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Potential Resistance Genes Conferred Tolerance to Fusarium Crown Rot in Wheat

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Abstract

Fusarium crown rot (FCR), primarily caused by various Fusarium pathogens, especially *Fusarium graminearum*, is a serious soilborne disease that significantly affects wheat production in arid and semi-arid regions worldwide. This study evaluated eight wheat genotypes for their resistance to FCR Disease incidence and severity were assessed, revealing that the varieties Arz (0.03 ± 0.57) and Hiddab (0.13 ± 0.11) showed the highest levels of resistance. In contrast, there was a highly significant difference ($p < 0.0001$) between these resistant varieties and the susceptible genotypes Waha (2.26 ± 0.30) and Vitron (2.1 ± 0.1). Additionally, the influence of temperature on disease progression was analyzed. While mycelial growth itself did not differ significantly ($p = 0.783$), it was significantly affected by temperature ($p < 0.0001$), suggesting that Fusarium spp. are capable of adapting to climatic variations. This research also highlights recent findings on two candidate genes conferring broad-spectrum resistance: *Sb1* and *Sb4*. PCR amplification produced amplicons of 880 bp and 750 bp, respectively. The nucleotide sequence similarity between these genes was 79.13% and 85.94%. Chromosomes 7D, 7DL, 4B, and 4BL were significantly associated with FCR resistance. The genetic map results indicated that the *Sb1* gene is linked to 6 markers and flanked by the marker TraesCS7D01G221000, while *Sb4* is located on chromosome arm 4BL, between markers Xgwm25I and RAC8758rep_c72961_977. These findings offer valuable insights into plant-pathogen interaction mechanisms and provide a theoretical foundation for developing sustainable genetically-based resistance in wheat.

Key words: Wheat; Resistance; Fusarium crown rot; Genes.

1. Introduction

Durum wheat *(Triticum durum)* and bread wheat *(Triticum aestivum)* are important sources of cereal food for humans (Enghiad *et al*., 2017) and the main staple crop in Algeria (Kara *et al*., 2020). Throughout its growth cycle, wheat faces various biotic stresses that significantly reduce grain yield and quality, particularly in bread wheat cultivars (Bakala *et al*., 2022). Phytopathogenic fungi that cause plant diseases are common and represent a major constraint to wheat production (Covarelli *et al*., 2015; Singh *et al*., 2016). Soil-borne diseases are also a major problem for global agricultural production, causing considerable economic losses in the productivity and quality of various key crops (Katan, 2017). Several species of Fusarium are pathogenic to cereals (Mielniczuk and Skwarylobednarz, 2020) and occur worldwide in dry and semi-arid wheat-growing regions (Kazan and Gardiner, 2018).

Fusarium Crown rot (FCR), caused by *Fusarium graminearum*, is a destructive fungal disease that significantly impacts major cereal crops such as wheat, barley, and sorghum at various stages of plant development (Zhou *et al*., 2020; Liu *et al*., 2021; Feng *et al*., 2023). Common symptoms of FCR include browning and necrosis at the stem base or lower leaf sheath, often accompanied by the formation of premature whiteheads (Chen *et al*., 2022). Yield losses due to FCR can be severe, with reductions of up to 35% in the Pacific Northwest region of the USA (Poole *et al*., 2012), 45% in Iran (Saremi *et al*., 2007), 16,29% in China (Zhang *et al*., 2022) and 43% in Turkey (Tunali *et al*., 2008). Long-term environmental changes have had a significant impact on crop diseases (Cohen and Leach, 2020), mainly high temperatures, which limit yield and affect the different growth stages of the plant (Jagadish, 2020), regulate fungal growth, and also affect the pathogenicity of plant pathogenic fungi (Ezrari *et al*., 2021). Infected grains secrete toxic metabolites that damage the grain directly and can accumulate in stored grain (Leslie *et al*., 2021). Trichothecenes are an important group of mycotoxins produced by fungi belonging to the genus Fusarium, including deoxynivalenol (DON) (Moretti *et al.,* 2019). On average, DON can be found in 57% of wheat samples worldwide (Mielniczuk and Skwarylobednarz, 2020). These substances exhibit neurotoxic and teratogenic properties and can cause acute and chronic immune disorders in humans and animals (Piacentini *et al.,* 2019).

Polymerase chain reaction (PCR) has become a practical tool for the identification of Fusarium species, offering a faster alternative to morphological methods. DNA sequencing, especially targeting the ITS region, remains a reliable approach for species identification.

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Given the evolving nature of pathogens, identifying novel resistance (R) genes is crucial for improving disease resistance in wheat (Ren *et al.,* 2017). However, this is complex for traditional breeding approaches (Zhang *et al.,* 2020). Resistance mechanisms have been linked to transcription factors, transport and detoxification genes, and metabolic pathways such as phenylpropanoid and glutathione biosynthesis (Powell *et al.,* 2017; Qiao *et al.,* 2021). Genome-wide association studies (GWAS) have proven effective in identifying novel genes/Quantitative Trait Loci (QTLs) linked to Fusarium resistance. Recent studies have mapped resistance loci across multiple wheat chromosomes including 1A, 1B, 2A, 2B, 2D, 3B, 4B, 5D, 6A, 6B and 6D (Bainsla *et al.,* 2020; Su *et al.,* 2021; Yang *et al.,* 2021). GWAS using the wheat 660K SNP array in 435 introgression lines uncovered 12 SNPs within a 0.5-Mb genomic region (Yang *et al.,* 2021). These findings underscore the value of GWAS in guiding the development of resistant wheat cultivars adapted to future agricultural demands. The objective of this study was to determine the degree of resistance of different genotypes of wheat to FCR and to contribute to the search for new potentially resistant genes to reduce the loss of wheat quality and productivity. The aim was also to see how different temperatures affect the mycelial growth of different fungal isolates.

2. Materials and methods

2.1. Plant material.

Eight wheat genotypes with different levels of resistance to FCR were used. They were supplied by the National Center for Control and Certification of Seeds and Plants of Algiers (CNCC). The characteristics of these genotypes are shown in Table 01.

Table 01: Characteristics of the different wheat varieties involved in the study (Abdelguerfi and Laouar, 2000; Ykhlef and Djekoun, 2000).

Variety	Origin	Pedigree	Grain species	Agronomic characteristics	Productivity	Adaptation zone
Core	Italy	Platani/Gianni	Durum wheat	Medium straw	High productivity	Mountain areas
Vitron	Mexico	Turchia-77/3/JORI-69(SIB)/	Durum wheat	Medium red grain	Medium productivity	Mountain areas And Saharan areas
Chen's	CIMMYT-ICARDA	Ichwa'S'/Bit 'S'CD 26406	Durum wheat	short straw, early growth cycle	High productivity	Arid, semi-arid and Saharan areas
Anforeta	Italy	EG-83/BEL-118	Bread wheat	Light grain, strong tillering, long red ears	High productivity	Mountain areas
Waha	Syria	Plc/Ruff//Gta's/3/Rolette CM 17904	Durum wheat	Light amber to red, medium	High productivity	Mountain areas and inland plains. Semi-dwarf
Gta dur	Mexico	Crane/4/PolonicumPI$_{185309}$//T.glutinen/2* Tc60/3/Gll	Durum wheat	Large and red grain	Medium productivity	Semi-arid climates Moderate drought
Hiddab	Mexico	HD1220/3*Kal/Nac CM40454	Bread wheat	early variety with medium straw and long spikes	High productivity	Melanized brown soil with vertic character
Arz	Mexico	Mayo54E/Lr//Tac/3/	Bread wheat	An upright plant with a long stem. Pyramid-shaped spike, colourful and loose with reddish grain	High productivity	Alluvial red brown soil with weak vertic character

2.2. Fungal strains.

A series of strains were isolated from durum wheat spikes and crowns collected at the INPV in El Tarf (North-Eastern Algeria). The selected plant parts were superficially disinfected in a 2% sodium hypochlorite solution for 5 minutes, rinsed three times with sterile distilled water for 5 minutes each time, and then dried with sterile paper. The disinfected fragments were transplanted onto PDA (Potato-Dextrose-Agar) medium at a rate of four pieces per Petri dish and incubated at 25°C for 7 days (Leslie and Summerell, 2006). Only typical colonies with Fusarium characteristics were selected. Colonies of *F. graminearum* were characterized by rapid growth and variable mycelial coloration, ranging from salmon pink to dark red. The macroconidia are falcate (crescent-shaped) and contain 3 to 7 transverse septa. Microconidia and chlamydospores were absent.

2.3. Molecular identification of the fungal species.

DNA was extracted from a single spore culture using the commercial NucleoSpin Plant II kit (Macherey-Nagel, Germany). The protocol was followed as described in the user manual. PCR was performed using two universal primer combinations described by Gardes and Bruns (1993) ITS1/ITS4 (CTTGGTCATTTAGAGGAAGTAA/TTCCTCCGCTTATTGATGC) and Carbone and Kohn (1999) EF-728F/EF-2 (CATYGAGAAGTTCGAGAAGG/GGGARGTACCAGTSATCATGTT). The reaction mixture, consisting of 25 µl, contained 5 µl of distilled water, 2 µl of genomic DNA (25ng/ µl), 5 µl of Promega Taq buffer (1X), 0.2 µl of Promega Taq Polymerase (1U), 0.2 µl of dNTP (0.2mM), 1.5 µl of MgCl2 (1.5mM), and 1 µl of each primer (0.5mM). The amplification program started with denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for ITS primers and 52°C for EF primers for 30 seconds,

extension at 72°C for 45 seconds, and a final elongation step at 72°C for 7 minutes. The amplification products were visualized by adding 10 µl of PCR products to a 1.5% agarose electrophoresis gel. Migration of DNA fragments was monitored through ethidium staining (0.5 µg/ml). The DNA was then visualized and captured under UV light using the Gel Doc system from Biorad (USA). PCR products were purified using the PCR Clean-up and Gel Kit NucleoSpin® from Macherey-Nagel (Germany), following the manufacturer's guidelines. The purified products were sequenced using the Sanger technique (Sanger *et al.,* 1977), with the PCR primers used to amplify the fragments of interest and the BigDye v3.1 kit from Applied Biosystems. The resulting sequences were cleaned and corrected using Chromas software, then compared with reference sequences published in the NCBI genome banks to determine the species of the sequenced isolate.

2.4. Conducting the experiment.

Single-spore isolates of the species *F. graminearum* were used to prepare the inoculum. The technique used for inoculation is that described by Hajieghrari (2009), which consists of inoculating the soil directly with mycelial explants. The wheat seeds were disinfected for 5 minutes in 2% sodium hypochlorite, rinsed in three successive baths of sterile distilled water, and dried on sterile Joseph paper. The seedlings were sown at a rate of 5 seeds per pot (three pots experiments were conducted), to a depth of 3 cm, in a substrate containing two-thirds soil and one-third sterilized compost. The pots were placed randomly in a greenhouse. The temperatures recorded from inoculation to the appearance of symptoms are shown in Figure 01 using the GraphPad Prism v10 program. The observed climatic data were obtained from https://power.larc.nasa.gov/.

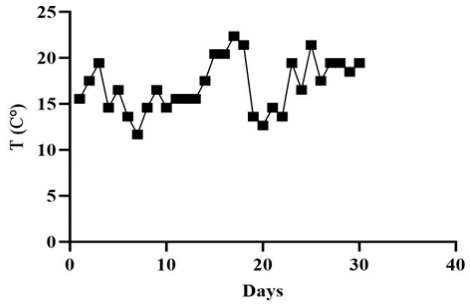

Figure 01. Histogram representing the variation of temperature in the region of Algiers during the month of April 2019.

2.5. Disease incidence.

Symptoms were assessed at the three-leaf stage, and the percentage of crown browning was used according to the Grey and Mathre (1984) 4-point scale as follows:

0 = No symptoms.

1 = Browning of 1 to 25% of the crown surface.

2 = Browning of 26 to 50% of the crown surface.

3 = Browning of more than 50% of the crown surface.

The disease index (DI) was calculated using the following formula:

$$DI = (0 \times F0 + 1 \times F1 + 2 \times F2 + 3 \times F3) / N$$

DI is the disease index, F represents the number of plants for each grade of the rating scale ranging from 0 to 3, and N is the total number of plants used.

2.6. Estimation of the in vitro mycelial growth rate of F. graminearum.

The effect of temperature range (15°C, 20°C, 25°C and 30°C) on mycelial growth of *F. graminearum* was determined following the protocol established by Ward *et al.* (2008) based on measuring fungal colony diameter. *F. graminearum* isolates were first grown on potato dextrose agar (PDA) medium for 7 days at 20-22°C in a culture chamber under continuous white light. Mycelial explants of 6 mm diameter were removed from these cultures using a cutter, and then cut into small pieces. Each isolate of *F. graminearum* was cultured in a Petri dish (8.6 cm in diameter) containing PDA medium. Petri plates were incubated in the dark for 24 hours at various temperatures (15°C, 20°C, 25°C and 30°C) and then radial growth was measured. The experiments were performed in three replicates. (Table 02).

Table 02. *F. graminearum* isolate used during this test.

Fusarium code	Species	Isolation Organ	Variety	Year
F1	*F. graminearum*	Crowns	Durum wheat (Siméto)	2018
F2		Spikes		2017
F3	*F. graminearum*	Crowns	Durum wheat (Vitron)	2018
F4		Spikes		2019
F5	*F. graminearum*	Crowns	Durum wheat (Siméto)	2018
F6	*F. graminearum*	Crowns	Bread wheat (Anza)	2017
F7		Crowns		2019
F8	*F. graminearum*	Crowns	Durum wheat (Siméto)	2017
F9		Spikes		2019
F10	*F. graminearum*	Crowns	Durum wheat (Vitron)	2018
F11	*F. graminearum*	Spikes	Durum wheat (Ammar6)	2017
F12		Crowns		2018
F13	*F. graminearum*	Crowns	Durum wheat (Vitron)	2017
F14		Crowns		2017
F15	*F. graminearum*	Crowns	Bread wheat (Anza)	2019
F16		Spikes		2019
F17	*F. graminearum*	Spikes	Durum wheat (Siméto)	2019
F18	*F. graminearum*	Crowns	Bread wheat (Maouna)	2017
F19		Crowns		2018
F20	*F. graminearum*	Spikes	Durum wheat (Siméto)	2019
	F. graminearum		Durum wheat (Vitron)	
	F. graminearum		Bread wheat (Maouna)	
	F. graminearum		Bread wheat (Anza)	
	F. graminearum		Durum wheat (Ammar6)	
	F. graminearum		Bread wheat (Anza)	
	F. graminearum		Bread wheat (Maouna)	
	F. graminearum		Durum wheat (Siméto)	
	F. graminearum		Durum wheat (Ammar6)	

2.7. DNA extraction from wheat plants and PCR amplification of potential resistance genes.

DNA was extracted from fresh and mature greenhouse-grown wheat leaves using a GF-a Plant DNA Extraction Kit. PCR was performed in a thermal cycler, the iCycler™ (BIO-RAD, USA). Using two universal primer combinations described by Rivkin *et al.* (1999): the *Sb1* gene (F-CCACAGTATGTGACTACTTC/R-AGCCAAATCCATTTGCAATG) and the *Sb4* gene (F-ACAATTTCTTTCCCAATCTC/R-CTTCAAAGACCAACACTG). The reaction mixture had a total volume of 25 µl and consisted of 5 µl of distilled water, 2 µl of genomic DNA (25ng/ µl), 5 µl of Promega Taq buffer (1X), 0.2 µl of Promega Taq polymerase (1U), 0.2 µl of dNTP (0.2 mM Promega), 1.5 µl of MgCl2 (1.5mM Solis BioDyne), and 1 µl of each primer (0.5mM Eurogentec). The amplification program began with denaturation at 95°C for 3 min, then continued with 40 cycles of denaturation at 95°C for 30 s, and annealing at 72°C for 30 s for the primers for the *Sb1* and *Sb4* genes after the 40 amplification cycles, and finally the elongation step at 72°C for 5 min.

The amplification products were revealed after electrophoresis on a 1.5% agarose gel in 50 ml TAE (40 mM Tris-HCL, 20 mM acetic acid, 1 mM EDTA, PH 8). Migration was preceded by coloration in an ethidium bromide bath (0.5 µg/ml). Genomic DNA was then visualized and photographed using a gel documentation system (Biorad Laboratories, Inc., CA, USA). The size of each fragment was estimated using a 100-bp DNA ladder size marker (Solis BioDyne, Tartu, Estonia). Purification of PCR products was carried out using the NucleoSpin® Gel and PCR Clean-up Kit from Macherey-Nagel (Germany), according to the manufacturer's instructions, which contained 40 µg of template DNA, 2 µl of the appropriate PCR primer, 10 µl of water, and 2 µl of BigDye Terminator v3.1 Ready Reaction Mix (Applied Biosystems). Two forward and two reverse sequences for each sample were aligned using DNASTAR Lasergene software (ver. 17.3.0.61) in order to obtain a composite sequence. The sequences were compared with the sequences of the closest accessions.

2.8. Statistical analysis.

The data were analyzed using SPSS ver. 20.0 (Chicago, IL, USA). An analysis of variance (ANOVA) incorporating Tukey's post-hoc test, and Pearson's correlation coefficients was used to determine the correlation between the different variables.

2.9. Map construction.

MG2C v2.1 (Jiangtao *et al.,* 2021) was used to assemble the genetic linkage map of the reported resistance genes. GWAS has been widely used to identify QTLs using 832 polymorphic Diversity Arrays Technology (DArT) markers, and high-throughput SNP toolkits are now available for GWAS on various traits of wheat (Sun *et al.,* 2020). Molecular markers, SNPs and QTLs controlling resistance, as well as wheat genotypes with variable resistance, were collected from previous publications and grouped in Table 03. Stable QTLs with large effect or linked with designated genes were labeled with asterisks and highlighted.

Table 03. Genetic loci controlling wheat resistance.

QTL name	Associated markers/ SNPs	Resistant wheat germplasms	References
Sb1*	7DS: Xgwm295, csLV34	Saar	Lilemo *et al*., 2013
Qsb	7DS: wPt-7654, gdm88	Saar	Lilemo *et al*., 2013
Qsb.bhu-7D	7DS: Xgwm111, Xgwm1168	Ning 8201	Kumar *et al*., 2010
Qsb.bhu-7D	7DS: Xgwm111, Xswm008	Chirya 3	Kumar *et al*., 2010
Qsb	7D: Xgwm437	19HRWSN6, 30SAWSN5	Tembo *et al.,* 2017
Qsb	7D: wPt-664459	(PI 477878), Soprimo (PI 479890), CI 10112 (PI 78814), Florentino (PI 565255), AW 6635A/86 (PI 572693), IWA8611737 (PI 625572), NW56A (PI 429667)	Adhikari *et al*., 2012
Qsb	7D: TraesCS7D01G067000, TraesCS7D01G081100, TraesCS7D01G221000	N. A.	Tomar *et al*., 2020
Sb4*	4B: TraesCSB01G295400.1	Zhongyu1211, GY17	Zhang *et al*., 2020
Qsb.sdsu-4B.1	4B: Excalibur_rep_c79414_306	OK05122, OK05723W, Venango	Ayana *et al*., 2018
Qsb.sdu-7B.1	4B: S4B_554842477	7HTWSN-4513, Aust-8, SB12-6703	Jamil *et al.,* 2018
Qcr/Rht1*	4BL: Xgwm165, Xgwm251	Kukri, 2-49 (Gluyas Early/Gala), Janz	Wallwork *et al.,* 2004 ; Collard *et al*., 2005,2006
Qcr	4BL: RAC875_rep_c72961_977	TX69A509.2//BBY/FOX/3/GRK//NO64/PEX/4/CER/5/KAUZ//ALTAR 84/AOS, ID800994.W/MO88	Pariyar *et al*., 2020

3. Results

3.1. Molecular characterization of fungal pathogen.

The strain was first identified on the basis of the morphological characteristics of the mycelium. After observing the conidia under the microscope and comparing them with the characteristics described by Leslie and Summerell (2006), the strain was found to be consistent with the description. However, the preliminary identification based on colony morphology was not sufficient to determine the species precisely. Molecular analysis was based on sequencing of the ITS region of the ribosomal DNA gene and the elongation factor 1 alpha (EF1) gene. The Fusarium sequences obtained from amplification of the conserved ITS region of the ribosome were compared with data from the National Center for Biotechnology Information (NCBI) using BLAST (http://www.ncbi.nlm.nih.gov/BLAST). The molecular results showed that the strain identified was represented by *F. graminearum*, with a percentage of 99.81% for the partial sequence of the ITS and 99.60% for the partial sequence of TEF1. The fragment sizes were 636 bp for ITS and 490 bp for TEF1 (Figure 02).

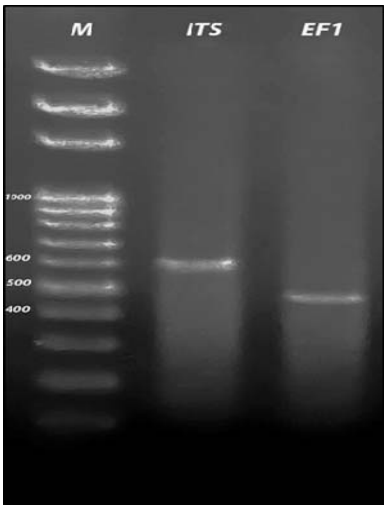

Figure 02. Electrophoretic profile of PCR amplicons targeting the TEFα and ITS regions from *Fusarium spp.* isolates resolved on 1.5% agarose gel. ITS: 636 bp; EF: 490 bp.

3.2. Pathogenicity and aggressiveness of F. graminearum on wheat.

Disease symptoms started to appear one week after inoculation, with one or two leaves dying, depending on the resistance/susceptibility of the wheat cultivars. Analysis of variance from this experiment showed significant variability in aggressiveness ($p < 0.05$) (Table 04) depending on the wheat genotype. Different levels of resistance and symptom severity were observed during the Fusarium infection. Disease indices showed that the varieties Waha (2.26 ±0.30) and Vitron (2.1 ±0.1) exhibited symptoms of high susceptibility (reduced growth, yellowing of leaves, wilting, and death of plants within ten days of inoculation). The Chen's and Core varieties, on the other hand, showed moderate susceptibility with infection rates of (1.53 ±0.30) and (1.40 ±0.72), respectively, with a slight delay in growth causing wilting and yellowing of plants around 25 days after inoculation. Conversely, the cultivar Arz was the most resistant with an average index of (0.03 ±0.57), followed by Hiddab (0.13 ±0.11), then Anforeta (0.86 ±0.50), and finally Gta dur with an average of (0.80 ±0.72). The Levene statistic was used to determine the variance homogeneity. The ratio test indicated that the variances were homogeneous at 3.20. In this experiment, the un-inoculated control plants showed no symptoms. Tukey's post-hoc analysis (multiple comparisons test) was used to determine the differences between the eight wheat genotypes according to disease index. The analysis showed highly significant differences between the variety Waha and the two varieties Hiddab and Arz ($p < 0.0001$). This confirms previous results showing that Arz and Hiddab are the most resistant to the aggressiveness of the fungus.

Table 04. Analysis of the variance of the disease index according to wheat varieties

Cultivar	Average of the IM	Standard deviation	Standard error	Confidence interval 95 % for the average		Min	Max
				Lower bound	Upper bound		
Core	1.400	0.7211	0.4163	-.391	3.191	0.8	2.2
Vitron	2.100	0.1000	0.0577	1.852	2.348	2.0	2.2
Chen’s	1.533	0.3055	0.1764	0.774	2.292	1.2	1.8
Anforeta	0.867	0.5033	0.2906	-.384	2.117	0.4	1.4
Waha	2.267	0.3055	0.1764	1.508	3.026	2.0	2.6
Gta dur	0.800	0.7211	0.4163	-0.991	2.591	0.2	1.6
Hiddab	0.133	0.1155	0.0667	-0.154	0.420	0.0	0.2
Arz	0.033	0.0577	0.0333	-0.110	0.177	0.0	0.1

3.3. Effect of temperature on mycelial growth.

The impact of temperature on mycelial growth was examined over a range of temperatures. F4, F9, and F17 had the highest mycelial growth (0.7cm each), while isolates F3, F10, and F19 had the lowest growth (0.1cm each). At a temperature of 20°C, strains F8 and F9 had the most significant growth (1.6 and 1.7 cm, respectively), whereas isolate F19 (0.5cm) showed low growth at this temperature (as shown in Figure 03). At a moderately high temperature of 25°C, strain F9 still had the highest mycelial growth (2 cm), while strains F6, F18, F19, and F11 had smaller diameters (1.1, 1.2, and 1.3 for the last two). The data also showed that some isolates appeared to be better adapted to warmer temperatures, while others were better adapted to lower temperatures. For example, at 30°C, strains F9 and F17 produced the largest mycelial growth (2.5cm), while strains F18 and F19 produced the lowest mycelial growth (1.8cm). This suggests that strain F9 could grow easily at a wide range of temperatures. All isolates used in the study were grown on PDA medium at the optimum temperature of 30°C. Two-factor analysis of variance (ANOVA) revealed that mycelial growth did not differ significantly between isolates ($p = 0.783$) but was significantly influenced by temperature ($p < 0.0001$) (Table 05).

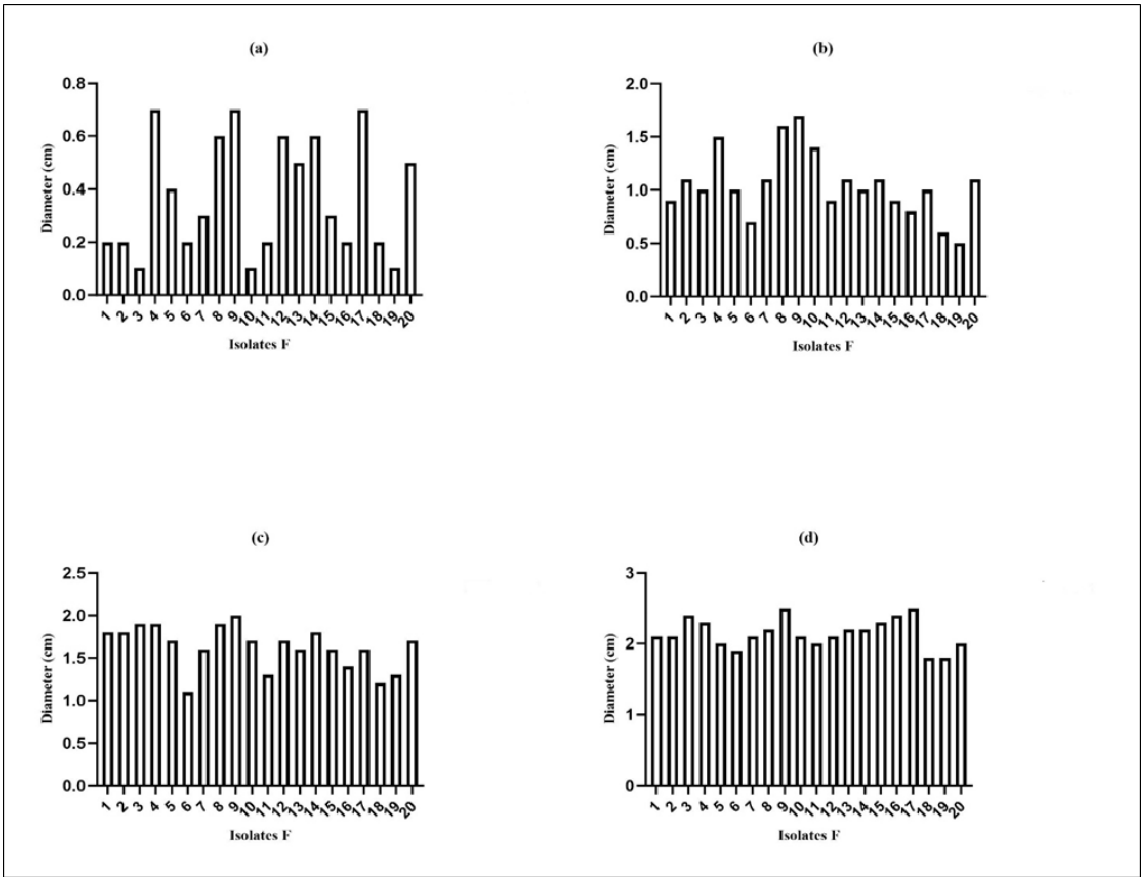

Figure 03. Diameter histograms based on the evolution of radial mycelial growth at different temperatures in a group of Fusarium isolates. **(a):** 15°C; **(b):** 20°C; **(c):** 25°C; **(d):** 30°C.

Table 05. Analysis of variance of mycelial growth rate of isolates as a function of temperature

Source of Variation	ddl	Medium Squares	F Value	P Value
Temperature	2	6.056	89.847	0.000
Isolate	19	0.049	0.0577	0.783
Temperature* Isolate	0	-	-	-
Error	57	0.067	-	-
Total	80	-	-	-

ddl: data definition language; F: fisher value

Tukey's post-hoc analysis (multiple comparisons test) was used to determine significant differences between growth means by temperature. Mycelial growth showed a significant difference between temperatures ($p<0.0001$). In general, mycelial change was more variable as temperature varied. The results of the final measurements are listed in Table 06.

Table 06. Post-hoc analysis of variance in mycelial growth rate of isolates between temperatures.

(I) Temperature	(J) Temperature	Average difference (I-J)	Standard error	P value	Confidence interval 95 % for the average	
					Lower bound	Upper bound
	T20°C	-0.6800*	0.0792	0.000	-0.888	-0.472
T15 °C	T25°C	-1.2600*	0.0792	0.000	-1.468	-1.052
	T30°C	-1.7800*	0.0792	0.000	-1.988	-1.572
	T15°C	0.6800*	0.0792	0.000	0.472	0.888
T20 °C	T25°C	-0.5800*	0.0792	0.000	-0.788	-0.372
	T30°C	-1.1000*	0.0792	0.000	-1.308	-0.892
	T15°C	1.2600*	0.0792	0.000	1.052	1.468
T25 °C	T20°C	0.5800*	0.0792	0.000	0.372	0.788
	T30°C	-0.5200*	0.0792	0.000	-0.728	-0.312
	T15°C	1.7800*	0.0792	0.000	1.572	1.988
T30 °C	T20°C	1.1000*	0.0792	0.000	0.892	1.308
	T25°C	0.5200*	0.0792	0.000	0.312	0.728

3.4. PCR amplification of resistant genes.

PCR amplification of the genes of resistant varieties (Hiddab, Arz, Anforeta, and Gta dur) and susceptible varieties (Vitron, Waha, Chen's, and Core) produced fragments of the expected size of 880 bp and 750 bp representing the *Sb1* and *Sb4* genes, respectively, using two specific primers (Figure 04). The *Sb4* gene is visible in almost all susceptible and resistant varieties, while the *Sb1* gene was detected only in one variety (Arz).

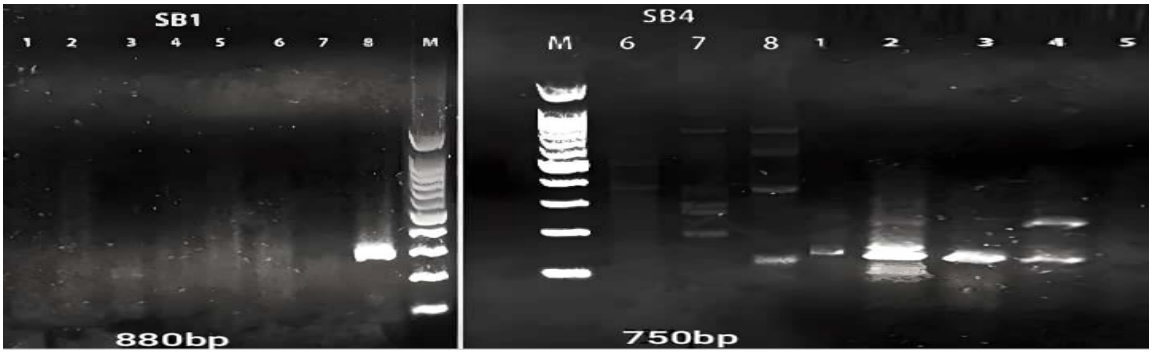

Figure 04: PCR-amplified products of the *Sb1* and *Sb4* genes from the eight wheat genotypes using two designed primers. Migration profile on 1.5% agarose gel, M = 100 bp molecular weight marker. 1: Core; 2: Vitron; 3: Chen's; 4: Waha; 5: Gta dur; 6: Anforeta; 7: Hiddab; 8: Arz.

The partial nucleotide sequences of the *Sb1* gene (880 bp) and the *Sb4* gene (732 bp) in the eight wheat genotypes tested were aligned and compared. BLAST sequence analysis showed that the *Sb1* gene studied had an identity of 79.13%. For the *Sb4* gene, the BLAST of partial nucleotides corresponded to 85.94%. Therefore, multiple alignments of the two genes revealed positional differences in the nucleotide sequences, as shown in Figure 05.

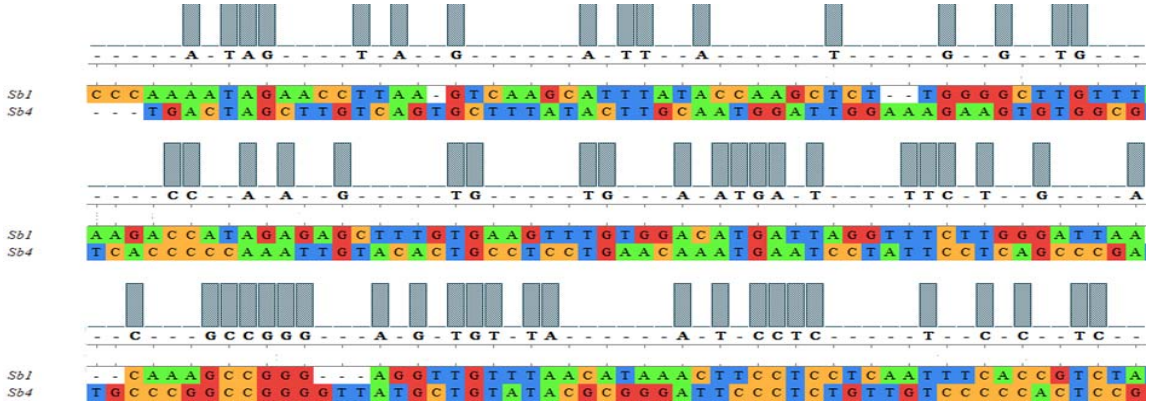

Figure 05. Nucleotide sequence alignment of *Sb1* and *Sb4* genes using DNASTAR Lasergene software. The alignment highlights positional differences in the nucleotide sequences providing a comparative analysis of variations between the two genes.

The nucleotide percentages of the two fragments are shown in Table 06. The average molecular weight of the dsDNA (497.989 kDa) was twice that of the ssDNA (248.640 kDa). In addition, the percentage frequencies of the four nucleotides, A, T, C, and G, were observed and presented in Table 07.

Table 7. Nucleotide percentages for the two R genes (*Sb1* and *Sb4*).

Genes	Molecular weight		A		C		G		T		GC	
	ssDNA	dsDNA	freq	%	freq	%	freq	%	freq	%	freq	%
Sb1	271.296	543.743	225	25.6	230	26.1	201	22.8	224	25.5	431	49.0
Sb4	225.984	452.235	196	26.8	147	20.1	150	20.5	239	32.7	297	40.6
Average	248.640	497.989	421	26.1	377	23.4	351	21.8	463	28.7	728	45.2

3.5. Genetic mapping of the candidate genes Sb1 and Sb4 conferring tolerance to FCR.

Analysis of the chromosome map based on chromosomal location and marker (table 03) showed that the *Sb1* gene is located on chromosome 7D associated with 6 markers; it is delimited in the genetic region of 0.536 Mbp and is flanked by the TraesCS7D01G221000 markers at an interval of 1.818 Mbp and TraesCS7D01G081100 at an interval of 0.478 Mbp. The *Sb4* gene is located on the long arm of chromosome 4B, which contains 4 markers. It is delimited in the 5.81Mbp genetic region on arm 4BL between markers Xgwm25I and RAC8758rep_c72961_977 at a genetic interval of 5.686 Mbp and 6.170 Mbp, respectively (Figure 06).

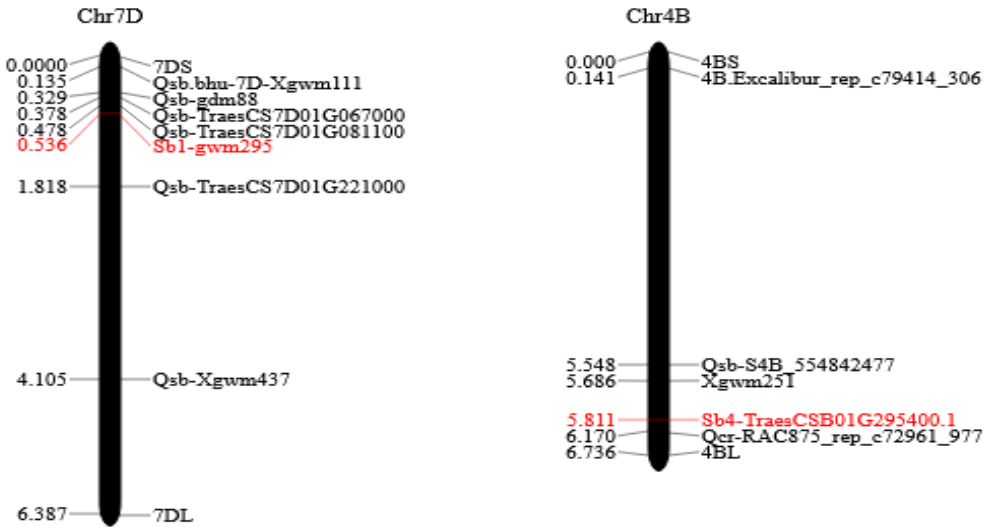

Figure 06. Genetic and physical maps of the predicted genes *Sb1* and *Sb4*. Molecular markers were collected from previous publications. (https://wheat-urgi. versailles.inra.fr/) were used to find the homologous sequences in the Chinese Spring genome assembly. The genetic distance (physical position) is mentioned on the left-hand side of each chromosome (in units of 100,000,000 bp).

4. Discussion

This research presents data on FCR infection, comparing the responses of eight of the most resistant and susceptible wheat genotypes. The results showed significant variability in aggressiveness (p =0.05) and a difference in disease severity between varieties, which is similar to the findings of Schaafsma and Hooker (2007). The authors reported that varietal factors significantly determine the presence or absence of disease in wheat. The Waha (2.267) showed high infection rates for FCR, which is consistent with the findings of Bencheikh *et al.* (2020), who found that the Waha variety was more susceptible to infection by the Fusarium pathogen. Vitron (2.100) exhibited a high infection rate, indicating significant susceptibility to *F. graminearum.* Nevertheless, the variety demonstrated remarkable agronomic performance, confirming the results reported by Haddad *et al.* (2021). Classified within cluster C1, Vitron belongs to the group of the most productive varieties under rainfed conditions in the eastern high plateaus of Algeria. This suggests a certain physiological compensation capacity in response to Fusarium attacks. However, Chen's (1.533) and Core (1.400) varieties showed moderate susceptibility. Previous authors Bencheikh *et al.* (2020) reported that Chen's has gained in popularity in recent years, reaching an occupancy rate of 7%. According to Haile *et al.* (2019), bread wheat is naturally more resistant than durum wheat due to its hexaploidy nature. Nevertheless, for our work, Arz (0.033) and Hiddab (0.133) showed low infection rates, while the genotypes Anforeta (0.867) and Gta Dur (0.800) showed moderate resistance. Some wheat varieties in Algeria do not have sufficient data on their level of tolerance to FCR, which is the case for Anforeta and Hiddab. Although, there are no wheat varieties that are completely resistant to FCR, the use of varieties with some degree of resistance is the most reliable and cost-effective method for controlling this disease (Francesconi *et al.* 2019; Gebremariam *et al.* 2020).

The increase in temperature levels would imply a significant rise in plant disease pressure, and consequently, a decrease in grain yield (Romero *et al.* 2022). The effect of temperature on mycelial growth of Algerian isolates of *F. graminearum* revealed a significant difference in thermal optima of development between isolates (p <0.0001) and in temperature × isolate interactions. The isolates used in this study were able to grow on PDA medium at an optimum temperature of 30°C. These results are consistent with those obtained by Manstretta and Rossi

(2016), who found that the optimal mycelial growth of *F. graminearum* occurs between 28 and 30°C. No relationship was observed between the optimal temperatures for mycelial growth of the isolates and their geographical origins, suggesting that thermal adaptation may not be strongly influenced by the origin of the isolates. The development indices of *F. graminearum* were higher at 25°C and 30°C, highlighting that the isolates used in this study may be better adapted to elevated temperatures. This could indicate a potential shift in the thermal tolerance of certain strains. Similar findings were reported by Magan *et al*. (2003), who demonstrated that *F. graminearum* exhibits faster growth and greater competitiveness compared to other Fusarium species at similar temperatures. Interestingly, mycelial growth was also observed at a low temperature of 15°C, indicating that this species retains a degree of adaptability to cooler conditions. This observation aligns with results from Hope *et al.* (2005), who reported active growth of *F. graminearum* within a temperature range of 15-25°C. Furthermore, Manstretta and Rossi (2016) noted that while the fungus can grow across a broader temperature spectrum 5-30°C, its capacity to reach full maturity is generally limited to 20-25°C range. These variations in thermal behavior suggest that different Fusarium isolates may exhibit growth optima possibly influenced by environmental adaptation (Nazari *et al.* 2014). They also noted that each Fusarium species has a specific temperature range at which disease expression is maximized. Under controlled conditions, Hay *et al.* (2021) found that warm nights at high levels can increase the aggressiveness of *F. graminearum* in wheat, further emphasizing the influence of thermal conditions on pathogenicity.

The present study aimed to identify potential R genes conferring FCR resistance in eight wheat genotypes, encompassing both newly introduced and older varieties, through molecular characterization. Targeted amplification and sequencing of the *Sb1* and *Sb4* genes revealed positional differences and base substitutions among the studied genotypes, suggesting genetic variation that may contribute to disease resistance.

The findings showed that *Sb4* gene was present in nearly all genotypes, except for two moderately resistant cultivars, 'Chen's' and 'Gta dur'. This observation aligns with Liu *et al*. (2021), who identified *Sb4* gene in highly resistant lines 'Zhongyu1211' and 'GY17', locating the gene within a 1.19cM interval on chromosome 4BL. The widespread presence of *Sb4* gene supports its role in broad and durable resistance, making it a valuable target for resistance breeding programs. The *Sb1* gene was detected in the highly resistant variety 'Arz', which corresponds to the findings of Lillemo *et al.* (2013), who identified *Sb1* gene in wheat line 'Saar' on chromosome 7DS. This gene is located near the Lr34 locus, which is also associated with resistance to yellow leaf tip necrosis (Ltn+). This proximity suggests that *Sb1* gene may be part of a broader defense system in wheat.

Sequencing results showed a nucleotide identity of 79.13% for *Sb1* and 85.94% for *Sb4*. Multiple sequence alignment revealed several base substitutions (transitions and transversions), along with insertions and deletions ranging from 1 to 57 amino acids. These results are partially consistent with those of Ma *et al.* (2012), who reported base substitutions in the *Sb4* gene in maize. However, the sequence differences observed in this study were smaller than those reported by Enyew *et al*. (2022) for the *Sb1* gene in the genetic background of the genotypes.

Comparative analysis with previous QTLs and GWAS studies supports the significance of *Sb1* and *Sb4* genes. Several QTLs linked to resistance against Spot Blotch and FCR have been identified on chromosome 4B and 7D (Gurung *et al*., 2014; Zhu *et al*., 2014; Singh *et al*., 2018; Su *et al*., 2021). The findings are consistent with Tomar *et al*. (2020), who reported major QTLs on chromosome 7D between SNP markers TraesCS7D01G221000 and TraesCS7D01G081100. Similarly, Yang *et al*. (2021) identified a QTL on chromosome 4B derived from the 'Lang' genotype, associated with FCR resistance in recombinant inbred lines. Although these studies have identified significant resistance loci, more stable and broadly effective genes are still needed. The simultaneous detection of *Sb1* and *Sb4* genes in variety 'Arz' highlights the potential for gene pyramiding to improve long-lasting resistance. As suggested by Wang *et al*. (2018), efficient gene integration strategies can accelerate resistance improvement. Future research should focus on cloning these genes and understanding their functional roles, which is crucial for developing wheat cultivars with improved resistance to FCR and minimizing yield and quality losses caused by this devastating disease.

5. Conclusion

This research provides information on the impact of FCR on wheat. Few cultivars show relatively high resistance to infection. Therefore, the selection of resistant wheat cultivars may be an effective control measure to manage this devastating fungal disease. This work has shown that *F. graminearum* can grow over a wide range of temperatures, enabling it to survive in a variety of seasonal and extreme temperature conditions. Genetic improvement of wheat resistance to this disease requires further research into the genes that control resistance. Efforts should also be made to explore resistance- related QTLs.

Conflict of interest

The authors cited have no financial or personal conflicts of interest that could have influenced the research presented in this article.

References

Abdelguerfi A and Laour M. 2000. Wheat genetic resources in Algeria. Past, present, and future. Wheat symposium: issues and strategies. 1st international symposium on the wheat grain OAIC. Algiers, Algeria, Pp 133-145.

Adhikari TB, Gurung S, Hansen JM, Jackson EW and Bonman JM. 2012. Association mapping of quantitative trait loci in spring wheat landraces with resistance to bacterial leaf streak and spot blotch. *Plant Genome*, **5**: 1–16.

Ayana GT, Ali SS, Sidhu JS, Hernandez JL, Turnipseed BB and Sehgal SK. 2018. Genome-wide combination study for spot blotch resistance in hard winter wheat. *Front Plant Sci*., **9**: 926.

Bainsla NK, Phuke RM, He X, Gupta V, Bishnoi SK, Sharma R, *et al*. 2020. Genome-wide combination study for spot blotch

resistance in Afghan wheat germplasm. *Plant Pathol*., **69**: 1161–1171.

Bakala H, Mandagal K, Ankita Sarao L, Srivastava P. 2022. **Breeding wheat for biotic stress resistance: achievements, challenges and prospects**. Current trends in wheat research. IntechOpen.

Bencheikh N, Rouag W, Mamache I and Belabed. 2020. Initial report of *Fusarium equiseti* causing crown rot and damping-off on durum wheat in Algeria. *Arch of Phytopathol Plant Prot*., **53**: 915-931.

Carbone I and Kohn L. 1999. An approach for designing primer sets for speciation studies in filamentous ascomycetes. *Mycol*., **91**: 10-2307/3761358.

Chen A, Islam T, Ma Z. 2022. An integrated pest management program for managing Fusarium head blight disease in cereals. *J Integr Agric*., **21**: 3434-3444.

Cohen SP and Leach JE. 2020. Warm temperature-induced plant disease sensitivity: more than the sum of its parts. *Curr Opin Plant Biol*., **56**:235-241.

Collard BC, Grams RA, Bovill WD, Percy C, Jolley R, Jolley A, *et al.* 2005. Improvement of molecular markers for crown rot resistance in wheat: mapping of QTLs for seedling resistance in a '2-49'× 'Janz' population. *Plant Breed*., **124**: 532-537.

Collard BC, Jolley R, Bovill WD, Grams RA, Wildermuth GB and Sutherland MW. 2006. Validation of QTL mapping and marker confirmation for partial seedling resistance to crown rot in wheat line '2-49'. *Crop Pasture Sci*., **57**: 967–973.

Covarelli L, Beccari G, Prodi A, Generotti S, Etruschi F, Juan C, Ferrer E and Manes J. 2015. Fusarium species, chemotype representation, and trichothecene infection of durum and bead wheat in an area of central Italy. *J Sci Food Agric*., **95**: 540-551.

Enghiad A, Ufer D, Countryman A and Thilmany D. 2017. A summary of the fundamentals of the global wheat market in the climate era. *Int J Agron*., **39**: 31897.

Enyew M, Feyissa T, Carlsson AS, Tesfaye K, Hammenhag C and Geleta M. 2022. Population structure and genetic variability of Sorghum (*Sorghum Bicolor (L.) Moench*) accessions as indicated by single nucleotide polymorphism markers. *Front Plant Sci*., **12**: 799482.

Ezrari S, Radouane N, Tahiri A, Amiri S, Lazraq A, Lahlahi R. 2021. Environmental effects of temperature and water potential on mycelial growth of *Neocosmospora solani* and *Fusarium spp.* causing dry root rot of citrus. *Curr Microbiol*., **78**: 3092-3103.

Feng C, Xu F, Li L, Zhang J, Wang J, Li Y, *et al*. 2023. Biological control of Fusarium crown rot of wheat with Chaetomium globosum 12XP1-2-3 and its effects on rhizosphere microorganisms. *Front Microbiol*., **14**: 1133025.

Francesconi S, Mazzagalia A, Balestra GM. 2019. Different inoculation methods affect components of Fusarium head blight resistance in wheat. *Phytopathol Mediterr*., **58(3)**: 679-691.

Gardes M and Bruns T. 1993. ITS Primers with increased specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. *Mol Ecol*., **2**: 113-118.

Gebremariam ES, Karakaya A, Erginbas-Orakci G, Dababat AA, Paulitz TC. 2020.Assessment of the seedling resistance of spring wheat lines to *Fusarium Culmorum*. *J Agric Sci*., **26**: 87-93.

Grey W and Mathre D. 1984. Reaction of spring barleys to common root rot and its effect on yield components. *Can J Plant Sci*., **64**:245-253.

Gurung S, Mamidi S, Bonman JM, Xiong M, Brown-Guedira G and Adhikari TB. 2014. Genome-wide association study finds new quantitative trait loci linked to resistance to several leaf spot diseases of spring wheat. *PLoS One*., **9(9)**: e108179.

Haddad L, Bachir A, Benmahammed A, Bouzerzour H. 2021. Durum wheat *(Triticum turgidum ssp durum)* improvement during the past 67-year in Algeria: Performance assessment of a set of local varieties under rainfed conditions of the eastern high plateaus. *Jordan J Biol Sci*., **14(2)**: 327-336.

Haile JK, N'Diaye A, Walkowiak S, Nilsen KT, Clarke JM, Kutcher HR, Steiner B, Buerstmayr H, Pozniak CJ. 2019. Fusarium head blight in durum wheat: recent status, breeding directions, and future research prospects, *Phytopa*thol., **109 (10)**: 1664-1675.

Hajieghrari B. 2009. Wheat crown and root rotting fungi in Moghan area, Northwest of Iran. *Afr J Biotechnol*., **8(22)**: 6214-6219.

Hay WT, McCormick SP, Vaughan MM. 2021. Effects of atmospheric Co2 and temperature on wheat and corn susceptibility to *Fusarium graminearum* and deoxynivalenol contamination. *Plants*, **10(12)**: 2582.

Hope R, Aldred D and Magan N. 2005. Comparison of environmental factors for development and production of deoxynivalenol by *F. culmorum* and *F. graminearum* on wheat grain. *Lett Appl Microbiol*., **40**: 295–300.

Jagadish SV. 2020. Thermal stress during flowering in cereals - impacts and adaptation methods. *New Phytol*., **226 (6)**: 1567-1572.

Jamil M, Ali A, Gul A, Ghafoor A, Ibrahim A, and Mujeeb-Kazi A. 2018. Genome-wide association studies for spot blotch *(Cochliobolus sativus)* tolerance in bread wheat using genotyping-by-sequencing. *Phytopathol*., **108**: 1307–1314.

Jiangtao C, Zhiyuan L, Yuhe S, Oluwaseun A, Xinru W, Qian W, Guanshan L. 2021. MG2C: a user-friendly online tool for drawing genetic maps. *Mol Hortic*., **1**: 16.

Kara K, Rached-Kanouni M, Mnasri S, *et al*. 2020. Genetic variability assessment in bread wheat *(Triticum aestivum)* grown in Algeria using microsatellites SSR markers. *Biodiversitas*, **21:** 2638-2644.

Katan J. 2017. Disease caused by soil borne pathogens: biology management and challenges. *J Plant Pathol*., **99(2)**: 305-315.

Kazan K and Gardiner D. 2018. Fusarium crown rot caused by *Fusarium Pseudograminearum* in cereal crops: latest advances and future prospects. *Mol Plant Pathol*., **19(7)**: 1547-1562.

Kumar U, Joshi A, Kumar S, Chand R, and Roder M. 2010. Quantitative trait loci for resistance to spot blotch induced by Bipolaris sorokiniana in wheat *(T. aestivum L.)* lines 'Ning 8201' and 'Chirya 3'. *Mol Breed*., **26**: 477–491.

Leslie JF and Summerell BA. 2006. **Laboratory Manual of Fusarium**. Blackwell Publishing Professional. New Jersey, USA.

Leslie JF, Moretti A, Mesterházy Á, Ameye M, Audenaert K, Singh PK, Richard-Forget F, Chulze SN, Del Ponte EM, Chala A, *et al.* 2021. Key actions at global level for the management of mycotoxins in wheat and other small grains. *Toxins*., **13**:725.

Lillemo M, Joshi AK, Prasad R, Chand R and Singh RP. 2013. QTLs for spot blotch resistance in the bread wheat line Saar coincide with the biotrophic disease resistance loci Lr34 and Lr46. *Theor Appl Genet*., **126**: 711–719.

Liu C, Guo W, Zhang Q, Fu B, Yang Z, Sukumaran S, Cai J, Liu Y, Zhai W and Wu X. 2021. Genetic determination of mature plant resistance to sharp eyespot using an updated genetic map of niavt14× xuzhou25 winter wheat recombinant inbred line population. *Plant Dis*., **105(4)**: 997-1005.

Liu W, Ye T, Jagermeyr C, Muller S, Chen X, Liu X, Shi P. 2021. Future climate change significantly alters interannual wheat yield variability over half of harvested areas. *Environ Res Lett*., **16(9)**: 094-045.

Ma J, Zhang C, Liu Y, Yan G and Liu C. 2012. Improving Fusarium crown rot resistance of durum wheat by introgressing chromosome segments from hexaploid wheat. *Euphytica*., **186 (1)**: 67-73.

Magan N, Hope R, Cairs V and Aldred D. 2003. Post-harvest fungal ecology: influence of fungal infection and mycotoxin accumulation in stored grain. *Eur J Plant Pathol*., **109**: 723-730.

Manstretta V and Rossi V. 2016. Temperature and moisture influence on the evolution of *Fusarium graminearum perithecia* in maize stalk residues. *Appl Environ Microbiol*., **82(1)**: 184–191.

Mielniczuk E and Skwarylo-Bednarz B. 2020. Fusarium Head Blight, Mycotoxins and techniques for their reduction. *Agro*., **10(4)**: 509.

Moretti A, Pascale M and Logrieco A. 2019. Mycotoxin risks from a climate change perspective in Europe. *Tr. Food Sci Technol*., **84**: 38–40.

Nazari L, Pattori E, Terzi V, Morcia C and Rossi V. 2014. Impact of temperature on infection, development, and mycotoxin formation by *Fusarium langsethiae* and *F. sporotrichioides* in durum wheat. *Food Microbiol*., **39**: 19-26.

Pariyar S Erginbasorakci G, Dadshani S, Chijioke O, Leon J, Dababat A, *et al*. 2020. Revealing the genetic complexity of Fusarium crown rot resistance in wheat. *Sci Rep*., **10**: 3200.

Piacentini K, Rocha L, Savi G, Carnielli-Queiroz L, De Carvalho Fontes L and Correa B. 2019. Evaluation of Toxigenic Fusarium strains and their Mycotoxins in Brewing Barley Grains. *Toxins*., **11** :31.

Poole GJ, Smiley RW, Paulitz TC, Walker CA, Carter AH, See DR, Garland-Campbell K. 2012. Identification of quantitative loci (QTL) for resistance to Fusarium crown rot (*Fusarium pseudograminearum*) in multiple assay environments in the pacific Northwestern US. *Theor Appl Gen*., **125**: 91-107.

Powell J, Carere J, Fitzgerald T, Stiller J, Covarelli L, Xu Q, *et al.* 2017. The Fusarium crown rot fungus, Fusarium *pseudograminearum*, activates a series of transcriptional and metabolic changes in bread wheat *(Triticum aestivum L.). Ann Bot*., **119**: 853–867.

Qiao F, Yang X, Xu F, Huang Y, Zhang J, Song M, *et al.* 2021. Quantitative proteomic analysis based on TMT reveals the defense mechanism of wheat against the crown rot pathogen *Fusarium pseudograminearum*. *BMC Plant Biol*., **21**: 82.

Ren Y, Hou W, Lan C, Basnet B, Singh R, Zhu W, Cheng X, Cui D and Chen F. 2017. QTL screening and nested assembly mapping for mature plant resistance to powdery mildew in two bread wheat populations. *Front Plant Scie*., **8**: 1212.

Rivkin M, Vallejos C, Eduardo and Mcclean P. 1999. Disease-resistance related sequences in common bean. Gen. Natl. *Res. Counc. Can*., **42**: 41-7.

Romero F, Cazzato S, Walder F, Vogelgsang S, Bender SF, Van der Heijden MG. 2022. Humidity and high temperature are important for predicting fungal disease outbreaks worldwide. *New Phytol*., **234**: 1553-1556.

Sanger F, Nicklen S, Coulson AR. 1977. DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci. U S A*, **74 (12)**: 5463-7.

Saremi H, Ammarellou A and Jafary H. 2007. Incidence of wheat crown rot disease of wheat caused by the novel soil fungal species *Fusarium Pseudograminearum* in North West Iran. *Pak J Biol*., Sci.**10**:3606-12.

Schaafsma AW and hooker DC. 2007. Climatic models to prevent the occurrence of Fusarium toxins in wheat and maize. *Inter. J. Food Microbiol*., **119 (1-2)**: 116-125.

Singh P, He X, Sansaloni C, Juliana P, Dreisigacker S, Duveiller E *et al*. 2018. Spot blotch resistance in two mapping collections of common wheat is controlled by multiple QTL of minor impacts. *Inter J Mol Sci*., **19**: 4054.

Singh R, Singh P, Rutkoski J, Hodson D, He X, Jorgensen L *et al*. 2016. Disease influence on wheat yield potential and prospects for genetic control. *Annu Rev Phytopathol*., **54**: 303-322.

Su J, Zhao J, Zhao S, Li M, Pang S, Kang Z, *et al*. 2021. Genetics of resistance to common root rot (spot blotch), Fusarium crown rot, and sharp eyespot in the grain of wheat. *Front Genet*., **12**:699342.

Sun C, Dong Z, Zhao L, Ren Y, Zhang N, Chen F. 2020. The Wheat 660K SNP array demonstrates great potential for marker assisted selection in polyploid wheat. *Plant Biotechnol J*., **18**: 1354–1360.

Tembo B, Sibiya J, Tongoona P, and Tembo L. 2017. Approval of microsatellite molecular markers associated with resistance to *Bipolaris sorokiniana* in wheat *(Triticum aestivum L.). J Agric Sci*., **155**: 1061–1068.

Tomar V, Singh R, Poland J, Singh D, Joshi A, Singh, P, *et al.* 2020. GWAS study and genomic prediction of spot blotch disease in wheat *(Triticum aestivum L.)* using genotyping by sequencing. *Res Square [Preprint]*.

Tunali B, Nicol J, Hodson D, Uckun Z, Buyuk O, Erdurmus D, Hekimhan H, Aktas H, Akbudak M and Bagci S. 2008. Root and crown rot fungi associated with spring, facultative, and winter wheat in Turkey. *Plant Dis*., **92**: 1299-306.

Wallwork H, Butt M, Cheong J, and Williams K. 2004. Resistance to crown rot in wheat identified through an improved method for screening adult plants. *Aust Plant Pathol*., **33**: 1–7.

Wang M, Wang S, Liang Z, Shi W, Gao C and Xia G. 2018. From genetic reserve to genome editing: gene exploitation in wheat. *Trends Biotechnol*., **36 (2)**: 160-172.

Ward TJ, Clear RM, Rooney AP, O'Donnell K, Gaba D, Patrick S, Starkey DE, Gilbert J, Geiser DM, Nowicki TW. 2008. An adaptive evolutionary shift in Fusarium head blight pathogen populations is driving the rapid spread of more toxigenic *Fusarium graminearum* in North America. *Fungal Gent Biol*., **45**: 473-484.

Yang X, Zhong S, Zhang Q, Ren Y, Sun C, and Chen F. 2021. A loss-of-function of the dirigent gene TaDIR-B1 enhances tolerance to Fusarium crown rot in wheat. *Plant Biotechnol J*., **19**: 866–868.

Ykhlef N and Djekoun A. 2000. Hydric stress, photochemical activity and drought resistance in durum wheat *(T. durum Desf.)*. Wheat Symposium: issues and strategies. 1st international symposium on the wheat grain OAIC. Algiers, Algeria, Pp 133-145.

Zhang P, Guo G, Wu Q, Chen Y, Xie J, Lu P *et al*. 2020. Identification and fine mapping of spot blotch (*Bipolaris sorokiniana*) tolerance gene *Sb4* in wheat. *Theor Appl Genet*., **133**: 2451–2459.

Zhang Q, Men X, Hui C, Ge F, Ouyang F. 2022. Wheat yield losses from pests and pathogens in China. *Agric Ecosyst Environ*., **326**: 107-821.

Zhou F, Li D, Song Y, Fan Y, Guan Y, Song P, Wei Q, Yan H, Li C. 2020. Biological characteristics and molecular mechanism of Fludioxonil resistance in *Fusarium graminearum* in China. *Plant Dis*., **104**: 2426-2433.

Zhu X, Qi L, Liu X, Cai S, Xu H, Huang R, Li J, Wei X and Zhang Z. 2014. The wheat transcription factor, an ethylene response factor that mediates host responses to both *Rhizoctonia cerealis*, a necrotrophic pathogen, and to freezing stress. *Plant Physiol*., **164(3)**: 1499-514.