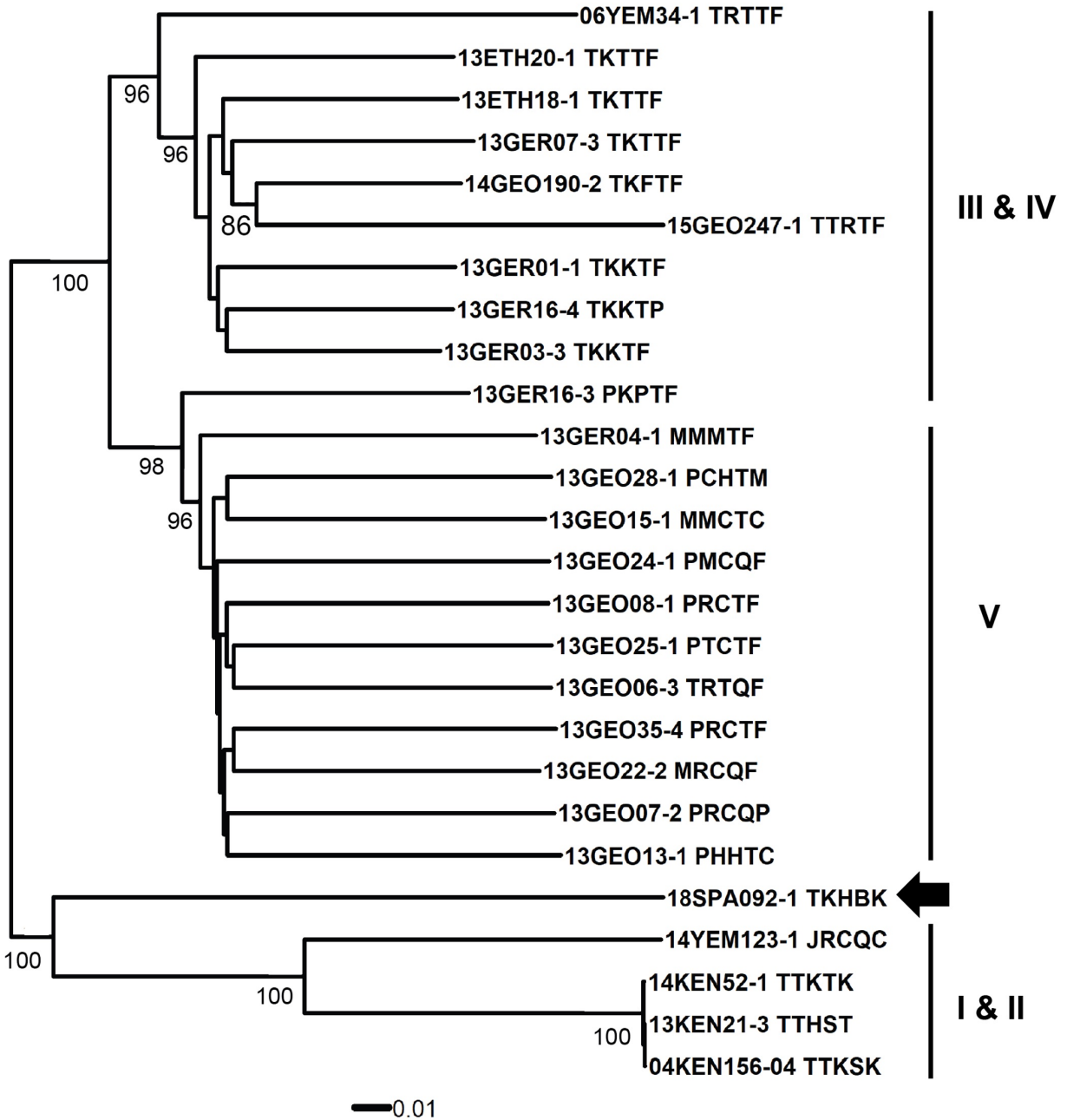
FIGURE 1. Infection types on wheat lines carrying *Sr31*, *Sr33*, *Sr53*, and *Sr59* when evaluated against race TKHBK at the seedling stage.

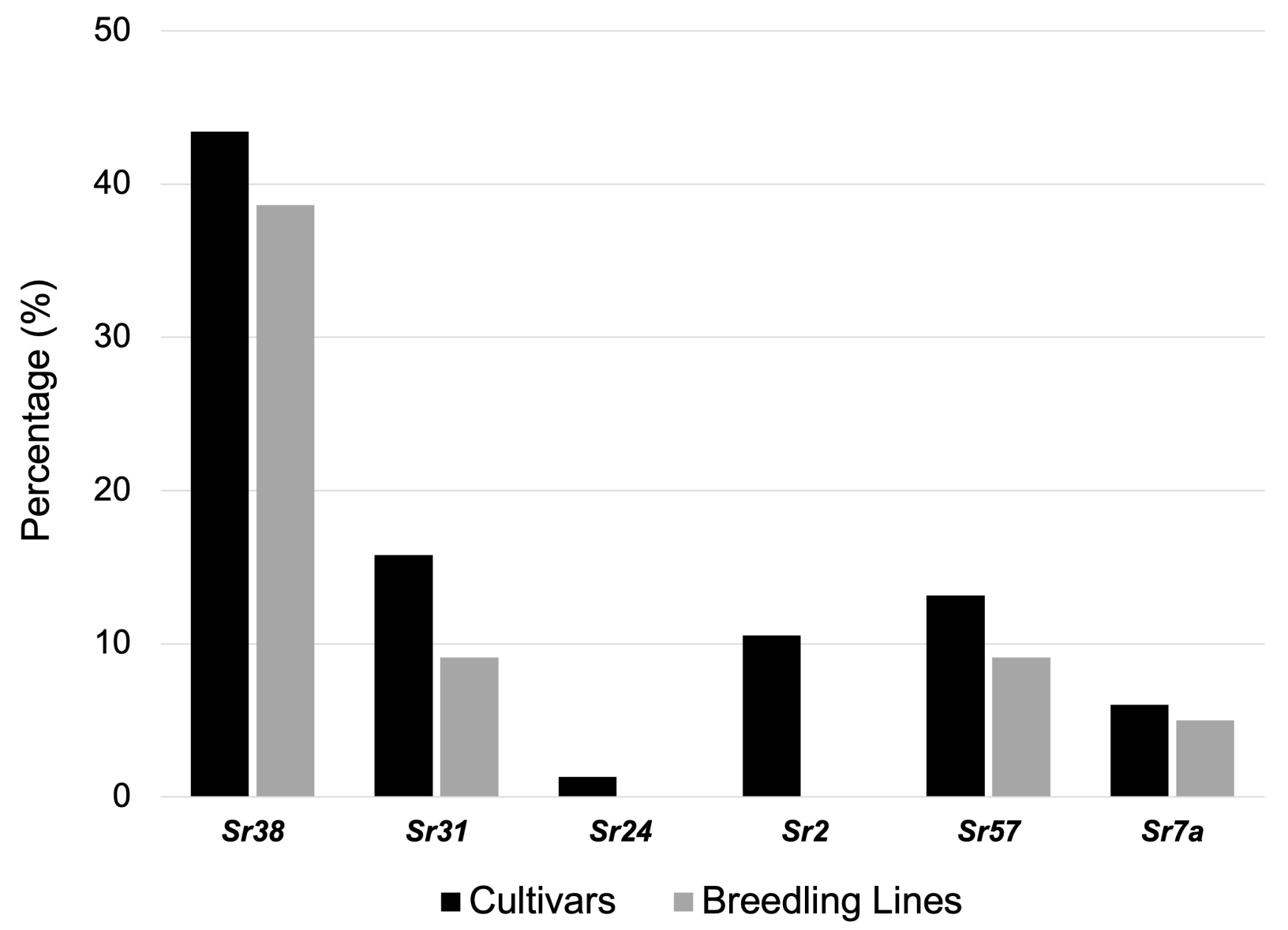
FIGURE 2. Neighbor-joining phylogenetic analysis of isolate 18SPA092-1 from Spain and 25 reference isolates based on 1,838 single-nucleotide polymorphic loci. Isolate 18SPA092-1 is indicated with a black arrow.

FIGURE 3. Percentage of Spanish bread wheat cultivars and breeding lines that tested positive for the DNA marker of the stem rust resistance genes *Sr38*, *Sr31*, *Sr24*, *Sr7a*, *Sr2*, and *Sr57*.

FIGURE 4. Percentage of Spanish durum wheat cultivars and breeding lines that tested positive for the DNA marker of the effective alleles *a*, *b* and *c* of the stem rust resistance gene *Sr13*.


TKTTF
TKHBK
Sr31/6*
LMPG
(Sr31)
Sr31/6*
LMPG
(Sr31)
DK42-2
(Sr31)
LineE/
Kavzaz
(Sr31)
CSID5405
(Sr33)
N6200-117
(Sr53)
N101
(Sr59)


06YEM34-1 TRTTF
13ETH20-1 TKTTF
13ETH18-1 TKTTF
13GER07-3 TKTTF
14GEO190-2 TKFTF
15GEO247-1 TTRTF
13GER01-1 TKKTF
13GER16-4 TKKTP
13GER03-3 TKKTF
13GER16-3 PKPTF
13GER04-1 MMMTF
13GEO28-1 PCHTM
13GEO15-1 MMCTC
13GEO24-1 PMCQF
13GEO08-1 PRCTF
13GEO25-1 PTCTF
13GEO06-3 TRTQF
13GEO35-4 PRCTF
13GEO22-2 MRCQF
13GEO07-2 PRCQP
13GEO13-1 PHHTC
18SPA092-1 TKHBK
14YEM123-1 JRCQC
14KEN52-1 TTKTK
13KEN21-3 TTHST
04KEN156-04 TTKSK
96
96
86
100
98
96
100
100
100
III & IV
V
I & II
0.01


50
40
30
20
10
0
Percentage (%)
Sr38
Sr31
Sr24
Sr2
Sr57
Sr7a
Cultivars
Breedling Lines

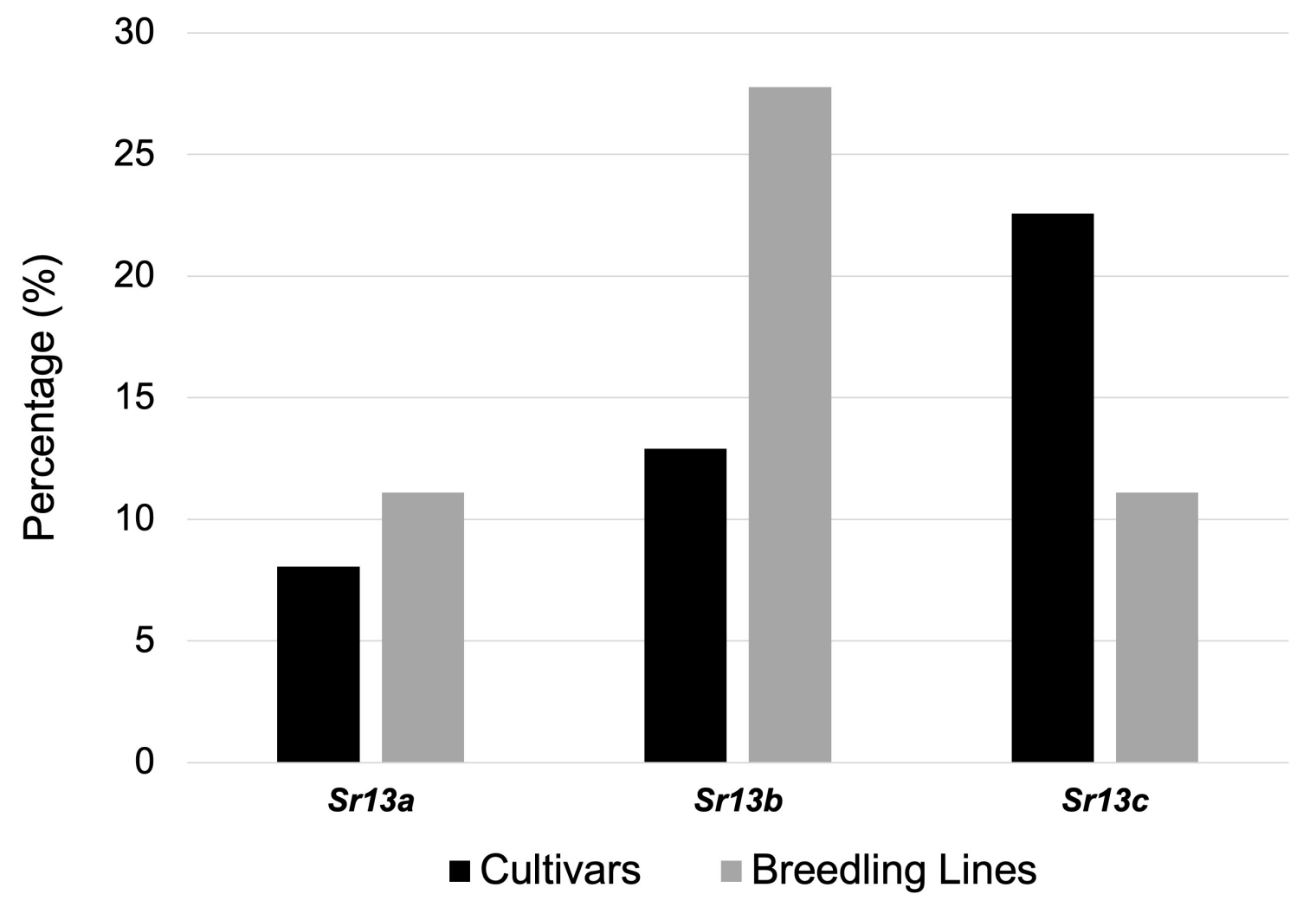

30
25
20
15
10
5
0
Percentage (%)
Sr13a
Sr13b
Sr13c
Cultivars
Breedling Lines

TABLE 1. Infection types observed in the 20 differential set lines caused by races TKTTF and TKHBK of *Puccinia graminis* f. sp. *tritici* collected in Spain in 2018

Line	**Gene**	**TKTTF (18SPA055-1)**	**TKHBK (18SPA092-1)**
ISr5-Ra	*5*	4	4[a]
CnS_T_mono_deriv	*21*	3+	3+
Vernstine	*9e*	3+	3+
ISr7b-Ra	*7b*	3+	3+
ISr11-Ra	*11*	2-	2-
ISr6-Ra	*6*	3+	33+
ISr8a-Ra	*8a*	4	3+
CnSr9g	*9g*	4	4
W2691SrTt-1	*36*	3+	0;
W2691Sr9b	*9b*	3+	3+
BtSr30Wst	*30*	33+	2-
Combination VII	*17+13a*	2+	2+3
ISr9a-Ra	*9a*	4	2-
ISr9d-Ra	*9d*	4	2-
W2691Sr10	*10*	4	11-;
CnsSrTmp	*Tmp*	3+	2-;
LcSr24Ag	*24*	2-	2
Sr31/6*LMPG	*31*	2-	33+
VPM-1	*38*	33+	33+
McNair 701	*McN*	4	4

[a] Infection types (ITs) observed on seedlings at 14 days after inoculation using the 0-4 scale according to Stakman et al. (1962), where ITs of 0, ;, 1, 2 or combinations thereof are considered as a low IT and ITs 3 or higher are considered as a high IT.

TABLE 2. Infection types observed on lines carrying stem rust resistance genes produced by races TKTTF and TKHBK of *Puccinia graminis* f. sp. *tritici* collected in Spain in 2018

Line	**Gene**	**TKTTF (18SPA055-1)**	**TKHBK (18SPA092-1)**
DK42-2	*31*	n.d. [a]	33+ [b]
Line E/Kavkaz-2	*31*	n.d.	3+
NA101/MgSr7a	*7a*	13C	;1
ST464	*13a*	2	2-
SwSr22T.B.	*22*	2-	2-
Agatha/9*LMPG	*25*	2+	2-
Eagle	*26*	2-	2-;
73,214,3-1/9*LMPG	*27*	;1-	;
ER5155	*32*	2	22-
CSID5405	*33*	2-	3+
Mq(2)5XG2919	*35*	0;	;
W3563	*37*	;1	11+;
RWG1	*39*	2-	2-
RL6088	*40*	2	;
RWG34	*43*	1+3-	;
RWG35	*47*	2-	2-
AUS91434	*50*	2-	2-
TS1-38	*51*	;1-	;
F09-18-11	*52*	31C	11+
U6200-117	*53*	22-	3+
N101	*59*	22+	3+
TAM 107-1	*1RS^{Amigo}*	2-	;1-
8155-B1	*8155B1*	0;	1+13-
Satu	*Satu*	;	0;
Leeds	*9e,13b,+*	;	;
Iumillo	*9g,12,+*	;1N	;
Q21861A	*Rpg1,4,5*	0;	;

[a] n.d. = no data.

[b] Infection types (ITs) observed on seedlings at 14 days after inoculation using the 0-4 scale according to Stakman et al. (1962), where ITs of 0, ;, 1, 2 or combinations thereof are considered as a low IT and ITs 3 or higher are considered as a high IT.

TABLE 3. Infection types on universal susceptible bread and durum wheat lines produce by race TKHBK (isolate 18SPA092-1) of *Puccinia graminis* f. sp. *tritici*

Line / cultivar	Crop species	Infection type
Line E	Bread wheat	4 [a]
Morocco	Bread wheat	3+
Little club	Bread wheat	3+
Chinese Spring	Bread wheat	3+
W2691	Bread wheat	4
LMPG-6	Bread wheat	3
Baart	Bread wheat	4
Rusty	Durum wheat	2

[a] Infection types (ITs) observed on seedlings at 14 days after inoculation using the 0-4 scale according to Stakman et al. (1962), where ITs of 0, ;, 1, 2 or combinations thereof are considered as a low IT and ITs 3 or higher are considered as a high IT.

TABLE 4. Number and percentage of resistant bread wheat (*Triticum aestivum*) cultivars and breeding lines from Spain evaluated against eight races of *Puccinia graminis* f. sp. *tritici*

	Cultivars		Breeding lines	
Race	**Number**	**%**	**Number**	**%**
TKHBK	51	67.1	40	90.9
TKTTF	29	38.2	20	45.5
TTRTF	53	69.7	22	50.0
TTKSK	13	17.1	12	27.3
TTKTT	14	18.4	10	22.7
JRCQC	67	88.2	43	97.7
TTTTF	51	67.1	14	33.3
QFCSC	113	90.1	44	100.0
All races	9	12.0	5	11.8

TABLE 5. Number and percentage of resistant durum wheat (*Triticum turgidum* ssp. *Durum*) cultivars and breeding lines from Spain evaluated against eight races of *Puccinia graminis* f. sp. *tritici*

	Cultivars		Breeding lines	
Race	**Number**	**%**	**Number**	**%**
TKHBK	62	100.0	18	100.0
TKTTF	39	62.9	10	55.6
TTRTF	47	75.8	15	83.3
TTKSK	38	61.3	9	50.0
TTKTT	37	59.7	9	50.0
JRCQC	32	52.5	9	50.0
TTTTF	37	59.7	10	55.6
QFCSC	42	96.8	18	100.0
All races	26	41.9	6	33.3

TABLE 6. Infection type observed in 120 bread wheat (*Triticum aestivum*) breeding lines and cultivars from Spain in seedling stage evaluations against races TKHBK, TKTTF, TTKK, TTKTT, TTRTF, JRCQC, and TTTTF of *Puccinia graminis* f. sp. *tritici*, gene postulation based on seedling phenotypes, and DNA marker confirmation for *Sr24, Sr31, Sr38, Sr7a*, and *Sr57* genes

	RACES									DNA Marker				
	TKHBK[a]	TKTTF	TTKSK	TTKTT	TTRTF	JRCQC	QFCSC	TTTTF	Gene postulated					
	18SPA092-1	13ETH18-1	04KEN156/04	14KEN58-1	14GEO189-1	09ETH08-3	06ND76C	02MN84A-1-2		*Sr24*	*Sr31*	*Sr38*	*Sr7a*	*Sr57*
08H075H1aee 1-1	0 [b]	3+	3+	3+	3+	2-	2-	4		-	-	-	-	-
08H075H1aee 1-2	0;	3+	4	3+	3+	2-	2-	4		-	-	-	-	-
09H061H5bfa 1	;11-	3+	4	3+	2+	0	2+	4		-	-	-	-	-
09H061H5bfa 2	;2-	3+	3+	3+	4	0	2	3+		-	-	-	-	-
07H007H15bda 1-1	0	;2-	3	3+	2-	;1	2-;	;	*Sr31*+	-	+	-	+	+
07H007H15bda 2-1	0;	;	3	3+	2-	0;	2-	;	*Sr31*+	-	+	-	+	+
12H544H1 1-2	;	3+	3+	3+	32+	;1-	2-	4		-	-	-	-	-
12H544H1 2-1	0	4	3+	3+	32+	;11-	2-	3+		-	-	-	-	-
08H027H1fdc-1-1	0;	;2-	3+	3+	3+	;	;	3	*Sr38*+	-	-	+	-	-
08H027H6ade-2-1	2-	2	3+	3+	13	0;	;	2-;	*Sr31*+	-	+	+	-	-
07H012H12fea-1-1-1	;	2+	2+	3	3+	2	2-	4		-	-	-	-	-
07H012H12fea-2-1-1	0;	2	2+	2+	3	2-	2-	33+		-	-	-	-	-
ES08H017L4aea-1	0	2-	4	3+	4	0;	;2-	3+	*Sr38*+	-	-	+	-	-
ES08H017L13aca-1	0	2-	4	3+	3+	0;	;2-	3+	*Sr38*+	-	-	+	-	-
14TH5506-1	0	4	3+	3	2-	2-	2-	4		-	-	+	-	-
09TH1001C2 bdf	;	2-	33+	3+	22-	;	2-;	2-	*Sr31*+	-	+	-	-	-
09TH1007C2 eba	0	4	4	2+3	4	;	2	4		-	-	-	-	-
09TH2047V8f	3+	4	4	3+	3+	4	4	4		-	-	-	-	-
09TH3011V2b	0;	2-	3+	3+	3	23	;	3C		-	-	-	-	-
09TH3034V6aa	33+	2+	2+	2+	3+	2-	;2-	2		-	-	-	-	-
09TH3035V1ab	33+	22+	2	2+	2+	0;	;	2-		-	-	+	-	-
ES08H025L6fbe	0;	3+	3+	3	13	31	;	2+		-	-	-	-	+
15TH5534H1	0;	;2-	2-	2-	2	;	;1-	;1-		-	-	+	-	-
10H042H5acf	0;	2	2+3	2+3	2-	2-	2-	2+		-	-	-	-	-
10H070H2fdc	0	4	33+	33+	3+	1+1;	;	3+	*Sr38*+	-	-	+	-	-
10H071H4afc	0	4	4	3+	3+3	0;	;	3+	*Sr38*	-	-	+	-	-
10H071H9baf	;	4	4	3+	33+	0;	;	4	*Sr38*	-	-	+	-	-
10H004H8abf	;1-	22+	2	2+	22-	2-;	;	2		-	-	+	-	-
10H062H8bfa	2-	4	33+	3	2+	11-;	2	3+		-	-	-	-	-
11H501H3 1-1-1	2	4	3+	3+	33+	1+1;	1-;	4	*Sr38*+	-	-	+	-	-
11H501H3 1-1-2	2-	4	4	3	33+	1+1;	11-;	4	*Sr38*+	-	-	+	-	-
ES06H136C-32-3-1-1-1-1	0;	2-	2+	3	2	;	2-	0		-	-	-	+	-
ES06H136C-32-3-1-1-1-2	0;	2	2+	3	2+	;1-	;	0;		-	-	-	+	-
06H034	0	4	33+	3+	3+	2-	2-	4		-	-	-	-	-
11H505 H2	2	2+3	2+3	2+3	2	;1-	;	2+		n.d. [c]	n.d.	n.d.	n.d.	n.d.
11H501 H3	2-	4	3+	3+	3+	1+1;	;11+	3+	*Sr38*+	n.d.	n.d.	n.d.	n.d.	n.d.
08H027 H1fdc	0	2-	3+	3+	3+	;	;	3	*Sr38*+	n.d.	n.d.	n.d.	n.d.	n.d.
09H036H6faa	0;	2	22+	2+	2+	2-	;	3;		-	-	-	+	-

10H062H8bba	2-;	4	3	3-	2+	0;	2	3+		-	-	-	-	-
08H27H6ade	;1-	3+	3+	3+	2+	;	;	4	*Sr38*+	-	-	+	-	+
08THES2162	0;	4	3+	3+	3+	0;	;	4	*Sr38*+	-	-	+	-	-
06TB06	3+	4	4	3+	3+	0	;11+	4	*Sr38*	-	-	+	-	-
11H505H2	2	2+	2+	2+	2	;11-	;	2		-	-	+	-	-
ES06H107WSL6	3+	4	4	3+	3+	33+	2	4		-	-	-	-	-
BALIVIAL	2-	2-	3+	3+	2+	2	2-;	2-;	*Sr31*+	-	+	-	+	-
BALSAMINA	2-	2-	3+	3+	2	2	2-	2-;	*Sr31*+	-	-	-	+	-
BANCAL	0;	2-	22+	22+	2-	2-	2-	;2-		-	-	-	-	-
BANDOLÍ	;	2-	3+	3	2-	0;	;2-	2-	*Sr31*+	-	+	-	-	-
BANER	0	3+	3+	3+	33+	2-	2-	4		-	-	-	-	-
BARBOL	2-	2-	2+	2+	2-	2-	2	2-		-	-	-	-	-
BAULA	0;	2-	3+	3	22-	2-	2-	2-	*Sr31*+	-	+	-	-	-
CATEDRAL	2	4	3+	3	3+	2	2	4		-	-	-	-	-
MAPEÑA	2-;	3+	33+	2+	2+	2	2	3		-	-	-	-	-
MONTCADA	22+	4	3+	2+	2+	2	3+	4		-	-	-	-	-
OSONA	22+	2-	3+	3+	2-	2-	;	2-	*Sr31*+	-	+	-	-	-
PEÑALON	0;	2-	3+	3+	2-	2-	;	2-	*Sr31*+	-	+	-	-	-
POTENCIANO	2	3+	4	3+	33+	2+	2-	4		-	-	-	-	-
BABUI	2	4	3+	3+	33+	2	2	4		-	-	-	-	-
BADINA	;	;	3+	3+	2-	0	0;	;	*Sr31*+	-	+	-	+	+
TRAMUNTANA	2-	2-	3	3+	2+3	2-	;	3+		-	-	-	-	-
VICTORINO	0;	2-	2+	3+	2-	2-	;	2-	*Sr31*	-	+	-	-	-
Variety 01	2-	2-	3+	3	2	0;	;	2	*Sr31*+	-	+	-	-	-
Variety 02	;	22+	2+3	2+	2	;	0;	2		-	-	+	-	-
Variety 03	;	3+	33+	3+	2+	2+	2-	2+		-	-	-	-	-
Variety 04	0	3+	3+	3	2+2	0;	;	4	*Sr38*+	-	-	+	-	-
Variety 05	0;	32+	3+	3+	3+	;	;	3+	*Sr38*	-	-	+	-	-
Variety 06	22-	4	3+	3+	3+	2-;	11-;	4	*Sr38*+	-	-	+	-	-
Variety 07	0;	4	3+	3+	22+	;1-	;	4	*Sr38*+	-	-	+	-	-
Variety 08	0;	2	2-	2-	2+	;	0;	0		-	-	+	-	-
Variety 09	0	22-	2-	2-	2-	;	;	2		-	-	+	-	+
Variety 10	2-	11+;	3+	3+	2	;1-	;	;1	*Sr31*+	-	+	-	+	+
Variety 11	3+	3	2	2	33+	;1-	;1	2		-	-	+	-	-
Variety 12	2-	2	2-	2-	2	;1-	;	2-		-	-	-	-	-
Variety 13	2-;	4	4	3+	22+	;	;	4	*Sr38*+	-	-	+	-	-
Variety 14	2+	4	3+	3+	2	31+;	;13	3+		-	-	-	-	-
Variety 15	;2-	2-	3+	3	2-	2-	2-	2-	*Sr31*+	-	+	-	-	-
Variety 16	2	2-	3+	3+	2-	2-	2-	2-	*Sr31*+	-	+	-	-	+
Variety 17	;	33+	3+	2+	2+	;	2-	33+		-	-	-	-	-
Variety 18	2	3+	4	3+	3+	33+	2+	3+		-	-	-	-	-
Variety 19	3	4	3+	3+	3+	1;	;	31	*Sr38*	-	-	+	-	-
Variety 20	3	3	4	3+	22+	0	;1-	33+	*Sr38*+	-	-	+	+	-
Variety 21	2-	3+	3+	3+	3+	11-;	;	4	*Sr38*+	-	-	+	-	-
Variety 22	2-	4	3+	4	3+	;	;	4	*Sr38*+	-	-	+	-	-
Variety 23	3+	4	4	3+	2+	22-	3+	4		-	-	-	-	+
Variety 24	;2-	4	3+	3+	3+	;	;	4	*Sr38*+	-	-	+	-	-
Variety 25	33+	2	2	2+3	3+	;	;	2-		-	-	+	-	-
Variety 26	2+	3+	3+	3	22+	;	11+;	3+	*Sr38*+	-	-	+	-	-
Variety 27	3+	4	3+	3+	3+	31;	1+1;	3+	*Sr38*	-	-	+	-	-

Variety 28	3+	4	3+	3+	3+	;1-	;1	3+	*Sr38*	-	-	+	-	-
Variety 29	2	4	3+	3+	22+	1+1;	;	3	*Sr38*+	-	-	+	-	-
Variety 30	2+3	4	3+	3+	3+	;	3+	3		-	-	-	-	+
Variety 31	4	4	33+	3+	2	11+;	;1+	3+	*Sr38*+	-	-	+	-	-
Variety 32	3	4	3+	3+	2+	13;	;	33+	*Sr38*+	-	-	+	-	-
Variety 33	3	4	3+	3+	3+	3;	;11+	4	*Sr38*	-	-	+	-	+
Variety 34	3	4	3+	3+	22+	31;	;1+1	3+	*Sr38*	-	-	+	-	-
Variety 35	4	4	4	3+	33+	13;	11+;	4	*Sr38*	-	-	+	-	+
Variety 36	3+	4	4	3+	3+	0;	;11+	4		-	-	-	-	-
Variety 37	33+	3	4	3+	22+	11+3;	;11+	4		-	-	-	-	-
Variety 38	2	3+	3+	3+	22+	11+;	;1+	3+	*Sr38*+	-	-	+	-	-
Variety 39	;	2-	3	3+	2-	;2-	;	11-C	*Sr31*+	-	-	-	+	-
Variety 40	3+	4	3+	3+	2+	3+	;	3+		-	-	-	-	-
Variety 41	3+	4	4	3+	2	0;	3;	3+	*Sr38*+	-	-	+	-	-
Variety 42	4	4	4	3+	2+	4	4	3+		-	-	-	-	-
Variety 43	3+	4	3+	3+	2	3+	4	3+		-	-	-	-	-
Variety 44	3	4	3+	3+	2+	33+	31;	33+		-	-	-	-	-
Variety 55	;	4	4	3+	31	;13	;	3+		-	-	+	-	-
Variety 46	32+	3+	4	3+	3+	;	;	1+1C		-	-	-	-	-
Variety 47	2-	2-	2+3	3	2+	;	;	;1		-	-	+	-	+
Variety 48	3+	4	3+	3+	3+3	3+	4	3+		-	-	-	-	-
Variety 49	2-;	3+	3+	3+	2+	;1	0;	31		-	-	+	-	-
Variety 50	2-	2-	3+	3+	0;	;	;	0;	*Sr31*+	-	+	+	-	-
Variety 51	2-	2	2-	2-	22-	;11+	;2-	2-		-	-	+	-	-
Variety 52	2	2-	2-	2+3	2-	2-	2-	2-	*Sr24*	+	-	+	-	-
Variety 53	2-	2-	2-	2-	2	1;	;	2-		-	-	-	-	-
Variety 54	4	4	3+	3+	2	4	4	3+		-	-	-	-	-
Variety 55	3	4	4	3	2+	3	33+;	4		-	-	-	-	-
Variety 56	4	4	3+	3+	3+	1+1;	;11+	4	*Sr38*	-	-	+	-	-
Variety 57	;	1;	3	3	2+	11-;	;2=	;1		-	-	-	+	+
Variety 58	2-	3+	3+	3	2+	2	;11+	13		-	-	-	-	-
Variety 59	3+	4	4	3+	22+	3;	31;	3+		-	-	-	-	-
McNair 701	4	4	4	4	4	4	4	4						

[a] Race nomenclature based on Jin et al. (2008).

[b] Infection types (ITs) observed on seedlings at 14 days post-inoculation using a 0-4 scale according to Stakman et al. (1962), where ITs of 0, ;, 1, 2, or combinations thereof are considered as a low IT and ITs of 3 or higher are considered as a high IT. C denotes extensive chlorosis.

[c] n.d. = no data.

TABLE 7. Infection type observed in 80 durum wheat (*Triticum durum* ssp. *durum*) breeding lines and cultivars from Spain in seedling stage evaluations against races TKHBK, TKTTF, TTKK, TTKTT, TTRTF, JRCQC, and TTTTF of *Puccinia graminis* f. sp. *tritici*, gene postulation based on seedling phenotypes, and DNA marker confirmation for *Sr13* alleles *a*, *b*, and *c*

	RACE									DNA
	TKHBK[a]	TKTTF	TTKSK	TTKTT	TTRTF	JRCQC	QFCSC	TTTTF		marker
Cultivar/line	18SPA092-1	13ETH18-1	04KEN156/04	14KEN58-1	14GEO189-1	09ETH08-3	06ND76C	02MN84A-1-2	Gene postulated	*Sr13a, b, c*
CB017	2-	3+	4	3	2-	4	;	3+		-
CB021	;	2-N	2-N	2-	2-	2	2-;	;N2-		b
CB023	;	2	2-	2-	2-	2	2-	;N2=		b
CB047	2-	3+	4	3+	2+3	3+	;	3		-
CB049	2-	2	2-	2-	2-	2	;	2-		c
CB072	2+	4	4	3+	2+	4	;	3+		-
CB086	;1	2-	2-	2	2+3	3+	;	2-	*Sr13b*	b
CB171	;	2-	2-	2-	2+3	3+	;	;1-	*Sr13b*	b
CB172	11-;	3+	3+	3	22+	2+3	;1-	2+		-
CB193	11-;	4	4	3+	3+	4	;1-	3+		-
CB194	22+	4	4	3+	2+	4	;	3+		-
CB232	;	2-	2-	2-	2-	2	2-	2		a
CB235	11+;	2-	2-	2	3	4	;	2-	*Sr13b*	b
05D278 D1be	;2-	2	2-	2	;2-	2	;	2-		c
08D010 D10cab	;	2	2-	2-	2	2	;	2		a
07D057 D4fba	2-;	3+	4	3	2-	1+3;	;	4		-
09D066 D8cab	;	2-	3	3	2+	2	;	3+		-
09d069 d1dcf	;1	3+	33+	3-	3	3	;1	4		-
BOLENGA	;2-	2-	2-	2-	2-	2	;	;2+		a
BOLIDO	2-;	2	2-	2-	2	2+	;	2		a
BOLO	;	2-	2	2	2	2	;	2-		c
BOMBASI	2-;	2	2	2	2	22+	;	2-		c
BORGIA	;	2+	4	3+	2+	2+	22-	3+		-
BORLI	11-;	2-	2-	2	2-	2	;	1;		c
ALTARAOS	;1-	2-	2	2	2-	2	;	2-		c
HISPASANO	11-;	33+	4	3	2+	33+	;	3		-
SEMOLERO	2;	33+	3+	3+	2+	32+	;	33+		-
VALIRA	2-;	2	2-	2-	2	22+	;	2-		-
VITRONERO	;1-	;1-	;1	0;1-	2+	1+C	;1-	;		-
ANCALEI	1;	2	2	2-	22+	33+	;1-	2-	*Sr13a/c*	c
HUALITA	;	2	2-	2-	2	2	;	2		a
CIRNO C 2008	1+1;	4	3+	3+	3+	3+	;	3+		-
Variety 01	2-; [b]	22+	2-	2-	2	2+	;	2-		a
Variety 02	;11+	3+	3+	3+	3+	4	3+	4		-
Variety 03	2-	22+	2-	2	2-	2	2-	2		-
Variety 04	2-;	3+	3+	2+3	2+	4	;	2+		-
Variety 05	1+1;	3+	2-	3-	3	4	2-;	3+		-

Variety 06	;	;N	2-N	2-	2-	2+	2-	2		b
Variety 07	;1	2-	2-	2-	32+	3+	2-;	2-	*Sr13b*	b
Variety 08	22-	2-	2	2	22+	22+	2-;	22-		c
Variety 09	0;	3	3+	3	2	3+	2-	4		-
Variety 10	11-;	2	2	2	2	2+	;	2-		c
Variety 11	1;	4	4	3-	32+	4	;2-	4		-
Variety 12	2-;	2	4	3	33+	2	;2-	3		-
Variety 13	2-	3+	3+	3	3+	3+	;	3		-
Variety 14	;1-	2-	22-	2	22-	2	;1-	11-		c
Variety 15	;1-	2	2-	2	2	2+	2-	2-		c
Variety 16	;1-	2	3+	3+	2+	2-;	2-	1+1		-
Variety 17	11-;	3+	3	3	2+	2+	;	3		-
Variety 18	;11-	3+	3	3	22+	3+	;	3+		-
Variety 19	;	2	3+	3	2+	22+	;	2		-
Variety 20	;11+	3+	3+	3+	2+3	3	;2-	33+		-
Variety 21	11+;	3+	3	3+	33+	33+	2-	3+		-
Variety 22	;11+	;	;	13	2+3	3+	;2-	4		-
Variety 23	;	0;	;2-	0;	2	2+	;	;2-		b
Variety 24	;	2	2	2	3	4	;	2-;	*Sr13b*	b
Variety 25	22-	3+	3+	3+	3+	3+	;	3+		-
Variety 26	;1-	2	2-	2	2	2+3	1-;	2	*Sr13a/c*	c
Variety 27	1+3-	3+	3+	3+	3+	3+	2	3+		-
Variety 28	;1-	2	2-	2-	2-	2	;	0		c
Variety 29	;1-	33+	2-	2-	2+	3+	2-	2-;		a
Variety 30	;1-	2	2-	2-	3+	4	;	2-	*Sr13b*	b
Variety 31	;11-	2-	2-	2-	2	2	;	2-		b
Variety 32	2-;	3+	4	3	3+	3+	;2-	4		-
Variety 33	;	2	2-	2-	2	22+	;	;		c
Variety 34	;1-	2	2-	2-	22-	2	;	2-		c
Variety 35	2	4	3+	3+	2+3	4	;	3+		-
Variety 36	;1	3+	4	3+	2+3	32+	3+	4		-
Variety 37	2	3+	4	3+	2+	3+	;	3+		-
Variety 38	11-;	22-	2-	2	2	4	;	;	*Sr13a/c*	b
Variety 39	1;	3+	4	3+	3+	3+	11+	4		-
Variety 40	;	2-	2-	2-	2+	2+	;	2-		-
Variety 41	;2-	3+	3+	3	33+	33+	;	3		-
Variety 42	;	22-	1+;	2	2-	22+	;	2-		-
Variety 43	;11+	2-	2-	2	2+	3+	2-	2-	*Sr13b*	b
Variety 44	;	11-	;	0;	33+	3+	;	3+		-
Variety 45	;11-	2	2-	2	2	2+	;	2-		c
Variety 46	1+;	22-	2-	2	3+	4	;	2-		b
Variety 47	;1-	2	2-	2	2	2+	;	2-		-
Variety 48	2	3+	33+	3	2+	3	;2-	4		-
McNair 701	4	4	4	4	4	4	4	4		

[a] Race nomenclature based on Jin et al. (2008).

[b] Infection types (ITs) observed on seedlings at 14 days post-inoculation using a 0-4 scale according to Stakman et al. (1962), where ITs of 0, ;, 1, 2, or combinations thereof are considered as a low IT and ITs of 3 or higher are considered as a high IT. N denotes excessive necrosis.

Supplementary Table S1. SNP genotypic clades of 25 reference isolates of *Puccinia graminis* f.

Isolate	Race	Clade	Reference
04KEN156-04	TTKSK	I	Olivera et al., 2015
13KEN21-3	TTHST	I	Newcomb et al., 2016
14KEN52-1	TTKTK	I	Newcomb et al., 2016
14YEM123-1	JRCQC	II	Olivera et al., 2015
06YEM34-1	TRTTF	III-A	Olivera et al., 2015
15GEO247-1	TTRTF	III-B	Olivera et al., 2019
13ETH18-1	TKTTF	IV-A-1	Olivera et al., 2015
13GER07-3	TKTTF	IV-A-2	Olivera Firpo et al., 2017
13ETH20-1	TKTTF	IV-B	Olivera et al., 2015
13GER16-3	PKPTF	IV-C	Olivera Firpo et al., 2017
13GER01-1	TKKTF	IV-D	Olivera Firpo et al., 2017
13GER16-4	TKKTP	IV-E-1	Olivera Firpo et al., 2017
13GER03-3	TKKTF	IV-E-2	Olivera Firpo et al., 2017
14GEO190-2	TKFTF	IV-F	Olivera et al., 2019
13GER04-1	MMMTF	V	Olivera Firpo et al., 2017
13GEO28-1	PCHTM	V	Olivera et al., 2019
13GEO15-1	MMCTC	V	Olivera et al., 2019
13GEO24-1	PMCQF	V	Olivera et al., 2019
13GEO08-1	PRCTF	V	Olivera et al., 2019
13GEO25-1	PTCTF	V	Olivera et al., 2019
13GEO06-3	TRTQF	V	Olivera et al., 2019
13GEO35-4	PRCTF	V	Olivera et al., 2019
13GEO22-2	MRCQF	V	Olivera et al., 2019
13GEO07-2	PRCQP	V	Olivera et al., 2019
13GEO13-1	PHHTC	V	Olivera et al., 2019

. sp. *tritici* .