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Mediterranean durum wheat: Seedling resistance to Ug99 and recent emergent stem rust races

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Abstract

The re-emergence of wheat stem rust disease, caused by *Puccinia graminis* Pers. f. sp. *tritici* (Eriks and E. Henn.) (*Pgt*), has recently been reported in Europe and North Africa. The prevalence of virulent *Pgt* genetic groups in Mediterranean basin countries, combined with the limited number of characterized resistance sources in durum wheat (*Triticum turgidum* L., ssp. *durum* (Desf.) Husn.) germplasm, poses a serious threat to durum wheat production. In this study, we evaluated a collection of Mediterranean wheat accessions, mainly durum wheat, for seedling stage resistance to two Ug99 races (TTKTT and TTKSK) and the recently emerged and prevalent races TTRTF (aka Sicily race) and TKKTF. Although 24% of the genotypes exhibited resistant responses, phenotyping screening showed significant variation in seedling responses to the different races. Specifically, 27% and 30% of the genotypes were resistant to TTRTF and TTKSK, whereas only 17.5% and 18% exhibited resistance to TKKTF and TTKTT, respectively. Only 9.4% of genotypes ($n = 13$) exhibited resistance or intermediate responses to all four tested races. Wheat accessions from Portugal, France, and Spain showed the highest resistance frequencies, ranging from 30% to 50%. Molecular analysis revealed the presence of the resistance gene *Sr13* in 13 genotypes, eight of which originated from Tunisia (one landrace and seven varieties). The study demonstrates the importance of Mediterranean durum wheat accessions as sources of novel and diverse genetic resistance to the predominant races

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Abbreviations: APR, adult plant resistance; ITs, infection types; KASP, kompetitive allele-specific PCR; PCR, polymerase chain reaction; *Pgt*, *Puccinia* f. sp. *graminis tritici*; *Sr gene*, stem rust resistance gene; STS, sequence tagged site.

in the Mediterranean region, especially to the TTRTF, TKKTF, and Ug99 lineage races.

Plain Language Summary

Wheat stem rust, a serious fungal disease, has recently reappeared in Europe and North Africa. The reemerged fungal population threat durum wheat production with limited identified resistance. In this study, we evaluated a collection of Mediterranean wheat accessions for resistance to stem rust races, including two from the Ug99 genetic group. About 24% of the accessions showed resistance, with the highest rates found in wheat from Portugal, France, and Spain. These results highlight the value of Mediterranean durum wheat as a source of resistance to face the disease spread in the region and worldwide.

1 | INTRODUCTION

The emergence of wheat stem rust, one of the most historically devastating wheat diseases, has recently been reported across several regions in Africa, Asia, and Europe (Hovmøller et al., 2023; Patpour et al., 2022, 2024; Saunders et al., 2019). This disease is caused by *Puccinia graminis* Pers. f. sp. *tritici* (Eriks and E. Henn.) and occurs on a wide range of hosts, such as bread wheat (*Triticum aestivum* L.), durum wheat (*Triticum turgidum* L., ssp. *durum* (Desf.) Husn.), barley (*Hordeum vulgare* L.), and triticale (x *Triticosecale* Wittmack) (Roelfs, 1985).

The genetic diversity of the pathogen and the various races identified to date confirm its broad virulence spectrum, which challenges the long-term effectiveness of the deployed resistance genes (Roelfs & Martens, 1988; Szabo et al., 2022; Zhong et al., 2009). Over the past decades, extensive genetic research has led to the identification and deployment of >70 stem rust resistance genes (*Sr genes*) in elite wheat cultivars, which have contributed significantly to disease management (Ellis et al., 2014; Jost et al., 2023; McIntosh et al., 1995; Megerssa et al., 2020). Most of these *Sr genes* are race specific (qualitative) and are classified as major genes (*R genes*) that confer all-stage resistance, following Flor's gene-for-gene model (Flor, 1971). However, the effectiveness of these monogenic resistances is often compromised by the emergence and spread of new *Puccinia* f. sp. *graminis tritici* (*Pgt*) races with novel virulence profiles (Ellis et al., 2014; Leonard & Szabo, 2005). Adult plant resistance (APR) genes confer race-non-specific and partial resistance, providing an alternative strategy for disease management. These genes are quantitative and polygenic and generally act additively, contributing to more durable resistance over time (Ellis et al., 2014). Unlike *R genes*, APR genes are expressed during the later stages of plant development and usually confer moderate to minor resistance, resulting in a "slow-rusting" phenotype (Ellis et al., 2014). While many *Sr genes* have been identified in hexaploid wheat (*Triticum aestivum* L.) and its wild relatives, only a few have been identified in tetraploid wheat. Major *Sr genes* identified in tetraploid backgrounds include *Sr13*, *Sr7a*, *Sr8b*, *Sr9*/*SrWeb*, *Sr11*, *Sr12*, *Sr14*, *Sr17*, *Sr8155-B1*, *Sr28/Sr16*, *Sr22*, *Sr49*, and the APR gene *Sr2* (Bansal et al., 2015; Singh et al., 2015; Wang et al., 2023). Among them, *Sr13* has been associated with 40%–60% of durum wheat resistance to Ug99 races (Singh et al., 2011). Despite this contribution, the emergence and spread of unrelated Ug99 lineages that are virulent to *Sr13b* and several other effective resistance genes have raised significant concern for wheat production, particularly in durum wheat-growing areas (Bhattacharya, 2017; Olivera et al., 2012).

Durum wheat is a staple crop in the Mediterranean Basin with major economic and cultural value, accounting for approximately 50% of global production (Martínez-Moreno et al., 2022). Its end-use products, such as pasta, couscous, semolina, and bulgur, are deeply integrated into the regional diet and serve as daily staples. Climate change (e.g., drought and heat stress) and the emergence of fungal diseases, including stem rust, increasingly threaten durum wheat production (Ben M'Barek & Ghaffary, 2021). Recent surveys have revealed the increasing reoccurrence of wheat stem rust disease and the prevalence of three races, namely, TKTTF, TKKTF, and TTRTF, across Mediterranean countries, including Spain, Italy, France, Morocco, and Tunisia (Abdedayem et al., 2023; BGRI, 2020; Patpour et al., 2022). In 2016, a severe stem rust outbreak in Sicilian durum wheat fields was attributed to the spread of the TTRTF race (Bhattacharya, 2017). This race demonstrated a broad virulence spectrum, overcame >20 *Sr genes*, and showed combined virulence to *Sr13b* + *Sr9e* as well as other effective genes such as *Sr21, Sr35, Sr44, Sr45*, and *Sr50*, which confer resistance against

Ug99 lineage races (Patpour et al., 2020; Singh et al., 2015). In Tunisia, the re-emergence of wheat stem rust disease has been reported since 2018, with three races, TTRTF, TKTTF, and TKKTF, detected at varying frequencies across wheat-growing areas (Abdedayem et al., 2023). The widespread occurrence of these races indicates that cultivated durum wheat varieties are susceptible to newly emerging *Pgt* populations. In addition, sexually reproducing *Pgt* populations with diverse virulence spectra have recently been reported in Europe (Rodriguez-Algaba et al., 2022, 2024; Villegas et al., 2022), further intensifying the threat to durum wheat production. These developments reinforce the need to identify novel resistance sources and diversify genetic resistance backgrounds in breeding programs.

To address these challenges, tetraploid wheat germplasm has been increasingly explored as a reservoir of novel resistance genes against stem rust (Kumar et al., 2021; Letta et al., 2013, 2014; Li et al., 2021; Mago et al., 2022; Marone et al., 2022; Megerssa et al., 2020; Nirmala et al., 2017). Advanced genetic and genomic approaches have been applied to facilitate gene characterization, introgression, and deployment in breeding programs (Jost et al., 2023). Multiple molecular markers linked to *Sr genes*, including simple sequence repeats (Haile et al., 2013), cleaved amplified polymorphism sequence (Mago et al., 2011), and kompetitive allele-specific PCR (KASP) markers (Pradhan et al., 2023; Rahmatov et al., 2016), have been identified and applied for resistance characterization and marker-assisted selection (MAS). Despite these advances, phenotypic screening remains a crucial step in breeding for resistance. Field-based trials and controlled-environment greenhouse assays are key to accurately distinguishing between qualitative and quantitative resistance, validating candidate genes, and guiding effective genomic applications, notably, *Sr genes* stacking/pyramiding (Dracatos et al., 2023; Ellis et al., 2014; B. Zhang et al., 2019).

This study evaluated a diverse panel of durum wheat for stem rust resistance at the seedling stage. The tested panel included durum wheat landraces and improved varieties from Algeria, France, Italy, Portugal, Spain, and Tunisia, as well as modern bread wheat varieties and one triticale variety from Tunisia. Additionally, the presence of *Sr* genes was assessed using molecular markers.

2 | MATERIALS AND METHODS

2.1 | Plant material and *P. graminis* f. sp. *tritici* races

A Mediterranean collection of wheat accessions was selected for seedling stage evaluation under controlled conditions in this study. The panel consisted of 126 durum and 11 bread wheat accessions, mostly landraces ($n = 78$), and one triticale variety (Bicentenario). These accessions originated from Algeria ($n = 12$), Italy ($n = 11$), France ($n = 11$), Spain ($n = 13$), Portugal ($n = 9$), and Tunisia ($n = 82$) (Figure 1a). The Tunisian subset consists of 53 landraces and 28 improved varieties, among which nine durum and eight bread wheat varieties are widely cultivated in Tunisia. In Table S1, detailed information is provided regarding accession names, geographical origins, pedigrees, and improvement status.

Core Ideas

- Mediterranean durum wheat landraces and improved varieties are resistant to Ug99 and recently emerged races.
- The resistance gene *Sr13* has been positively identified in several cultivated Tunisian durum varieties.

The entire panel was evaluated against four *Pgt* races: TTRTF and TKKTF, which are predominant in the Mediterranean region and belong to Clades III-B (Olivera et al., 2019) and IV-F (Patpour et al., 2022), respectively, and TTKTT and TTKSK, which belong to Clade I (Ug99 lineage) (Olivera et al., 2015; Szabo et al., 2022). The virulence profiles of the four races in the seedling assays are summarized in Table 1. Two isolates of the TTRTF race, originating from Tunisia (sampled in 2021) and Italy (sampled in 2018), were included to investigate the virulence variability within the same race.

2.2 | Seedling resistance assay

Six to eight seeds of each accession were sown in pots with a 1:1 Pindstrup Substrate peat mix containing slow-release plant nutrients (Pindstrup Mosebrug A/S). Five seedling sets of the Mediterranean collection were grown, following a complete randomized block design, in spore-proof cabins in the Global Rust Reference Center–Denmark (GRRC) greenhouse. At the 10-day seedling stage, the plants were inoculated following Patpour et al. (2022). Briefly, urediniospores of the five *Pgt* isolates, previously stored at −80˚C, were heat-activated at 42˚C for 5 min and suspended in 3 M Novec 7100 Engineered Fluid (3 M) prior to inoculation. *Pgt* spore multiplication of the five isolates was conducted on the susceptible wheat line Morocco.

The five seedling sets were inoculated separately using an airbrush spray gun (standard class, Revell GmbH), then incubated for 24 h in the dark in the dew chamber with high relative humidity (RH = 100%) at 18˚C. After incubation, the seedlings were transferred to the quarantine greenhouse with a 16-h photoperiod (16 h light/8 h darkness) using natural light

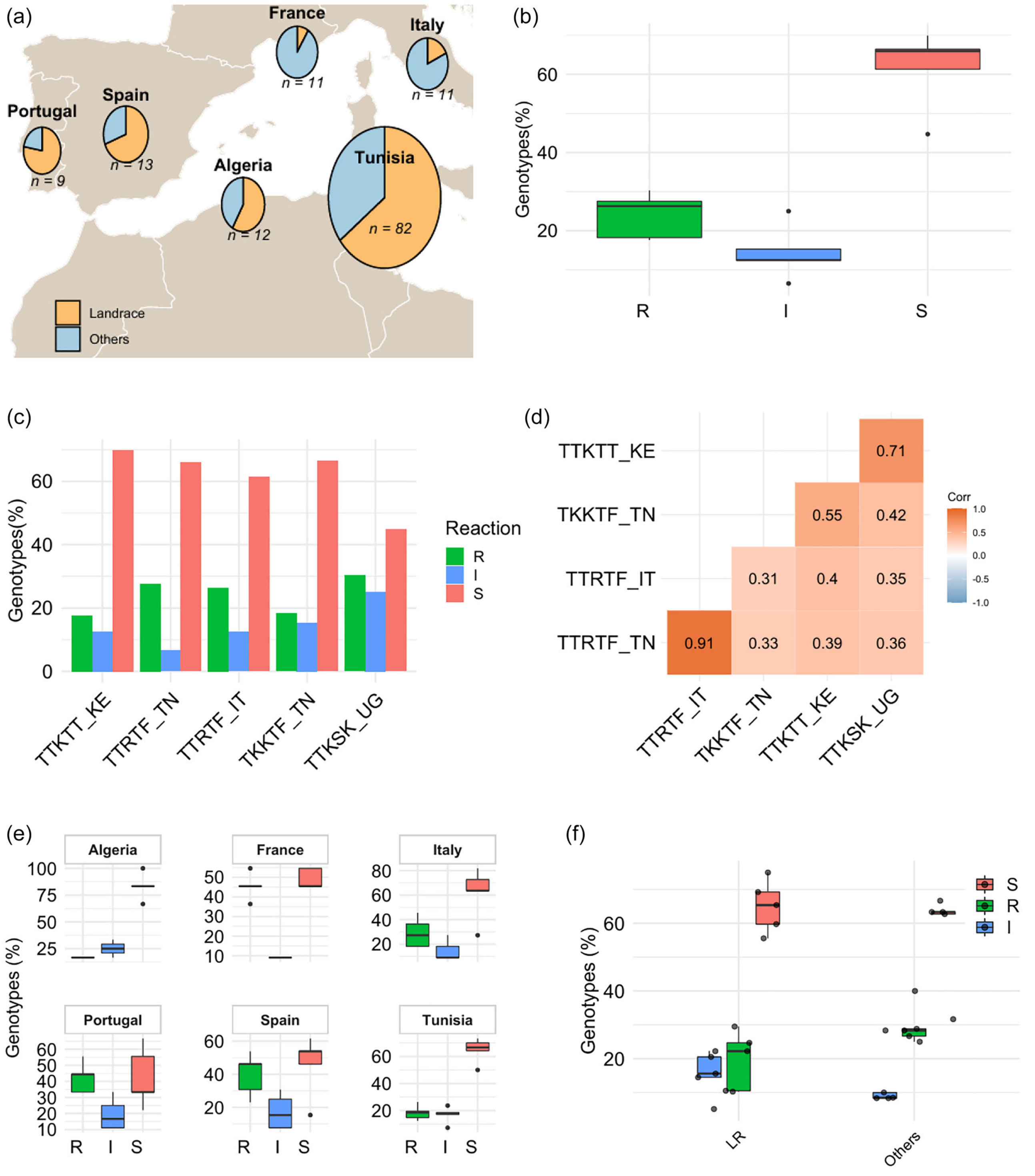

FIGURE 1 Seedling phenotypic data analysis of (a) a diverse collection of Mediterranean accessions composed of landraces and others (improved varieties, cultivars, breeding materials, and genotypes with unknown improvement status) to five *Puccinia* f. sp. *graminis tritici* (*Pgt*) isolates: TTKTT_KE (KE184a/22), TTRTF_TN (TN134/22), TTRTF_IT (IT16a/18), TKKTF_TN (TN101/19), and TTKSK_UG (UG244a/19). (b) The resistant (*R*), intermediate (*I*), and susceptible (*S*) seeding reaction rates and their (c) distribution among *Pgt* isolates. (d) Pearson correlation test of seedlings infection types between *Pgt* isolates. Seedling reactions (*R, I, and S*) rate variability across country of origin and (f) between landraces (LR) and remaining accessions (Others). *Infection types of ';', 0,;0, 1, 1−, 1+, 2−, and 2 were considered resistant* (*R*); infection types (*ITs*) *of 2+ and 3− were considered intermediate* (*I*); *and ITs of 3, 3+, and 4 were considered susceptible* (*S*).

TABLE 1 Isolates of *Puccinia graminis* f. sp. *tritici* used in this study, their race designation, Clade, country of origin, date of collection, and their virulence profile.

Isolates	Race	Clade	Country (date of collection)	Virulence	Avirulence
TN134/21 IT16a/18	TTRTF	III B	Tunisia (2021) Italy (2018)	*Sr5 Sr21 Sr9e Sr7b, Sr11, Sr6, Sr8a, Sr9g, Sr36, Sr9b, Sr17, Sr9a, Sr9d, Sr10,*	*Sr30, Sr24, Sr31*
TN101/19	TKKTF	IV F	Tunisia (2019)	*Sr5 Sr21 Sr9e Sr7b, Sr6, Sr8a, Sr9g, Sr9b, Sr30, Sr17, Sr9a, Sr9d, Sr10, SrTmp, Sr38, SrMcN*	*Sr11, Sr36, Sr24, Sr31*
KE184a/22	TTKTT	I	Kenya (2022)	*Sr5 Sr21 Sr9e Sr7b, Sr11, Sr6, Sr8a, Sr9g, Sr9b, Sr30, Sr17, Sr9a, Sr9d, Sr10, SrTmp, Sr24, Sr31, Sr38, SrMcN*	*Sr36*
UG244a/19	TTKSK	I	Uganda (2019)	*Sr5 Sr21 Sr9e Sr7b, Sr11, Sr6, Sr8a, Sr9g, Sr9b, Sr30, Sr17, Sr9a, Sr9d, Sr10, Sr31, Sr38, SrMcN*	*Sr36, SrTmp, Sr24*

Note: Virulence and avirulence profiles described based on the 20 North American stem rust differential sets.

supplemented with sodium lamps (200 µmol m^{-2} s^{-1}). The temperature was maintained between 18°C at night and 22°C during the day, with a relative humidity of 80%–90%. To prevent cross-contamination, each seedling set was placed in a separate spore-proof cabin.

Two weeks post-inoculation, infection types (ITs) were assessed on the first and second leaves using the 0–4 scale described by Stakman et al. (1962): ITs = 0, 0; 1, 1+, 2−, and 2 were considered as resistance reactions; intermediate reactions were assigned to seedlings with ITs 2+ and 3−; and susceptible reactions included ITs ranging from 3 to 4 (i.e., 3, 3+, and 4). The wheat line Morocco was used as a susceptible control.

2.3 | DNA extraction and molecular marker analysis

Plant genomic DNA was extracted from 100 mg of healthy leaf tissue collected from 10-day-old seedlings grown under greenhouse conditions. After 4 days in the freeze-dryer, the dried plant material was ground, and genomic DNA was extracted using the GeneJET Plant Genomic DNA Purification Kit (Thermo Fisher Scientific) according to the manufacturer's instructions. The quantity and quality of the extracted DNA were assessed using a DS-11 FX Spectrophotometer/Fluorometer (DeNovix Inc.). All-stage resistance genes *Sr13*, *Sr31*, and *Sr36*, as well as the APR genes *Sr2* and *Sr57*, were investigated for presence/absence in the collection of Mediterranean wheat accessions. The presence of seedling resistance genes *Sr13* and *Sr36* and the APR genes *Sr2* and *Sr57* (*Sr57/Lr34/Yr18/Pm38*) was assessed using KASP markers for molecular analysis. For *R gene Sr31*, the sequence-tagged site (STS) linked marker (*xiag95*) was used (Table S2).

The polymerase chain reaction (PCR) master mix for *Sr31*-linked STS marker was prepared using the Recombinant Taq DNA Polymerase TaKaRa Taq kit (Takara Biotechnology (Dalian) Co., Ltd.). A volume of 2 µL of the DNA was added to 23 µL of PCR master mix (1 µL forward + 1 µL reverse primers + 2.5 µL buffer + 2 µL dNTPs + 0.25 µL rTaq polymerase + 18.25 µL nuclear-free H2O). PCR amplification was conducted using an S1000 Thermal Cycler (Bio-Rad Laboratories) with the following program: 94°C for 3 min; 30 cycles of 94°C for 30 s, 55°C for 60 s, and 72°C for 70 s; and 60 s at 25°C (Mago et al., 2002). The PCR products were resolved by electrophoresis on a 2% agarose gel and visualized under UV light (UVP BioDoc-It 220 UV Imaging Systems, Analytik Jena).

For the KASP assay, each well of a 96-well plate was prepared with 5 µL of genomic DNA and 5 µL of PACE Genotyping Master Mix. Thermal cycling was performed using the following conditions: an initial denaturation at 94°C for 15 min, followed by 10 cycles of touchdown PCR consisting of 94°C for 20 s and 65°C to 57°C for 60 s, with the annealing temperature decreasing by 0.8°C per cycle. This was followed by 36 cycles of standard PCR at 94°C for 20 s and 57°C for 60 s, and fluorescence was read at 20°C. Thermal cycling and fluorescence detection were carried out using the CFX96 Touch Real-Time PCR Detection System (Applied Biosystems). Genotyping data were analyzed using Bio-Rad CFX Manager Software version 3.1 (Bio-Rad Laboratories).

2.4 | Data analysis

Three types of infection responses were classified using the Stakman scale (Stakman et al., 1962): Resistance (R), Susceptible (S), and Intermediate (I). Genotypes with ITs of 0, 0; 1, 1+, 2−, and 2 were classified as R reactions.

TABLE 2 Summary of analysis of variance (ANOVA) variance analysis for seedling infection responses to *Puccinia graminis* f. sp. *tritici* (*Pgt*) isolates, considering the effects of genotype, country of origin, and improvement status.

Source of variation	Df	Square sum	*F* value	*p* value
Isolate	4	92.3	5.777	0.000144***
Genotype	137	1739	5.157	<2e-16***
Country	5	240.3	12.032	3.89e-11***
Imp.status	1	30.6	7.661	0.005812**
Isolate:country	20	47	0.588	0.922215
Isolate:Imp.status	4	33.5	2.099	0.079422
Country:Imp.status	5	49	2.455	0.032343*

Abbreviations: DF, degree of freedom; Imp.status, Improvement status.
*Significant at the 0.05 probability level codes, **Significant at the 0.01 probability level. ***Significant at the 0.001 probability level.

Intermediate reactions were assigned to genotypes with ITs 2+ and 3−. S reactions included genotypes with ITs ranging from 3 to 4 (i.e., 3, 3+, and 4). For statistical analysis, including analysis of variance (ANOVA), Pearson correlation test, and heatmap generation, the ITs were converted to a linear scale as follows: 0, 1−, 1, 1+, 2−, 2, 2+, 3−, 3, and 3+ are coded as 0, 1, 2, 3, 4, 5, 6, 7, 8, and 9, respectively (Zhang et al., 2011). The symbol fleck (;) and IT 4 were converted to 0 and 9, respectively. All data analyses and visualizations were performed using R software version 4.1.2 (R Core Team, 2021). The "stats" package was used to perform ANOVA and correlation analyses. The "ggplot2" package (Wickham, 2009) was employed for data visualizations, and the heatmap was generated using the "pheatmap" packages (Kolde, 2010).

3 | RESULTS

3.1 | Seedling ITs

Seedling resistance assays of 137 Mediterranean wheat accessions and one triticale variety against five *Pgt* isolates representing four races, TTRTF (Clade III-B), TKKTF (Clade IV-F), and TTKSK and TTKTT (Clade I), revealed a broad range of phenotypic responses. Among them, 61% were susceptible (S), exhibiting high infection responses, while 14.7% displayed intermediate (I) reactions. A total of 24% of the genotypes showed low ITs, indicating R to the tested *Pgt* races (Figure 1b; Table S3). ANOVA revealed a highly significant variance effect of race, geographical origin of the isolates, genotype, and improvement status on infection responses (Table 2).

Among the isolates, the TTKTT race exhibited the highest virulence across the panel, with 69.3% of genotypes being susceptible, followed by TKKTF (66.2%) and the two TTRTF races (61.1%–65.7%), while the TTKSK_UG showed the lowest rate of susceptible genotypes (43%) (Figure 1c). A high correlation index ($r = 0.9$, $p < 0.05$) (Figure 1d; Table S4) was observed between the Italian and Tunisian TTRTF race isolates, with comparable resistant genotype rates (26.6%–27.8%).

Despite the high correlation between the two Ug99 races, TTKTT_KE and TTKSK_UG ($r = 0.7$, $p < 0.05$) (Figure 1d; Table S4), a greater difference in seedling reaction rates was observed. Thirty percent of the genotypes showed low ITs exhibiting resistance reactions to TTKSK_UG, while for TTKTT_KE, 17% of genotypes were resistant. Accessions from France, Spain, and Portugal exhibited high levels of resistance, with frequencies ranging from 20% to 45%, depending on the tested races (*Pgt* isolates) (Figure 1e). While it was below 20% for genotypes from Tunisia, except for race TTKSK_UG (25%) (Figure S1). Similarly, resistance rate to TTKSK_UG was observed reaching 35% in Italy, France, Portugal, and Spain. In contrast, the Algerian accessions, which included seven landraces and five cultivars, showed no resistance to TTKSK_UG, with responses divided between intermediate (33%) and susceptible (67%) reactions. Moreover, all latter accessions were susceptible to TTKTT_KE (Figure S1).

The variability in ITs between landraces and the remaining genotypes, which include commercial varieties, cultivars, breeding materials, and genotypes with uncertain improvement status (grouped as "Others" in Figure 1a), was clearly observed. Indeed, Mediterranean landraces exhibited greater variation in seedling responses (Figure 1f). The lowest resistance rate (10%) was observed for the two races TTKTT_KE and TKKTF_TN. In contrast, 22%, 25%, and 30% of accessions were resistant to TTKSK_UG, TTRTF_IT, and TTRTF_TN, respectively (Figure S2). For the remaining accessions (Others), resistance rates were more consistent across *Pgt* races (ranging from 25% to 29%), except for TTKSK_UG, which showed a higher resistance rate of 40% (Figure S2).

All modern durum and bread wheat varieties from Tunisia showed low to intermediate ITs to TTKSK_UG. However, bread wheat varieties, except for variety Tahent, exhibited a higher susceptibility to TTKTT, TTRTF, and TKKTF races in comparison to durum wheat varieties (Table 3). Certain durum wheat varieties, such as INRAT100, Karim, and Razzak, were susceptible to the TTRTF race while they exhibited resistance to TKKTF and the two Ug99 races (Figure 2). The imported durum variety Monastir (registered in 2012) showed high infection responses to the TKKTF and TTKTT races in addition to the TTRTF race (Figure 2).

The heatmap in Figure 2b illustrates the variability in accession responses to the five *Pgt* isolates (Table S3). Overall, 13 wheat genotypes, including six improved varieties (Tomclair, Mondur, Maali, Khiar, Diabolo, and Alex 281), two genotypes with uncertain improvement status (Safari and Razza

TABLE 3 Seedling infection types (ITs) on cultivated durum and bread wheat varieties from Tunisia when inoculated with the five *Puccinia* f. sp. *graminis tritici* (*Pgt*) isolates and the identified stem rust resistance genes (*Sr genes*).

Variety	Specie	TTKTT_KE	TTRTF_TN	TTRTF_IT	TKKTF_TN	TTKSK_UG	*Sr genes*[a]
Byrsa	BW	3	3	3	3+	2+	*Sr57*
Carthage	BW	3	3	3	1+	2−	*Sr57*
Dougga	BW	3+	3	3	2−	2+	–
Florence Aurore	BW	3+	3+	3+	3+	3+	–
Haidra	BW	3	3	3	3	2+	*Sr57*
Neapolis	BW	3	3	3+	3+	2+	–
Salammbo	BW	3	3+	3+	3+	2+	–
Tahent	BW	3	3−	3−	2+	2−	–
Utique	BW	3	3	3	3	2+	–
Dhahbi	DW	2	3+	3	2−	2−	*Sr13*
INRAT100	DW	2	3+	3+	1+	2+	*Sr13*
Karim	DW	2+	3	3+	2	2	*Sr13*
Khiar	DW	2−	2+	2−	2−	2−	*Sr13*
Maali	DW	2−	2+	2	1	2−	*Sr13*
Monastir	DW	3	3+	3	3+	2−	–
Nasr	DW	3	3	3−	3	2+	–
Razzak	DW	2	3	3	1	2	*Sr13*
Salim	DW	2	3	3−	1+	2−	*Sr13*

Note: ITs: Seedlings were scored based on a 0–4 scale (Stakman et al., 1962); "0"–"2"-: resistant reaction, '2+' and '3−': intermediate reaction, and '3'–'4': susceptible reaction.

Abbreviations: BW: Bread wheat; DW: Durum wheat.

[a] Results based on *Sr genes* detection using molecular linked markers.

181), five landraces (Preto Amarelo, Mahmoudi Glabre RP 2, Espanhol, Duro Africano, and Anafil Escuro), and the triticale variety Bicentenario showed low to intermediate ITs across all tested *Pgt* races (Figure 2; Table S3).

Fewer genotypes were susceptible to the tested isolates, including Castiglione Pubescente, Afili Romani, Scavuzza, and Beskri. Responses to the two TTRTF race isolates (TTRTF_TN and TTRTF_IT) were highly positively correlated; however, 17 genotypes exhibited reciprocal shifts in ITs, transitioning either between intermediate and susceptible reactions or between resistant and intermediate reactions.

3.2 | Molecular marker analysis for Sr genes

Neither the all-stage resistance gene *Sr36* nor the APR gene *Sr2* was detected in any tested accessions. Amplification of the STS marker linked to *Sr31* was only detected in the triticale variety Bincentenario. The bread wheat varieties Haidra, Byrsa, and Carthage also showed positive response to the KASP marker for the APR gene *Sr57* (Table 3).

The KASP marker *Sr13R/S_SNP* (MASWheat) was used for the detection of all-stage resistance gene *Sr13*. Only accessions that showed resistant or intermediate responses to the TTKSK race and exhibited a positive molecular detection for *Sr13* were considered. In total, 13 genotypes that showed low to intermediate ITs to TTKSK_UG tested positive for *Sr13R/S_SNP* (Table S3). However, their seedling reactions to the TTRTF isolates varied: six genotypes showed R responses, including the Tunisian landrace Derbassi and five improved varieties (Alex 281, Diabolo, Mondur, Safari, and Tomclair). Two durum wheat varieties, Khiar and Maali, exhibited I reactions, and five genotypes were S, including the Tunisian durum wheat accessions Karim, Razzak, INRAT100, Salim, and Dhahbi (Table 3; Table S3).

4 | DISCUSSION

The increasing occurrence of wheat stem rust in durum wheat fields across several European and North African countries and the emergence of new and highly virulent *Pgt* pathotypes have demonstrated the urgent need to enhance resistance breeding strategies. Host resistance remains a fundamental component of disease management, and its effectiveness depends on the continuous integration of novel and diverse resistance sources to combat the evolving virulence of the pathogen. Despite its potential, durum wheat's genetic

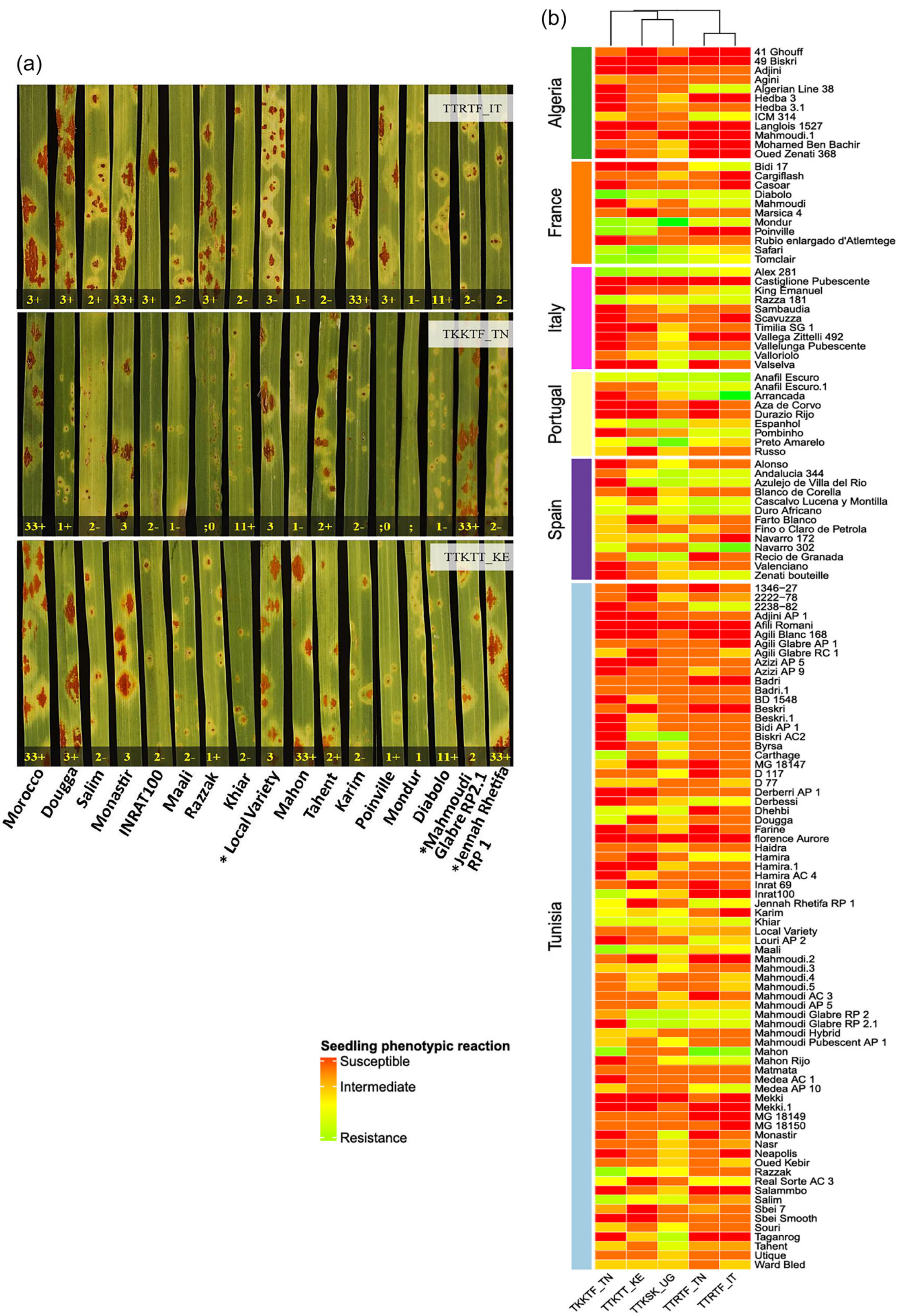

FIGURE 2 (a) Phenotyping seedling responses of improved wheat varieties and landraces variability to the three *Puccinia* f. sp. *graminis tritici* (*Pgt*) isolates: TTRTF_IT (IT16a/18), TKKTF_TN (TN101/19), and TTKTT_KE (KE184a/22). First leaves were scored 14 days post-inoculation using the 0–4 scale described by Stakman et al. (1962). Infection types (ITs, highlighted in yellow) of ';, 0,;0, 1, 1-, 1+, 2-, and 2′ were considered resistant; ITs of "2+ and 3−" were considered intermediate; and ITs of "3, 3+, and 4" were considered susceptible. *Used to

(Continues)

FIGURE 2 (Continued)
mention the landraces and wheat line Morocco used as a susceptible control. The durum wheat varieties Karim, Poinville, Razzak, and INRAT100 were resistant to TTKTT_KE and TKKTF_TN and susceptible to TTRTF_IT. While Khiar, Maali, and Salim showed resistance to intermediate reactions to TTRTF_IT. The widely cultivated variety in Tunisia, Monastir, exhibited susceptibility to the three races and landrace Jennah Rhetifa RP 1, Mahmoudi were resistant to TTRTF_IT. The bread wheat accessions Dougga, Local variety, and Mahon were susceptible to TTKTT-KE, except of Tahent, which showed intermediate ITs. (b) Heatmap of genotypes seedlings reactions to five *Pgt* isolates: TTKTT_KE (KE184a/22), TTRTF_TN (TN134/22), TTRTF_IT (IT16a/18), TKKTF_TN (TN101/19), and TTKSK_UG (UG244a/19). *The infection types 0, 1−, 1, 1+, 2−, 2, 2+, 3−, 3, and 3+ are coded as 0, 1, 2, 3, 4, 5, 6, 7, 8, and 9. The flecks* (;) *are converted to 0. 0–4 represents the resistance reactions, 5–6 for intermediate reactions, and the susceptible reactions represent the high infection level from 7–9. Genotypes with one missing seedling response were not included.*

diversity remains underutilized, as only a limited number of *Sr* genes have been identified and deployed from the durum wheat germplasm.

Phenotypic screening across a Mediterranean wheat panel revealed that, on average, 24% of the genotypes exhibited resistance reactions to stem rust. This indicated the presence of potential resistance sources within the Mediterranean panel conferring broad-spectrum resistance. However, when considering the different isolates, significant variability was observed, with resistance rates ranging from 17% to 30%. Only 9.4% of accessions showed low to intermediate ITs across all tested races.

Since the breakdown of *Sr31*, one of the most effective stem rust resistance genes by the TTKSK race (Pretorius et al., 2000), and the subsequent overcoming of multiple *Sr* genes (*Sr24, Sr36, Sr9h, SrTmp*, and *Sr8155B1*) by its variants (Jin et al., 2008, 2009; Patpour et al., 2016; Rouse et al., 2014), durum wheat has become a promising source of resistance to Ug99 races (Kumar et al., 2021; Letta et al., 2013, 2014; Marone et al., 2022; Megerssa et al., 2020; Nirmala et al., 2017). The widely deployed resistance gene *Sr13* is largely responsible for resistance to Ug99-related races in approximately 60% of cultivated durum wheat varieties worldwide (Singh et al., 2011). Several studies have also shown the substantial genetic diversity found in Mediterranean durum wheat germplasm (Krima et al., 2020; Ouaja et al., 2021; Rufo et al., 2019; Soriano et al., 2016), which strengthens its potential as a valuable reservoir of resistance that can be utilized in breeding programs to limit *Pgt* spread. The present study revealed that 30% of the evaluated panel exhibited resistance to the TTKSK_UG race, whereas only 17.6% showed resistance to the TTKTT_KE race. Compared to TTKSK, the TTKTT race exhibited additional virulence toward *Sr24* and *SrTmp* resistance genes. These two genes have been identified and widely deployed in bread wheat lines and cultivars from Australia, China, North and South America, and South Africa (Jin et al., 2008; Lopez-Vera et al., 2014; Sun et al., 2023; Xu et al., 2017). A previous study also reported *Sr24* postulation in eight German wheat lines (Flath et al., 2018). However, the presence of *Sr24* and *SrTmp* in the Mediterranean wheat germplasm has not yet been investigated. Most evaluated bread wheat accessions exhibited susceptible response to the TTKTT, TTRTF, and TKKTF races and an intermediate response to TTKSK. Phenotypic data showed that two commercial bread wheat varieties, Tahent and Carthage, were susceptible to TTKTT but resistant to TTKSK (Table S3), suggesting that their resistance may be associated with *SrTmp* and/or *Sr24*. The variety Carthage exhibited high ITs to the TTRTF race, indicating the likely presence of *SrTmp* rather than *Sr24*, whereas Tahent displayed an intermediate response to TTRTF, making it difficult to determine whether *Sr24* was involved. Moreover, phenotypic seedling data suggests the potential presence of *SrTmp* single in five bread wheat accessions that exhibited an intermediate reaction to TTKSK (ITs: 2+) (Utique, Salammbo, Neapolis, Haidra, and Byrsa) or were associated with *Sr11* (Carthage and Dougga) and *Sr30* (local variety). The landrace Mahon, which showed high ITs only to TTKTT and TTKSK, may possess resistance genes *Sr30, Sr31*, and *Sr11*. The APR gene *Sr57* (*Lr34/Yr18/Pm38/Bdv1/Sr57*) was detected by molecular analysis in the bread wheat varieties Carthage, Byrsa, and Haidra. However, adult plant evaluations are needed to confirm the presence of *Sr57* in these varieties.

Similarly, several durum wheat accessions contributed significantly to the observed variance in reactions between the TTKTT and TTKSK races (Table S3), suggesting the potential involvement of uncharacterized resistance sources. Previous studies have reported genetic resistance in durum wheat against Ug99 races (Aoun et al., 2021), including the *Sr8155B1* allele, which confers resistance to the TTKST race but is ineffective against TTKSK race (Nirmala et al., 2017). Further analysis using molecular markers and postulation of *Sr* genes is recommended to elucidate the genetic basis of resistance to Ug99 races and differentiate the genetic resistance basis across Mediterranean durum wheat germplasms.

The effective resistance gene *Sr13* was positively identified in twelve improved durum wheat varieties and in one Tunisian durum wheat landrace, Derbassi. *Sr13* was originally derived from the progenitor of durum wheat, *Triticum turgidum* subsp. *dicoccum* (emmer wheat), and has been detected in several durum wheat varieties, including Kronos, Langdon, Leeds, Altar 84 (Gill et al., 2021; Li et al., 2021), and Karim (Susanne Dreisigacker, personal communication, February 8, 2024). This suggests that the *Sr13* gene in Razzak likely originated from Karim, a parent in its pedigree. Similarly, the presence of Altar 84 in the pedigrees of Khiar and Salim suggests a likely introgression of *Sr13* into these cultivars. Altar 84 is widely used as a donor parent in breeding programs and carries the *Sr13c* allele (Gill et al., 2021). A recent study by Klindworth et al. (2025) showed the effectiveness of *Sr13a* and *Sr13c* alleles against the TTKTT, TTKSK, and TTRTF races. However, the varieties Karim, Razzak, and Salim exhibited high ITs to the TTRTF race, suggesting that they may carry the *Sr13b* allele rather than *Sr13c*. The French variety Diabolo has Leeds in its pedigree, a cultivar known to harbor the *Sr13b* allele (R2 haplotype) (Gill et al., 2021). *Sr13b* confers resistance to the TTKSK (W. Zhang et al., 2017) and TTKTT races but is ineffective against TTRTF (Klindworth et al., 2025). This suggests that Diabolo may possess additional resistance sources beyond *Sr13b*, as it exhibited resistance to all tested races, including TTRTF. Alternatively, Diabolo may carry either the *Sr13a* or *Sr13c* alleles, both of which confer resistance to the two Ug99 races and TTRTF (Klindworth et al., 2025). In addition to improved cultivars, *Sr13* has also been detected in certain landraces, particularly Ethiopian landraces, such as ST464 (PI 191365) and Camadi Abdu tipo #103 (PI 192168) (Klindworth et al., 2025). The Tunisian landrace Derbassi exhibited resistance to the TTRTF race (ITs: 2−/2), susceptibility to TTKTT (ITs: 3), and an intermediate response to TTKSK (ITs: 2+). The *Sr13d* allele was reported to confer similar response patterns to TTRTF and TTKTT but not to TTKSK (ITs: 3+) (Klindworth et al., 2025). This may indicate the presence of another resistance gene effective against TTKSK in addition to *Sr13d*. Further investigation of potential *Sr13* allelic variation, together with additional seedling assays against a broader range of races, is required to better characterize the genetic basis of the observed resistance.

Despite its effectiveness against Ug99 races, the breakdown of *Sr13* has been reported following the emergence of new races unrelated to the Ug99 lineage, such as JRCQC and TTRTF (Olivera et al., 2012; Patpour et al., 2020). Although JRCQC has not been detected in Europe or North Africa, the TTRTF race has been increasingly reported in several countries since the 2016 stem rust epidemic in Italy (Abdedayem et al., 2023; Bhattacharya, 2017; Patpour et al., 2022).

The Mediterranean wheat collection in this study was evaluated using two TTRTF isolates collected from Tunisia (2021) and Italy (2018). Seedling responses were highly correlated between the two isolates, although slight variations in ITs were observed for a few accessions. Similar variability in virulence profiles among isolates of the same race has previously been reported for TKTTF isolates from Germany and Ethiopia (Olivera Firpo et al., 2017). Furthermore, a recent *Pgt* population analysis from Georgia revealed intra-race virulence variation within TKKTF isolates collected in 2018 and 2014 (Olivera, Sikharulidze, et al., 2021). The results of this study showed that Tunisian genotypes are highly susceptible to the TKKTF race, even though it has been detected at low frequency in Tunisia (Abdedayem et al., 2023). The virulence of TKKTF has also been reported on European varieties, with 71% of the tested varieties showing susceptibility (Patpour et al., 2022). Given the widespread geographic distribution of *Pgt* since 2016 across Europe and North Africa, and the dominance of a limited number of genetically related races, identifying the virulence differences among isolates classified within the same race may reveal underlying evolutionary events that remain undetectable through standard race or clade classification. Such analysis can also provide a more comprehensive understanding of host-pathogen population dynamics and the evolution of virulence.

Most improved varieties, particularly Tunisian ones, were more susceptible to the TTRTF race, whereas landraces exhibited a higher rate of resistance. Tunisian durum wheat landraces represent a rich and diverse genetic pool (Ouaja et al., 2021; Robbana et al., 2019) and a valuable source of resistance to major fungal wheat diseases, such as Septoria tritici blotch (Ben M'Barek et al., 2022; Ferjaoui et al., 2022; Ouaja et al., 2020, 2023), tan spot (Laribi et al., 2023), and rust diseases (Bansal et al., 2015; Olivera, Bulbula, et al., 2021). Landraces such as Ward Bled, Hamira, and Jennah Rhetifa RP 1 exhibited low ITs against the TTRTF race, despite their susceptibility to Ug99 races. This highlights potential novel resistance sources that could be introgressed from landraces into elite varieties to mitigate the spread of TTRTF in the region. High resistance rates were also observed among accessions from Italy, France, Portugal, and Spain. Promising sources of stem rust resistance have previously been reported in durum wheat germplasm, including improved materials and landraces from Italy, Portugal, and Tunisia (Aoun et al., 2019; Olivera, Bulbula, et al., 2021).

The absence or limited detection of known *Sr genes* in genotypes displaying resistance to the tested *Pgt* races may indicate the presence of novel or uncharacterized sources of resistance. To better characterize the genetic basis of this resistance, additional pathogenicity assays using a broader panel of *Pgt* races, combined with molecular assays targeting a wider range of *Sr* resistance genes and their allelic variants, are recommended.

5 | CONCLUSIONS

Given the increasing threat of stem rust disease, there is an urgent need to strengthen breeding programs across Europe and North Africa by incorporating effective and durable resistance, particularly against the most virulent races in these regions. This study demonstrated that Mediterranean wheat landraces and improved wheat varieties represent valuable sources of resistance, including potentially novel and uncharacterized genes. These genetic resources confer resistance to both the Ug99 lineage and predominant regional races such as TTRTF and TKKTF. To fully exploit this diversity, further molecular characterization of *Sr genes*, combined with field-based evaluations across environments, is essential. Integrating multiple *Sr* genes through stacking or pyramiding supported by MAS and modern genomic tools will enhance resistance durability and reduce the risk of pathogen adaptation.

Overall, these findings underscore the importance of conserving and using genetic diversity and highlight the need for coordinated regional breeding efforts to develop wheat cultivars with broad-spectrum and long-lasting resistance to stem rust in the Mediterranean region and beyond.

AUTHOR CONTRIBUTIONS

Wided Abdedayem: Conceptualization; data curation; formal analysis; investigation; methodology; visualization; writing—original draft; writing—review and editing. **Mehran Patpour**: Data curation; investigation; methodology; validation; writing—review and editing. **Marwa Laribi**: Data curation; validation; writing—review and editing. **Amor Yahyaoui**: Project administration; supervision. **Sonia Hamza**: Supervision; writing—review and editing. **Mahbubjon Rahmatov**: Data curation; investigation; methodology; validation; writing—review and editing. **Sarrah Ben M'Barek**: Conceptualization; data curation; funding acquisition; investigation; project administration; resources; supervision; validation; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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SUPPORTING INFORMATION

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