Article

Characterization of Wheat Yellow Rust and Stem Rust Virulence in Southern Spain

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Abstract: Effective mitigation of the current threat from yellow rust and the potential threat from stem rust to wheat production in the south of Spain requires the characterization of the lineages/races currently present in the region. Results from this study clearly indicated that the main yellow rust lineages currently present in the south of Spain are PstS10, PstS13, and PstS14, to which several widely grown commercial cultivars are resistant. Even though stem rust is not yet present during the regular cropping season, the main lineages/races Clade IV-B and Clade IV-F, were identified, much like in most of Europe and parts of North Africa. The evaluation of differential series and special breeding lines with known genes under local conditions has indicated the availability of several genetic options that could be used in breeding/selection programs to provide effective levels of resistance to either disease in the future. However, in undertaking these efforts, it is important to consider not only the lineages currently present locally but also resistance options effective against lineages/races that are rapidly developing elsewhere and could very likely reach the south of Spain in the near future.

Keywords: *Puccinia graminis*; *Puccinia striiformis*; resistance; Warrior race

1. Introduction

Wheat is one of the world's leading cereal grains and represents a staple food for more than one-third of its population, contributing more calories and proteins than any other cereal [1]. It is the most cultivated crop worldwide, with an annual area of 221 Mha (million ha), and third in grain production, after maize and rice, with 771 Mt (million t) in 2021/22 [2]. It provides 21% of the calories and 20% of the protein for more than 4.5 billion people in 94 developing countries [3,4]. According to Choudhary et al. [5], wheat production needs to be increased annually by 2% in the coming years [6], representing double the rate of increase currently being achieved [7,8]. Wheat yields are, on average, around 20% lower than the current crop potential because of biotic and abiotic factors. Among the factors that affect wheat production and prevent the crop from realizing its yield potential are wheat rusts, which can cause significant yield losses in susceptible cultivars and can be difficult to control chemically in some areas of the world [9,10]. There are three types of rust affecting wheat: leaf rust, yellow rust, and stem rust, and all three can be most effectively controlled through genetic resistance. While fungicides can be excellent means of control, they are not always available in large wheat-growing areas worldwide and are not the most environmentally friendly option [11,12].

Wheat yellow (or stripe) rust is caused by the fungus *Puccinia striiformis* Westend. f. sp. *tritici* (Pst), a pathogen generally prevalent in temperate regions with cool and wet weather conditions (Figure 1) [13]. In the susceptible plant, chlorotic flecks typically appear after six to eight days from infection, whereas sporulation (characteristic yellow-orange uredinia appearing in long, narrow stripes on leaves) starts approximately from 12 to

14 days under favorable conditions [14], eventually leading to the desiccation of the leaves. Urediniospores germinate rapidly in optimum dew conditions and a temperature between 7 and 12 °C, with ideal disease development conditions (from infection to sporulation) between 12 and 15 °C [15]. Global losses inflicted by the disease can reach 20 Mt annually, representing an estimated US$ 1 billion [10,16,17]. Wheat stripe rust has been reported in more than 60 countries, and evidence suggests a significant geographical expansion in the last 50 years [16]. According to McIntosh et al. [18], 67 yellow rust resistance genes (*Yr1* to *Yr67*) and 42 with temporary designations have been reported. Since the 2000s, aggressive lineages of Pst adapted to higher-temperature climates have spread to warmer regions of the world that were previously less affected by this disease [19]. Although populations of yellow rust appear to be clonal in Europe, Australia, and North America, there are significant levels of genetic diversity within some pathogen populations [13]. Such polymorphic populations are evident in western China and Central Asia, consistent with their reported center of diversity in the Himalayan and nearby regions, where sexual recombination appears to be common [19,20]. Races from that region, such as Warrior or Warrior- reached Europe in recent years, bringing virulence to important resistance genes present in European wheat cultivars [21]. These linages were reported in Spain starting in 2014 and, more specifically, in the southern region of the country in 2015 [11].
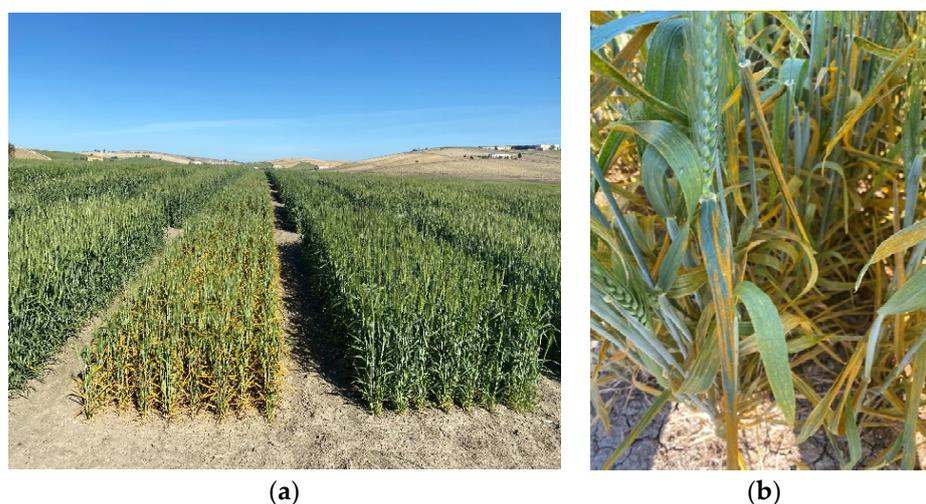

(**a**)   (**b**)

Figure 1. Yellow rust in bread wheat cultivars at the Jerez field (2022). (**a**) Susceptible (left, cv. Califa) vs. resistant bread cultivar (right, cv. Conil) in field plots; (**b**) Symptoms on bread wheat susceptible plants (leaves).

Wheat stem rust, caused by the basidiomycete *Puccinia graminis* Pers. f. sp. *tritici*, was historically considered the main wheat rust disease due to its devastating impact on grain yield and quality, in some cases even with losses of more than 50% (Figure 2) [22,23]. Stem rust did not appear frequently in European countries in recent decades, thanks to effective control, starting in the 1950s–1960s [24], resulting from the widespread use of host resistance (*Sr2*, *Sr31*), the eradication of the alternate host, common barberry, and the release of early maturing cultivars descending from the Green Revolution [25]. In Eastern Africa, one of the most stem rust-prone areas in the world, the pathogen was well under control thanks to the deployment of the *Sr31* gene, which was very effective against the predominant stem rust races of the time [26]. However, the race TTKSK (syn. Ug99), detected in Uganda in 1998 and its subsequent variants, defeated the effectiveness of many designated and undesignated resistance genes, and losses due to Ug99 were reported to be as high as 90% [27]. Many *Sr* genes originated from tetraploid wheats (*Sr7a*, *Sr8b*, several alleles of *Sr9*, *Sr11*, *Sr12*, *Sr13*, *Sr14*, *Sr17*, and *Sr8155-B1*) [28,29]. Several of these genes are widely used in bread and durum wheat, contributing to the successful control of stem rust worldwide [30]. The *Sr13b* gene is a major basis for stem rust resistance in durum wheat

worldwide [22,31,32]. New stem rusts from different lineages have emerged in East Africa. Race TKTTF resulted in epidemics in Ethiopia in 2013, with yield losses close to 100% on the most grown cultivar, Digalu [33]. In 2013, wheat stem rust reappeared at several locations in Germany [34], along with sporadic incidences in southern Denmark, eastern Sweden, and the United Kingdom [35]. A more widespread outbreak was reported in Sicily in 2016, where thousands of hectares of both bread and durum wheat were affected [36]. Analyses of infected plant samples from Sicily suggested the presence of a new race in Europe, TTRTF [36], which carried virulence to *Sr13b* and is of particular relevance for durum wheat [37]. In southern Spain, this disease has been detected regularly since 2018, but with low severity. Stem rust was also detected, with higher severity, in the irrigated off-season wheat trials (August–September) in the south (Conil de la Frontera, Cadiz) and northeast of Spain (Lleida).
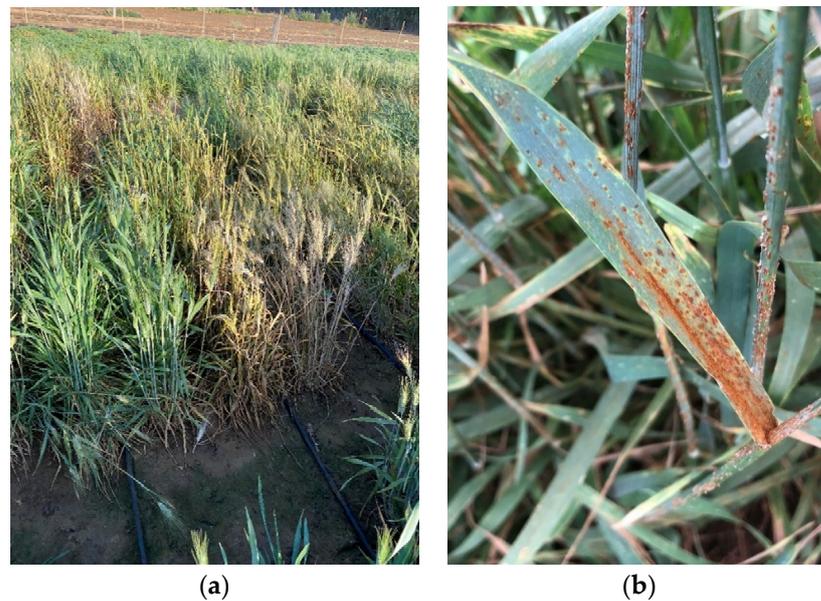

(**a**)　　　　　　　　　　　　　　　　　　(**b**)

Figure 2. Stem rust in durum wheat cultivars at the Conil field (2023). (**a**) Resistant (left, cv. Amilcar) vs. susceptible bread cultivar (right, cv. Athoris), in field plots; (**b**) Symptoms on susceptible plants (leaves and stems).

Given the increasingly threatening incidences of yellow and stem rust in Spain, there is a need to characterize and understand the virulence spectrum of the local lineages of these pathogens. This objective was addressed through field testing of differential lines and main cultivars in locations where both diseases occurred naturally during the period from 2016 to 2022 by sampling and subsequently analyzing several single pustule isolates of both pathogens collected from several locations during these seven years.

2. Materials and Methods

2.1. Locations, Plant Material, and Experimental Setup

This research was conducted at experimental sites used by Agrovegetal, a local breeding company, at four locations in southern Spain, namely, Escacena del Campo, near Huelva (37°27′20″ N; 6°21′49″ W), Jerez de la Frontera near Cadiz (36°42′14″ N; 6°10′11″ W), Ecija near Sevilla (37°32′23″ N; 5°6′42″ W), and Conil de la Frontera near Cadiz (36°18′30″ N; 6°5′13″ W). The first 3 locations were used for evaluations related to yellow rust and were sown during the regular season (December planting), while the fourth was dedicated to evaluations related to stem rust, with a summer, off-season sowing in June under irrigation. Field trials were conducted under rainfed conditions in winter sowings and weekly irrigated at the off-season site of Conil. Rainfall during the wheat growing season (November–May) across the years of the present study is shown in Table 1. Experiments

were carried out from 2016 to 2022, relying exclusively on natural infections. In all trials, entries were sown in twin rows of 1 m length using 4 g of seed per plot. The susceptible checks, Califa for yellow rust, and McNair and Tocayo for stem rust, were planted around their respective nurseries. Tejada and Calero were planted as resistant checks for yellow and stem rust, respectively.

The set of commercial varieties for yellow rust evaluation was planted in a total of 21 location/year combinations, but only the 14 location/year combinations are reported herein, as the other seven were characterized by very low or absent natural disease incidence. The set of yellow rust differential lines was planted in a total of 19 location/year combinations, with only 15 of those reported as having a high disease incidence. The 52 wheat entries in the yellow rust nurseries were divided into two sets: the old set of 35 genotypes with different *Yr* genes and a collection of 17 near-isogenic lines in the Avocet background. The 100 wheat entries for stem rust evaluations included (1) 34 advanced durum breeding lines selected by CIMMYT for their different responses to the African races of stem rust in Kenya and Ethiopia; (2) 6 advanced breeding lines with known resistance gene(s) introgressed at CIMMYT through marker-assisted selection; (3) 20 cultivars, landraces, or other sources of resistance to the African races used in the CIMMYT breeding program; and (4) 40 stem rust differential lines used to identify races according to their virulence (Supplementary Table S1).

Rust severity was also assessed in additional field trials independently sown by the Agrovegetal company at the same four locations in southern Spain for both yellow rust and stem rust. These trials included the commercial varieties of bread wheat (Arthur Nick, Tejada, Conil, Escacena, Califa), durum wheat (Amilcar, Euroduro, Athoris, Don Ricardo, Calero), and triticale (Bondadoso, Valeroso, and Trujillo).

2.2. Pathogen Isolation

Yellow rust and stem rust differentials in all field trials were infected naturally. Samples of both rusts were sent every year to the Global Rust Reference Center (GRRC) in Denmark [38], where phenotypic and/or genetic characterizations of single pustule isolates were carried out on each sample to identify the local lineages and/or races.

Table 1. Monthly precipitation (mm) during the wheat growing season in the three locations used for rainfed field trials.

Season [1]	Month	Escacena	Écija	Jerez
2015/16	Nov.	76	28	91
2015/16	Dec.	147	110	115
2015/16	Jan.	20	15	50
2015/16	Feb.	82	24	62
2015/16	Mar.	112	42	131
2015/16	Apr.	79	35	71
2015/16	May	3	5	1
2015/16	**Total**	**519**	**259**	**521**
2016/17	Nov.	24	80	47
2016/17	Dec.	37	120	56
2016/17	Jan.	112	41	67
2016/17	Feb.	48	57	34
2016/17	Mar.	124	24	125
2016/17	Apr.	117	24	82
2016/17	May	2	10	4
2016/17	**Total**	**464**	**356**	**415**
2017/18	Nov.	88	76	79
2017/18	Dec.	98	103	90

Table 1. *Cont.*

Season [1]	Month	Escacena	Écija	Jerez
2017/18	Jan.	110	67	65
2017/18	Feb.	23	56	15
2017/18	Mar.	34	45	18
2017/18	Apr.	14	34	5
2017/18	May	1	2	1
2017/18	**Total**	**368**	**383**	**273**
2018/19	Nov.	67	45	88
2018/19	Dec.	78	68	111
2018/19	Jan.	104	99	159
2018/19	Feb.	132	98	77
2018/19	Mar.	63	74	56
2018/19	Apr.	76	65	68
2018/19	May	34	19	23
2018/19	**Total**	**554**	**468**	**582**
2019/20	Nov.	36	59	86
2019/20	Dec.	78	87	75
2019/20	Jan.	69	57	112
2019/20	Feb.	35	76	47
2019/20	Mar.	15	14	87
2019/20	Apr.	32	22	54
2019/20	May	4	3	16
2019/20	**Total**	**269**	**318**	**477**
2020/21	Nov.	103	117	121
2020/21	Dec.	98	54	109
2020/21	Jan.	54	13	89
2020/21	Feb.	76	45	56
2020/21	Mar.	21	24	79
2020/21	Apr.	12	13	28
2020/21	May	5	2	10
2020/21	**Total**	**369**	**268**	**492**
2021/22	Nov.	95	107	113
2021/22	Dec.	86	68	70
2021/22	Jan.	72	89	53
2021/22	Feb.	61	75	97
2021/22	Mar.	43	48	65
2021/22	Apr.	60	46	51
2021/22	May	8	5	8
2021/22	**Total**	**425**	**438**	**457**

[1] Data from RIA [39]. Escacena data were from the La Palma del Condado station.

2.3. Plant Disease Evaluation

To determine the disease severity in each plot and the field response (type of reaction to the disease), the percentage of foliar area covered by uredinia, according to the modified Cobb scale, was recorded. In addition, the response to infection was scored according to Roelfs et al. [40]. The disease severity scores were converted into the AUDPC (Area Under Disease Progress Curve) [40], using the following formula:

AUDPC = D $[\frac{1}{2}$ (Y1 + Yk) + Y2 + Y3 + . . . + Yk-1], where D is the time interval (days between readings) and (Y1 + Yk) is the sum of the first and last scores. (Y2 + Y3 + . . . + Yk-1) is the sum of all intermediate disease scores. The AUDPC for each entry was converted into a relative percentage by using the AUDPC of the check, Atil/Local Red, as 100% [41]. To determine the difference between susceptible and resistant lines, a threshold of 30% (stem rust) or 20% (yellow rust) severity relative to the most susceptible line in each trial was set.

3. Results

3.1. Yellow Rust Responses

Based on a combination of DNA analyses conducted at the GRRC from samples collected as part of this study, observations made herein on the set of differentials, and independent reports from different Spanish locations, it appears that the predominant yellow rust lineages present in Spain are PstS4, PstS7, PstS10, PstS13, and PstS14. The virulence spectra of these lineages are shown in Table 2, according to the classification from GRRC [38].

Table 2. Virulence on *Yr* genes of the main lineages of yellow rust in Spain, according to GRRC.

Lineage	Virulence on *Yr* genes																			Name
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*15*	*17*	*24*	*25*	*27*	*32*	*Sp*	*Avs*	*Amb*	
PstS4		x				x	x	x		x			x							Triticale 2006
PstS7	x	x	x	x		x	x		x			x		x		x	x	x	x	Warrior
PstS10	x	x	x	x		x	x		x			x		x		x	x	x		Warrior -
PstS13		x				x	x	x	x								x			Triticale 2015
PstS14		x	x			x	x	x	x			x		x		x	x	x		

Virulence on *Yr1* and *Yr4* distinguishes between Warrior and the rest of the lineages, and virulence on cultivar Ambition is between PstS7 and PstS10. If virulence is observed in *Yr8*, the Warrior lineage is not present. If virulence on *Yr8* is observed in addition to virulence on *Yr10* and *Yr24*, lineage PstS4 is most likely present. Virulence on *Yr8*, but not on *Yr10* or *Yr24*, would be an indicator of the presence of PstS13 or PstS14. To distinguish between both latter lineages, we must observe the virulence in the differentials *Yr3*, *Yr17*, *Yr25*, *Yr32*, and *YrSp.* Virulence would indicate the presence of PstS14; avirulence would indicate the presence of PstS13.

During the period of the present study, the lineages detected in the various experiments varied from year to year and between locations within years. In Tables 2 and 3 below, the predominant lineages detected are shown for each combination location/year. In 2016, the first year the differential sets were evaluated as part of the present study, analyses conducted at the GRRC on samples collected from highly infected commercial bread wheat fields indicated that PstS10 (Warrior-) was the major lineage present. In 2017, no samples were sent to the GRRC, but from the set of differential responses, it could be deduced that the PstS13 and PstS14 races were the prevailing lineages present. In 2018, only Warrior- was detected in the field trials of the present study, but the presence of PstS7, PstS13, and PstS14 was independently reported in the country. In 2019, both differentials and DNA analysis again indicated the predominance of Psts13 and PstS14, with Warrior- also present. In 2020, only data from the Jerez location could be taken and indicated the presence of Warrior-, PstS13, and PstS14. In 2021, the presence of PstS14 was inferred from the evaluation of the set of differentials, and DNA analysis by GRRC [38] confirmed this assumption. In 2022, both results from the set of differentials and DNA analysis revealed the presence of PstS10 (Warrior-), PstS13, and PstS14.

Monitoring the reaction to yellow rust of key commercial cultivars of bread wheat, durum wheat, and triticale sown in southern Spain was initiated in 2016 by the Agrovegetal company. Results from these evaluations are presented in Table 3. While these evaluations relied exclusively on natural field infections, the generally high rust scores observed on the susceptible bread wheat cultivar Califa indicate that the environmental conditions, in most location/year combinations reported herein, were conducive to the development of the disease and that virulent races were always present. Similarly high infections, to a somewhat lesser extent, were observed on the susceptible bread wheat cultivar Escacena. The bread wheat cultivar Conil exhibited low to intermediate susceptibility reactions, mostly when Pst10 was present. The other two bread wheat cultivars, Tejada and Arthur Nick, consistently exhibited high levels of field resistance and virtually total immunity, as did all the

commercial durum wheat and modern triticale cultivars (Bondadoso and Valeroso). They were not at all affected by any of the lineage combinations present during the period of this study, in spite of the conducive environmental conditions. Very minor signs of infection, in some location/year combinations, were observed on commercial durum wheat cultivars starting in 2019 and on the triticale cultivar Bondadoso in 2022. However, infection levels were never high enough to be considered indicative of failing genetic resistance. The only case of emerging non-complete resistance, possibly intermediate susceptibility, is in the case of the durum cultivar Athoris. Old (from the 1980s) and now commercially obsolete, the triticale cultivar Trujillo was characterized, in most location/year combinations, by intermediate to high infection levels.

Table 3. Yellow rust severity data on commercial durum, bread wheat, and triticale cultivars grown in southern Spain.

	ESC 16 [1]	ECI16	JER16	ESC 17	JER17	ECI18	ESC19	ECI 19	JER19	JER20	JER21	ESC22	ECI 22	JER22
Yellow rust lineage [2]	PstS10	PstS10	PstS10	PstS13 PstS14	PstS13 PstS14	PstS10	PstS13 PstS14	PstS13 PstS14	PstS13	PstS10 PstS13 PstS14	PstS14	PstS10 PstS13	PstS10	PstS14 PstS13
Durum wheat cultivars														
Amilcar	0	0	0	0	0	0	0	0	2	1	2	0	0	5
Euroduro	0	0	0	0	0	0	0	0	1	1	1	0	0	3
Athoris	0	0	0	0	0	0	3	0	7	5	9	0	0	22
D. Ricardo	0	0	0	0	0	0	0	0	1	0	1	0	0	1
Calero	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Bread wheat cultivars														
Artur Nick	0	7	2	0	0	0	0	0	0	1	0	0	0	0
Tejada (rc) [3]	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Conil	1	23	30	0	0	30	27	0	13	7	0	3	14	0
Escacena	1	10	63	80	50	40	80	70	35	50	28	35	53	70
Califa (sc) [3]	9	47	87	90	73	80	90	90	70	90	43	90	73	90
Triticale cultivars														
Bondadoso	0	0	0	0	0	0	0	0	0	0	0	1	1	6
Valeroso	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trujillo	0	5	20	27	20	10	43	57	22	0	12	30	33	33

[1] Locations: ESC = Escacena del Campo; ECI = Ecija; JER = Jerez de la Frontera. The number indicates the year of trial (e.g., 16 = 2016). [2] Pst lineages according to GRRC [38]. [3] Califa and Tejada were the susceptible (sc) and resistant (rc) checks.

To obtain a preliminary indication of what type of genetic option could be useful in breeding against the yellow rust lineages prevailing in southern Spain, two sets of lines with known genes of resistance were evaluated at three locations for their adult plant field reaction from 2016 to 2022. An analysis of variance with genotypes and locations was performed, using years within locations as replications, and is presented in Table 4. Genotype and location were clearly important factors of variation, but the interaction between them was not significant, indicating that the genotypes' severity scores were rather correlated between locations.

The results of the severity scores on the sets of differential lines are presented in Table 5. The presence of *Yr2*, *Yr6*, and *Yr9* in a monogenic state was associated with high levels of field susceptibility in all years at all locations, with variability depending on the line's background in some cases, but independently from the lineages present. Of the 52 lines tested, 14 were resistant at all locations and years, even when weather conditions were very favorable to yellow rust, as indicated by the high severity scores on the susceptible lines. These lines must have been resistant to PstS10, PstS13, and PstS14 since these lineages were present at these locations during the time of the present study.

Table 4. Analysis of the variance of yellow rust severity scores of a set of 52 differential lines evaluated under natural field infection at three southern Spain locations from 2016 to 2022, using years of evaluation within location as replicates.

Source	Sum of Square III Type [1]	df	Mean Square	F	Significance
Corrected model	206,729	155	1334	6.1	0.000
Intersection	184,823	1	184,823	845.2	0.000
Genotype	176,911	51	3469	15.9	0.000
Location	12,019	2	6010	27.5	0.000
Genotype Location	23,074	102	226	1.0	0.398
Error	124,857	571	219		
Total	509,127	727			
Total corrected	331,586	726			

[1] R square = 0.623 (R corrected square = 0.521).

Consistent field resistance in the form of near immunity over years and locations and over genetic background (some genes were present in lines of different backgrounds) was associated with the presence of *Yr4*, *Yr5*, *Yr10*, *and Yr15*. Differential lines carrying the *Yr24/Yr26* and *Yr27* genes were of particular interest since they should display horizontal resistance. Indeed, the lines known to harbor these genes exhibited low levels of infection with slow disease progress, never reaching levels of high susceptibility in all trials, as slow-rusting genotypes theoretically should.

In some cases, lines containing the same genes displayed contrasting responses, depending on the genetic background carrying them. This was the case for lines carrying *Yr7*, *Yr8*, and *Yrsp*. The rest of the genes were associated with field reactions typical of race-specificity, with high infection in particular seasons and locations, depending on the lineages present, and with no infection in other instances. This was the case of lines carrying genes *Yr1*, *Yr3*, *Yr8* (in one background), *Yr17*, *Yr18*, *Yr25*, *Yr33*, and *YrA*.

3.2. Stem Rust Responses

Stem rust reaction in a set of 100 genotypes (2018–2022), as well as a set of commercial cultivars (2021–2022), was recorded in off-season summer trials, under irrigation, at the southern Spain location of Conil de la Frontera.

Results of race and DNA analyses indicate the presence of two races across the five testing seasons involved in the present work: Clade IV-B (TKTTF) and Clade IV-F (TKKTF), which is consistent with the GRRC information for Spain (Table 6). The key gene to differentiate Clade IV-B and Clade IV-F from the Sicilian race is *Sr13*. The Spanish races should be avirulent on this gene, while the Sicilian race is virulent. In the present study, lines carrying *Sr13* genes displayed resistance, albeit partial in its expression in some cases. Similarly, races from Clade IV-B and Clade IV-F could be differentiated from the Ug99 lineage based on virulence on both *Sr31* and *Sr36*. The line harboring *Sr31* was resistant to the Spanish races, while the line with *Sr36* shows susceptibility, confirming that the races present in this study were of Clade IV-B and Clade IV-F.

In Table 7, an analysis of variance is shown using genotype as a factor and years as replications. The differences between the lines were significant with respect to their severity, showing that the ranking of severity or resistance due to a particular gene remained similar over the years of the study.

Table 5. Yellow rust severity scores in two sets of differential lines evaluated under natural field infection at three southern Spain locations between 2016 and 2022.

Line	ESC 16 [1]	ESC17	ECI 17	JER 17	ECI 18	ESC 19	ECI 19	JER 19	JER 20	ESC21	ECI 21	JER 21	ESC 22	ECI 22	JER 22
	PstS10 [2]	PstS13 PstS14	PstS13 PstS14	PstS13 PstS14	PstS10	PstS13 PstS14	PstS13 PstS14	PstS13	PstS13 PstS14 PstS10	PstS13	PsTs14	PsTs14	PstS10 PstS14	PstS10	PstS14 PstS13
Yr1/6×Avocet S	30	0	0	0	40	0	0	0	2	0	0	0	30	30	0
Yr5/6×Avocet S	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Yr6/6×Avocet S	60	30	40	20	50	50	40	50	60	40	60	70	70	70	50
Yr7/6×Avocet S	60	20	40	50	50	60	40	20	70	20	50	60	70	70	70
Yr8/6×Avocet S	5	20	8	10	0	30	20	15	50	15	30	15	30	0	20
Yr9/6×Avocet S	50	40	80	50	60	70	70	10	60	20	70	60	60	90	70
Yr10/6×Avocet S	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Yr15/6×Avocet S	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Yr17/6×Avocet S	40	2	40	5	40	5	30	0	10	0	25	20	5	50	0
Yr18/6×Avocet S	40	5	80	30	30	30	40	0	20	5	60	70	15	60	30
Yr24/6×Avocet S	5	0	10	10	10	15	20	10	30	0	10	10	0	0	15
Yr26/6×Avocet S	15	2	20	5	5	5	15	5	20	0	10	20	0	0	5
Yr27/6×Avocet S	10	1	7	2	5	15	5	5	5	0	20	20	5	5	10
Yr32/6×Avocet S	50	20	40	10	20	40	20	10	30	2	40	70	10	50	20
YrSp/6×Avocet S	30	2	3	2	20	40	5	5	10	0	50	30	5	10	5
Jupateco R (Yr18)	5	5	15	15	5	20	10	0	10	5	20	30	0	5	40
Jupateco S	40	20	15	40	10	70	40	20	40	10	40	80	0	30	80
Avocet R (YrA)	30	20	20	40	5	80	50	40	50	5	60	90	60	30	70
Avocet S	70	40	80	50	15	90	70	10	60	10	70	70	40	90	60
Egret	15	40	10	30	20	70	50	5	15	15	50	70	0	40	80
Bowerbird	10	10	15	25	15	20	40	5	30	15	40	20	2	40	80
Brennan	5	5	2	10	5	10	5	0	0	0	40	10	0	10	5
Crusader	5	0	2	2	5	2	15	0	2	0	5	5	0	5	2
H45	30	30	50	60	30	20	80	2	20	20	60	60	2	80	60
Chinese 166 (Yr1)	15	1	1	0	5	0	1	0	2	0	0	0	2	50	5
Lee (Yr7)	15	2	30	50	10	40	20	0	50	2	50	20	5	30	70
H. Koben (Yr2, Yr6)	20	1	40	40	5	50	15	15	60	2	40	30	10	30	40
Vilmorin 23 (Yr3)	0	0	20	15	0	10	2	0	0	0	0	5	0	0	0
Moro (Yr10)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Strubes Dickkopf	1	0	30	10	1	40	10	0	0	0	40	10	0	10	20
Suwon 92/Omar	15	10	60	60	20	70	30	0	0	20	60	10	80	60	70
Clement (Yr2, Yr9)	5	0	30	5	1	15	2	0	0	2	15	2	20	0	5
T. spelta (Yr5)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hybrid 46 (Yr4)	0	0	0	0	0	0	0	0	0	0	0	0	0	10	5
Reichersberg42 (Yr7)	0	0	0	0	0	5	10	0	0	0	5	2	2	2	0
H. Peko (Yr2, Yr6)	0	0	15	5	0	30	20	2	15	0	5	15	0	0	20
Nord Desprez	10	2	5	5	5	30	50	10	10	0	40	30	0	5	20
Compare (Yr8)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
Carstens V (Yr32)	2	2	5	5	0	10	50	0	5	0	30	10	0	15	10

Table 5. *Cont.*

Line	ESC 16 [1]	ESC17	ECI 17	JER 17	ECI 18	ESC 19	ECI 19	JER 19	JER 20	ESC21	ECI 21	JER 21	ESC 22	ECI 22	JER 22
	PstS10 [2]	PstS13 PstS14	PstS13 PstS14	PstS13 PstS14	PstS10	PstS13 PstS14	PstS13 PstS14	PstS13	PstS13 PstS14 PstS10	PstS13	PsTs14	PsTs14	PstS10 PstS14	PstS10	PstS14 PstS13
S. Prolific (*YrSp*)	1	0	2	5	-	0	5	0	0	0	0	0	0	5	m
Heines VII	1	5	0	5	10	50	15	0	0	2	30	10	2	20	m
Avocet R (*YrA*)	30	30	5	30	20	90	70	10	50	30	70	80	30	60	70
Kalyansona (*Yr2*)	30	10	10	10	10	50	40	15	25	5	70	60	10	50	70
Trident (*Yr17*)	60	0	4	10	40	15	10	2	10	0	5	10	50	10	20
Yr15/6×AvS	0	0	1	0	0	0	0	0	0	0	0	0	0	2	0
Hugenoot (*Yr25*)	20	2	10	5	10	80	20	0	10	0	60	30	0	15	50
Selkirk (*Yr27*)	5	0	0	0	0	0	0	0	0	0	0	0	0	0	20
EGA Gregory (*Yr33*)	-	2	15	5	10	5	20	0	2	0	10	30	0	2	30
Ellison	10	0	1	1	10	10	15	0	2	0	5	5	2	0	0
Binnu	30	0	2	5	40	20	40	0	15	0	50	5	15	5	5
Breakwell	10	1	0	1	5	40	30	0	30	0	0	0	30	0	40
Tobruk	0	0	0	0	5	2	0	0	0	0	0	0	5	0	0

[1] Locations: ESC = Escacena del Campo; ECI = Ecija; JER = Jerez de la Frontera. The number indicates the year of trial (e.g., 16 = 2016). [2] Pst lineages according to GRRC [38].

Table 6. Virulence spectrum of the two main races of stem rust reported in southern Spain compared to that of a race from the Ug99 lineage and the Sicilian race.

Race Name (Origin)	Virulence on Known *Sr* genes Present in Wheat Differential Lines													
TKKTF (Spain)	5	21	*9e*	*7b*	-	6	*8a*	*9g*	-	*9b*	30	17	*9a*	*9d 10 Tm - - 38 McN -*
TKTTF (Spain)	5	21	*9e*	*7b*	-	6	*8a*	*9g*	36	*9b*	30	17	*9a*	*9d 10 Tm - - 38 McN -*
TTKSK (Ug99 lineage)	5	21	*9e*	*7b*	11	6	*8a*	*9g*	-	*9b*	30	17	*9a*	*9d 10 - - 31 38 McN -*
TTRTF (Sicily)	5	21	*9e*	*7b*	11	6	*8a*	*9g*	36	*9b*	-	17	*9a*	*9d 10 Tm - - 38 McN 13*

Table 7. ANOVA Table for stem rust AUDPC for 100 genotypes evaluated for 5 years (years considered replications) under natural infection at Conil, in the south of Spain, during the summer counter-season under irrigation.

Source	Sum of Squares	Df	Mean Square	F-Ratio	*p*-Value
Between groups	3.59	99	3.63	4.0	0.0000
Within groups	3.66	400	914,606		
Total (Corr.)	7.25	499			

　　　With regard to the reaction of commercial cultivars evaluated for two seasons (2021 and 2022), the results presented in Table 8 indicate that cultivars Athoris and Euroduro were susceptible. Cultivar Don Ricardo exhibited an intermediate and possibly useful level of resistance, while cultivars Amilcar and Calero show high levels of resistance. In bread wheat, cultivar Tocayo was very susceptible, as expected given that it was considered the susceptible check, and cultivar Arthur Nick exhibited a reaction that could be considered moderately to fully susceptible. Cultivar Tejada was characterized by an intermediate level of resistance, which was certainly interesting and potentially useful. Finally, the cultivars Conil and Escacena were found to have high levels of resistance to the prevailing races. The two modern triticale cultivars, namely Bondadoso and Valeroso, were completely immune to stem rust, while the old and obsolete cultivar Trujillo exhibited an intermediate level of resistance, clearly allowing for some development of the pathogen.

Table 8. Stem rust AUDPC data calculated for commercial cultivars in off-season field trials (summer under irrigation) under natural infection at the southern Spain location of Conil.

Cultivar	AUDPC		
	2021	**2022**	**Mean**
Durum Wheat			
Amilcar	14	0	7
Euroduro	945	780	863
Athoris	1400	1135	1268
Don Ricardo	70	78	74
Calero (rc) [1]	14	0	4
Bread Wheat			
Arthur Nick	574	642	608
Tejada	35	215	125
Conil	0	78	39
Escacena	0	16	8
Tocayo (sc) [1]	1260	1572	1416
Triticale			
Bondadoso	0	0	0
Valeroso	0	0	0
Trujillo	14	175	95

[1] Tocayo and Calero were susceptible (sc) and resistant (rc) checks.

　　　In order to assess the genetic resistance options available against the Spanish stem rust races, we have assembled a set of 100 genotypes, including the stem rust differential series, some breeding lines with known reactions to the African races of stem rust (based on CIMMYT data from the precision phenotyping platforms established in Ethiopia and Kenya), others with known resistance genes introgressed through marker-assisted selection at CIMMYT, and, finally, some sources of resistance used in the CIMMT program. This set was evaluated under natural infection during the counter-season under irrigation at the southern Spain location of Conil for five seasons (2018 to 2022). It was also evaluated at the Jerez location in 2022, when stem rust was observed at high incidence levels during the main

season. The results of the AUDPC calculated for each genotype are shown in Supplementary Table S2. In all seasons, the inoculum pressure was strong, the environmental conditions were favorable, and the stem rust severity was ultimately high, allowing for reliable discrimination between genotypes without the possibility of escape.

In the first sub-group of lines, genotypes 1 to 34, we can observe a general coincidence between the reaction to the Spanish races over the five seasons and the summary information from data collected in Ethiopia and Kenya for the same material. Several exceptions are indeed observed, but the general tendency is present. Lines with good resistance to the African races in Ethiopia and Kenya are, on average, more resistant in Spain than those that are susceptible to the African races. As the resistance to the African races decreases, the susceptibility to the Spanish races increases dramatically.

Within the lines with specific genes of resistance introgressed through marker-assisted selection, the results indicate that *Sr25* (line 35) and *Sr22* (line 38), in monogenic states, provide good to intermediate levels of resistance to the Spanish races, depending on the season. Lines with both *Sr22* and *Sr25* present together (lines 39 and 40) were characterized by the highest levels of resistance among this group. Lines with *Sr38* have shown variable reactions, from complete resistance (line 1) to intermediate resistance (line 36) to outright susceptibility (line 37). Among the sources identified by CIMMYT over the years for their resistance to the Ethiopian races (lines 41 to 60), some maintained high levels of resistance to the Spanish races, including modern cv. Amria (Morocco), CIMMYT breeding line 45, and old landrace type Iumillo (line 42). Another accession of Iumillo (line 54) shows high infection levels indicative of susceptibility, possibly indicating the existence of different biotypes within the landrace or misclassification of the seed source. Among the same group, other sources with good resistance in Ethiopia were characterized by intermediate, possibly interesting, levels of resistance to the Spanish races, including breeding line 46 and modern cvs. Kronos (USA), Westbred 881 (USA), Calero (Spain) and Carpio (Spain), old and tall cvs. Boohai (Ethiopia), Trinakria (Italy), and landrace type Khapli (India). One surprising result was the high level of susceptibility of landrace type Reichenbachii (Turkey), which is one of the sources of resistance most effective against the African races in Ethiopia and Kenya and used significantly in the CIMMYT breeding program. Finally, sources of resistance gene *Sr47* (USA), namely lines 56 to 58, exhibited promising levels of resistance, consistent with their field adult-plant reaction to the African races in Ethiopia. Among the stem rust differential lines, only line 96, carrying *Sr32*, exhibited high levels of resistance, albeit variable over years. Lines carrying genes *Sr27*, *Sr33*, and *Sr35* had intermediate resistance levels on average, again highly variable between years. The rest of the differential lines were characterized by intermediate to very high susceptibility levels.

4. Discussion

In southern Spain, wheat is produced primarily under rainfed, often drought-prone, conditions with highly variable, but generally low yields, depending primarily on the amount and distribution of rainfall during the cropping season (Table 1). In such an extensive and relatively high-risk production system, the economic viability of any crop requires the maximization of yield potential expression under the prevailing climate conditions in any given year and the minimization of production costs. Genetic control of rust diseases, possibly the biotic factor most likely to prevent maximum yield expression in the region, does both. Not only is it key to the economic viability of wheat, but it also reduces the environmental footprint of the crop and the entire production system. The first step in achieving effective and durable genetic control of rust diseases is the thorough characterization of the lineages/races of the pathogens present in the region and the adjacent areas. The second step involves assessing the previously characterized genetic options available (known resistance genes) as well as non-characterized resistance sources for their suitability against the prevailing lineages/races of the pathogen. The objectives of the present study were to collect such information and provide such an assessment for the south of Spain, with the ultimate goal of guiding breeding and variety selection programs serving the

region in their efforts to develop and deploy cultivars with effective and durable resistance to yellow and stem rust.

The present study relied exclusively on the natural infection of both rusts. This ensured that pathogen sampling for race analyses and genotyping involved lineages/races that were representative of those actually present and relevant in the region. The long duration of the study (seven years) also contributes to the robustness and relevance of our results. The results reported herein were exclusively from location/year combinations where disease incidence was sufficiently high and uniform to enable reliable differentiation between genotypes. Location/year combinations with too little or no disease incidence were not considered.

Results from samples taken from plots from the present study indicate that yellow rust lineages PstS10 (Warrior-), PstS13, and PstS14 prevailed in southern Spain in recent years. Reports from other areas of Spain also refer to the presence of PstS4 and PstS7 (Warrior). Some of these lineages were known to be present in the Guadalquivir valley [11], but they did not cause serious epidemics until 2015, when an outbreak in northern Spain was attributed to the Warrior race. This race, previously described in Denmark, France, England, Germany, and Sweden, circumvented the resistance of most commercial wheat cultivars in these countries. It is reported to have originated in the Himalayan region of Pakistan, subsequently spreading rapidly throughout the wheat area of Europe [42,43]. The region where our research was conducted was in fact one of the last to be reached by this race, probably because it was in the far southwestern corner of Europe with infrequent occurrences of favorable weather conditions. The newer lineages, PstS13 and PstS14, are probably those to pay more attention to, as their presence was generally associated with a slightly higher disease incidence on commercial cultivars evaluated in this study. This was particularly visible in the commercial durum wheat cultivars, with the appearance of some incidence, albeit at very low levels, during the last years of the study as PstS13 and PstS14 became more present. PstS14 was detected in 2016 in Morocco and Sicily (Italy) by the GRRC. This lineage (virulent on *Yr2*, *Yr3*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17*, *Yr25*, *Yr32*, and *YrSp*) was first detected in Spain in 2017 [38].

Monitoring the reaction of commercially grown cultivars of bread wheat, durum wheat, and triticale has been useful to provide an assessment of the risk facing the southern Spain wheat production sector and how urgent it is to mitigate it. The fact that highly resistant, locally adapted, and commercially successful cultivars exist for the three crops is a very positive outcome, as they provide immediate genetic solutions to the threat of yellow rust on wheat production in southern Spain, solutions that seed companies and growers have successfully deployed and that breeding entities could use as sources of resistance in their improvement programs.

While the number of commercial cultivars of each crop evaluated in this study may be too small to make generalized inferences on the resistance to yellow rust of the different species, our results clearly indicate that the durum wheat and triticale cultivars evaluated were much less affected by the southern Spanish lineages of yellow rust than bread wheat. Durum cultivars were, in most instances, immune to the disease, and even when some symptoms were present, generally starting in the 4th year of the study, they amounted to very low infection scores; too low to consider them susceptible. One possible exception may be cultivar Athoris, which closely approached a susceptible score, but only at one location in the last year of the study. Recently released commercial triticale cultivars also maintained near-immunity or immunity to yellow rust over the duration of the study. The old cultivar Trujillo, selected from a group of CIMMYT lines released in the early 1980s in several countries worldwide, has lost its resistance to yellow rust (and other diseases) decades ago in Mexico, Spain, and Tunisia (M.S. Gharbi, personal communication). While this was reflected during the duration of this study, Trujillo susceptibility levels remained inferior to those observed in the susceptible bread wheats. While resistant bread wheat cultivars Tejada and Arthur Nick exhibited resistance levels similar to those of resistant durum

wheat and triticale cultivars, susceptible cultivars Califa and Escacena were characterized by levels of susceptibility unseen in the other two species.

The generally better and, to a certain extent, more frequently seen genetic resistance of durum wheat and triticale compared to bread wheat observed in the small sample of cultivars evaluated in this study has been observed more widely in the south of Spain and worldwide. Among the germplasm of the three crops annually sent by CIMMYT to southern Spain for the past 20 years, highly susceptible durum or triticale lines were extremely rare, but highly susceptible bread wheat lines were regularly observed, albeit at a low frequency. Unpublished data collected from international nurseries distributed globally by CIMMYT to numerous cooperators worldwide indicate that the great majority of the CIMMYT durum wheat germplasm exhibits virtually generalized high-level resistance almost everywhere it is tested. On the other hand, a significant, even though generally low, frequency of susceptibility can be seen in the CIMMYT bread wheat germplasm distributed in the same international nurseries.

The results of this study and the information discussed in the above paragraph may objectively indicate less of an emergency to work on enhancing the genetic basis for resistance to yellow rust in durum wheat. However, recent developments in the pathogen's populations in Chile should serve as a call for vigilance for breeding programs working with this crop. In 2019, a never-before-seen yellow rust epidemic appeared in commercial durum wheat fields in central Chile, and, in the experimental fields of the national program, 70% of the lines evaluated as part of CIMMYT's international durum wheat screening nursery exhibited, atypically, high levels of susceptibility to the disease (I. Matus, INIA-Chile, personal communication), while the same germplasm maintained its virtually generalized resistance to yellow rust everywhere it was tested outside of Chile (data not published). Thousands of breeding lines from the CIMMYT program were subsequently sent to INIA Chile for evaluation of their reaction to the local race of yellow rust, confirming the very high frequency (close to 70%) of susceptible lines within this germplasm group. While there is no evidence so far that this race made it out of Chile, durum wheat breeders in the Mediterranean basin, including those providing germplasm to southern Spain, need to assess the risks associated with such a devastating race appearing in their region. The collaboration between INIA-Chile and CIMMYT to address this threat to the local durum production has also resulted in a proactive preventive breeding effort by CIMMYT to increase the frequency of resistance to the Chilean race in its germplasm pool.

Since the 1970s, a significant part of the spring bread wheat cultivars sown in southern Spain originated from the Mexico-based CIMMYT breeding program. A wide range of *Yr* genes were deployed by this program, such as *Yr6*, *Yr9*, and *Yr17*. These were quite common in the past, but their resistance has been overcome by new races. On the other hand, *Yr27*, a gene still used by CIMMYT and most likely present in the bread wheat material planted in Andalusia, is effective against the Warriors lineage [43–45]. In this study, this gene has provided intermediate resistance. The other genes that are worth mentioning in terms of their usefulness against the southern Spanish lineages are *Yr5*, *Yr10*, and *Yr15*, whose presence in the differentials series was associated with an immune reaction for the duration of the study. *Yr5*, located on chromosome 2BL, is effective against most yellow rust races worldwide and originated from *T. spelta* [46]. *Yr15* is another gene that is performing well all over the world, including against the Warrior races. It originated from wild emmer (*T. turgidum* ssp. *dicoccoides*) and was transferred to chromosome 1BS of bread and durum wheat. It has been recently cloned and found to encode a compound protein kinase-pseudokinase in tandem [47]. Because of their global effectiveness against most yellow rust races worldwide and the availability of reliable molecular markers that could facilitate their introgression in elite germplasm, these genes represent very attractive protective solutions and are bound to be used extensively by breeding programs worldwide and deployed over large production areas across continents. This could theoretically expose them to being circumvented by rapidly evolving pathogen populations, as seen in the past for many such genes. It is therefore useful to keep characterizing new sources of effective

resistance to yellow rust to facilitate their use in breeding and provide wheat breeders and variety developers with a more ample and diversified set of genetic options to mitigate the threat of yellow rust.

Unlike yellow rust, stem rust is not yet a commercial problem in Spain; however, in recent years, the pathogen occasionally appeared at the end of the regular wheat growing season in the south of the country. Historically, when the very late-maturing and generally stem-rust-susceptible landraces were grown extensively around the Mediterranean basin, the disease was not uncommon. It was a scenario of a susceptible host maintaining green tissue well into the late spring season (May–June), providing the late-appearing pathogen with favorable conditions to successfully infect the crop. With the complete displacement of these landraces by the earlier, fast-maturing semi-dwarf wheats from the Green Revolution, which were also mostly resistant to the older stem rust races in the region thanks to the effective genes *Sr2* and *Sr31*, the disease virtually disappeared and stopped being a yield-threatening concern. However, recent developments in the pathogen populations and more regular appearances of the disease around the Mediterranean basin, as presented in the introduction, have raised concerns and prompted us to consider stem rust as a potential emerging threat to wheat production in southern Spain.

All evaluations were again performed relying exclusively on natural infections that occurred during each of the five years of this study and developed into intense epidemics thanks to the weekly irrigations implemented for the summer sowing (off-season planting), which brings together green leaf tissue from susceptible hosts, a virulent pathogen, and favorable conditions for infection.

Based on the off-season field readings from this study and DNA analysis at the GRRC, the predominant groups present during the current study were Clade IV-B (TKKTF) and Clade IV-F (TKTTF) [38]. As reported by Abdedayem et al. [48], stem rust has recently reappeared in many parts of Europe, and Clade IV-B and Clade IV-F were found to be present in Spain, Italy, France, Morocco, and Tunisia. The presence of the Sicilian TTRTF race in southern Spain and during the present study was considered improbable as this race is virulent on *Sr13* [49], and the line harboring *Sr13* was always resistant (partial resistance) over the duration of this study. Molecular markers identified 37 strains collected in France in 2021 as part of the sampling campaign from RustWatch (European project coordinated by the GRRC aimed at characterizing the main rust races in Europe). All of them clustered into two genetic groups, namely Clade IV-B and Clade IV-F. These two groups were already detected in 2019 and 2020 in nurseries of the Île-de-France and in 2018 and 2019 in Italy. All this information strongly indicates that these are indeed the main stem rust races in Mediterranean Europe [50], and probably also in North Africa. According to DNA analyses of samples sent to the GRRC from all parts of Europe, the races of the Ug99 family have not reached Europe. This was confirmed in the case of southern Spain, as the local races and the Ug99 lineage differed in their virulence on *Sr24* and *Sr31* [51–53].

Another clear difference between yellow and stem rust resides in the level of expression of their resistance. While resistance to yellow rust was often expressed as a fully immune reaction (complete absence of pustules), such a reaction was very rare for stem rust (1 genotype out of the 100 evaluated in the present study), including when genes known to have major effects, such as *Sr22*, *Sr25*, and the combination of both, were present. None of the differential lines exhibited a near-immune reaction, indicating that no known *R*-gene would in fact provide such a "clean" reaction. This is consistent with the observations made by Ethiopian and CIMMYT scientists over many years, screening thousands of lines in Ethiopia at the Debre Zeit experimental site hosted by the Ethiopian Institute of Agricultural Research (EIAR). This different expression of resistance to stem rust is important to point out, as breeding programs around the Mediterranean basin have traditionally selected completely immune types with regard to leaf and yellow rusts. Those contemplating local resistance breeding efforts against stem rust will need to adjust their visual selection thresholds for what would constitute an effective and useful resistance level.

Results from monitoring the reaction to stem rust of the main cultivars grown in Spain clearly indicate that suitable commercial genetic solutions are immediately available to farmers in the south of Spain should this disease become an issue in the main season. While the probability of this happening may be considered low, it did indeed materialize during the present study at the Jerez location in 2022. Durum cultivars Amilcar, Don Ricardo, and Calero; bread wheat cultivars Tejada and even Conil; and triticale cultivars Bondadoso and Valeroso were consistently resistant to both yellow and stem rusts. While the commercial availability of locally adapted cultivars with such resistance is a very positive result, it is important to keep in mind the virulence spectrum of the races these commercial cultivars were resistant to, particularly with regard to the avirulence of the Spanish races on *Sr13*. Virulence on *Sr13*, a gene that has substantially contributed to the protection of durum wheat from several germplasm pools, while still absent in Spain, is present in the south of Italy, and it is unavoidable to assume that this virulence will soon reach the south of Spain. Results from screening a large collection of durum wheat lines from different germplasm pools against the race JRCQC (with combined virulence on *Sr9e* and *Sr13*) [54] revealed a very low frequency of resistance, reportedly 5.2% [55]. The very low frequency of resistance against the Ethiopian races with combined virulence on *Sr9e* and *Sr13* was consistently confirmed upon screening thousands of CIMMYT lines over years in Ethiopia (data not published). In this context, it becomes clear that the races currently present in Spain do not represent an unmanageable threat to the local wheat production, but that the most important and consequential threat would come from the eventual appearance of races with *Sr13* virulence. Any preventive breeding effort or variety selection program to provide stem rust-resistant cultivars for the south of Spain should aim at resistance to races with combined virulence on *Sr13/Sr9e*, and not resistance merely to the races currently present in Spain. This can be effectively addressed using recent CIMMYT germplasm that was pre-screened for resistance to the African races, either to the Ug99 lineages via the selection in Kenya for bread wheat or to the *Sr13/Sr9e* virulence via the evaluation of lines in Ethiopia for durum wheat. Results from the present study confirm the suitability of this approach, as many of the durum lines known to have acceptable levels of resistance in Ethiopia and Kenya were also resistant to the currently present races in the south of Spain.

In terms of the usefulness for the south of Spain of single genes or gene combinations known to provide good resistance to stem rust globally, *Sr22* and *Sr25*, in a monogenic state, were found to provide adequate protection against the Spanish races in the present study, while also known to be effective against the African stem rust races (data not published). *Sr22* originated from *T. monococcum* ssp. *boeoticum* and *T. monococcum*, and the translocated segment has been mapped on chromosome 7AL of wheat [56]. It has not been extensively used due to an associated yield penalty and a delay in the heading date [57]. In fact, the *Sr22* introgression line used in this study is agronomically inferior and, based on unpublished CIMMYT data, has limited yield potential and drought tolerance. It was therefore never distributed globally but was used as a new donor of the gene for subsequent crossing/selection to pyramid with other resistance genes while selecting for better agronomic types with the least possible yield penalty. *Sr25*, linked to *Lr19*, was transferred to bread wheat in a translocated segment from *Lophopyrum ponticum*, which was also mapped to chromosome 7AL. It has been extensively deployed in many spring bread wheat cultivars of the Middle and Lower Volga regions of Russia [58], with some presence in the CIMMYT bread wheat germplasm. When introgressed into durum wheat, this gene provides good levels of resistance to the Spanish races (as indicated in the present study) as well as to the Ethiopian durum races. The first generation of CMMYT lines with *Sr25* introgressed expressed variable levels of yield penalties, especially under drought. After two cycles of crossing/selection, new CIMMYT germplasm carrying *Sr25* with no yield penalty has been developed (data not published). The combination *Sr22* + *Sr25* is attractive to use in breeding as the loci for the two genes are very closely linked on chromosome 7AL. Once assembled in the correct genetic configuration, they should effectively be inherited together with very little probability of being separated in

further cycles of crossing/selection. The combination was found to be the most effective against the current Spanish races of stem rust, with reactions generally close to an immune response in the present study. Its use in spring bread wheat breeding programs in Russia was found to be associated with a higher ratio of dough tenacity to extensibility, lower flour strength, porosity, and bread volume [58]. In CIMMYT durum wheat backgrounds, the first generation of lines carrying both genes was characterized by severe yield and drought tolerance penalties; however, after two cycles of crossing/selection, the last generation of such lines has compensated for much of the yield or drought tolerance penalties, making them useful improved parents to use in any breeding programs.

Other genes, such as *Sr38*, with limited usefulness in bread wheat breeding against stem rust, may still be useful for durum wheat. Included in a translocated 2NS fragment from *T. ventricosum* onto bread wheat chromosome 2AS [59], it was extensively used for its triple resistance to rusts, as it carries genes *Lr37* and *Yr18* in addition to *Sr38*. This gene is now considered to have limited utility against stem rust in bread wheat. In the present study, it was carried by the cultivar Trident, which was found to be susceptible to the Spanish races. However, when introgressed into a durum background, it had a variable effect against the Spanish races, ranging from complete immunity to complete susceptibility. The original introgression line (line 36) had an intermediate level of resistance to the Spanish races, with a highly resistant field reaction to the African durum races in Ethiopia and Kenya. The other *Sr38* introgression line (line 37) had a rather susceptible reaction to the Spanish races and was characterized by an intermediate to susceptible reaction against the Ethiopian races, suggesting a possible recombination between the molecular marker used in the introgression and the actual gene. Line 1 of the set evaluated herein was a result of the introgression of *Sr38* via marker-assisted selection in a CIMMYT elite background that already had a good level of resistance to the African races of stem rust, hence its immune reaction, likely due to the additive effects of several genes, including *Sr38*. Rust-resistance genes, with limited to no usefulness in bread wheat but with significant value for durum wheat breeding, are not unheard of. A clear example is the *Lr14a* gene for leaf rust resistance. It is completely useless in bread wheat breeding, as most bread wheat leaf rust races are virulent on this gene. However, most races that are virulent on durum wheat are avirulent on the *Lr14a* gene, making it one of the most useful sources of genetic resistance in durum wheat for many years [60], until it was recently circumvented around the Mediterranean basin [61], but it continues to be highly effective everywhere else to this day.

The only other known and well-characterized genes to consider in developing/selecting stem rust-resistant cultivars for the south of Spain are *Sr27*, *Sr32*, and *Sr35*. These were characterized by very low levels of infection in the presence of the Spanish races (this study) and also provide good protection against the Ug99 lineage and derivatives [62–64]. *Sr27* is located on a segment from chromosome 3R of rye translocated onto chromosome 3A of bread wheat [63]. *Sr32* originated from *A. speltoides* and was transferred to chromosome 2DS in bread wheat [64]. Finally, *Sr35* is located on chromosome 3AL of bread wheat and was transferred from *T. monococcum* [62]. For several of the genes previously mentioned, their origin is a translocated segment from wild relatives of wheat, which will require an assessment of the likely yield or quality penalties that could come with the resistance genes, especially if they are to be transferred to durum wheat.

5. Conclusions

The results of the present study clearly indicate that the main lineages of yellow rust detected in recent years in southern Spain were PstS13, PstS14, and PstS10 (Warrior-), and those for stem rust were Clade IV-B and Clade IV-F, both avirulent on the important gene *Sr13*, as seen in most of Europe and North Africa, with the exception of southern Italy, with its Sicilian races harboring virulence on *Sr13*. For both diseases, the high and/or useful levels of resistance to the Spanish lineages/races exhibited by some of the most widely grown commercial cultivars of bread wheat, durum wheat, and triticale indicate that, in the short term, the threat to these crop productions may be effectively mitigated. Various

known resistance genes were shown to be potentially useful in breeding for resistance to either rust disease and could provide effective protection in the near future. However, future breeding efforts will need to consider not only the lineages/races currently present in Spain, but also the rapidly evolving pathogen populations in and outside the Mediterranean basin, implementing strategies that include resistance to linages/races currently absent locally but that could potentially come in and threaten wheat production in southern Spain in the near future.

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