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MATING TYPE CHARACTERIZATION OF *FUSARIUM CULMORUM* STRAINS CAUSING WHEAT CROWN ROT IN IRAQ

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A B S T R A C T

Fusarium crown rot (CR) of wheat is one of the most important diseases known from regions of the world where cereal crops are produced. In this study, we examined the mating types of *F. culmorum* CR strains isolated across Iraq, including more arid regions within the country. The result showed two mating compatibility (MAT) type idiomorphs of *F. culmorum* are present in Iraq, with 27.6% of the isolates examined representing the MAT-1 type and 72.4% representing the MAT-2 type. The MAT-1 type was commonly found among isolates from the mid-latitudes of Iraq, this being a warmer and drier region as compared to the country's northern region; however, it was also detected from one isolate from a northern site. The MAT-2 type, however, was more broadly distributed across Iraq and was also detected among many isolates from the mid-latitude region. Thus specific biogeographic patterns were not apparent for mating compatibility idiomorphs. The mating types characterized here were also compared to chemotypes known for these same isolates and characterized in a previous study. This comparison revealed the MAT-1 type was found only among DON chemotype isolates, while the MAT-2 type was found among both DON and NIV chemotype isolates. Thus, there does not appear to be a direct correspondence between chemotype and mating compatibility idiomorphs within Iraq. Overall, a broader sampling across the country with more isolates being examined at each site is advised.

Keywords: diversity, DON, *Fusarium*, MAT, NIV, PCR

INTRODUCTION

Fusarium spp. are well known fungal pathogens that cause chronic and destructive diseases of cereal grains. *Fusarium culmorum* is among the important pathogens causing crown rot (CR) and head blight (HB) of wheat (Obanor *et al.,* 2010; Matny 2015). These diseases are known to influence grain quality as they produce mycotoxins that are harmful to human and livestock (Najim 2013; Matny 2015). Studies suggest *F. culmorum* is replacing other species, such as *F. graminearum*, as the primary CR and HB strains in some parts of Europe, and this is likely due to climatic factors (Waalwijk *et al.,* 2003; Jennings *et al.,* 2004; Miedaner *et al.,* 2008). *Fusarium culmorum* is a common cause of CR in the Middle East (Motallebi *et al.,* 2015), including Iraq where it is widespread and potentially among the more aggressive CR causing strains found there (Matny *et al.,* 2012, 2016) *Fusarium* is an asexual fungus (anamorph) in the Ascomycota, with the ascospore producing sexual stage (teleomorph) known in the genera *Calonectria*, *Gibberella*, and *Nectria* (Booth 1981). Ascomycota species can be homothallic (self-fertile) or heterothallic (self-sterile), and mating compatibility in this phylum is controlled by a single mating compatibility locus (MAT) with two dissimilar (idiomorphic) alleles, MAT-1 and MAT-2 (Niessen 2007). Homothallic *Fusarium* species, such as *F. graminearum* (teleomorph = *Gibberella zeae*), have both alleles in a contiguous arrangement (Donnell *et al.,* 2004). *Fusarium culmorum*, on the other hand, is thought to be heterothallic (Kerényi *et al.,* 2004) and has

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only one allele, either MAT-1 or MAT-2 (Tóth *et al.,* 2004). The teleomorphic stage of *F. culmorum* has never been discovered; however, population studies suggest sexual recombination does occur in the species (Gargouri *et al.,* 2003).

In a previous study, we demonstrated the existence of distinct wheat CR associated chemotypes in Iraq, with the DON-type being widespread and the NIV-type restricted to mid-latitudes of the country (Matny *et al.,* 2016). Recent studies have shown a close to 1:1 distribution of *F. culmorum* idiomorphs across the globe, including various parts of the Middle East (Obanor *et al.,* 2010) and further suggesting sexual recombination. However, the distribution of mating types for Iraqi *F. culmorum* strains has not been investigated. Here we further characterize Iraqi strains of *F. culmorum*, using specific primers to determine mating types of isolates from CR diseased wheat collected across the country. We hypothesized that both MAT-1 and MAT-2 alleles would be detectable within *F. culmorum* isolates from Iraq and that they would also exhibit distinct biogeographic patterns, similar to those observed previously for *F. culmorum* CR associated chemotypes (Matny *et al.,* 2016).

MATERIALS AND METHODS

Wheat plants exhibiting crown rot symptoms were collected from various locations in north and central Iraq (Figure 1). *Fusarium* isolates were prepared using methods outlined previously (Matny *et al.,* 2016). Briefly, a 1 cm^2 of diseased tissue was harvested from each plant using a scalpel sterilized with 10% sodium hypochlorite (bleach). Individual tissue samples were placed onto 9 cm Petri dishes of potato dextrose agar (PDA) and were then incubated at 25˚ C for 5 days. A single spore was removed from each *Fusarium* isolate exhibiting a unique morphology and was then used to inoculate a new Petri dish in order to prepare pure cultures following the method of Leslie and Summerell (2006). Sequences of the marker gene *translation elongation factor 1 alpha* (TEF-1α) gene were used to confirm the species identity of each morphotype recovered (see below).

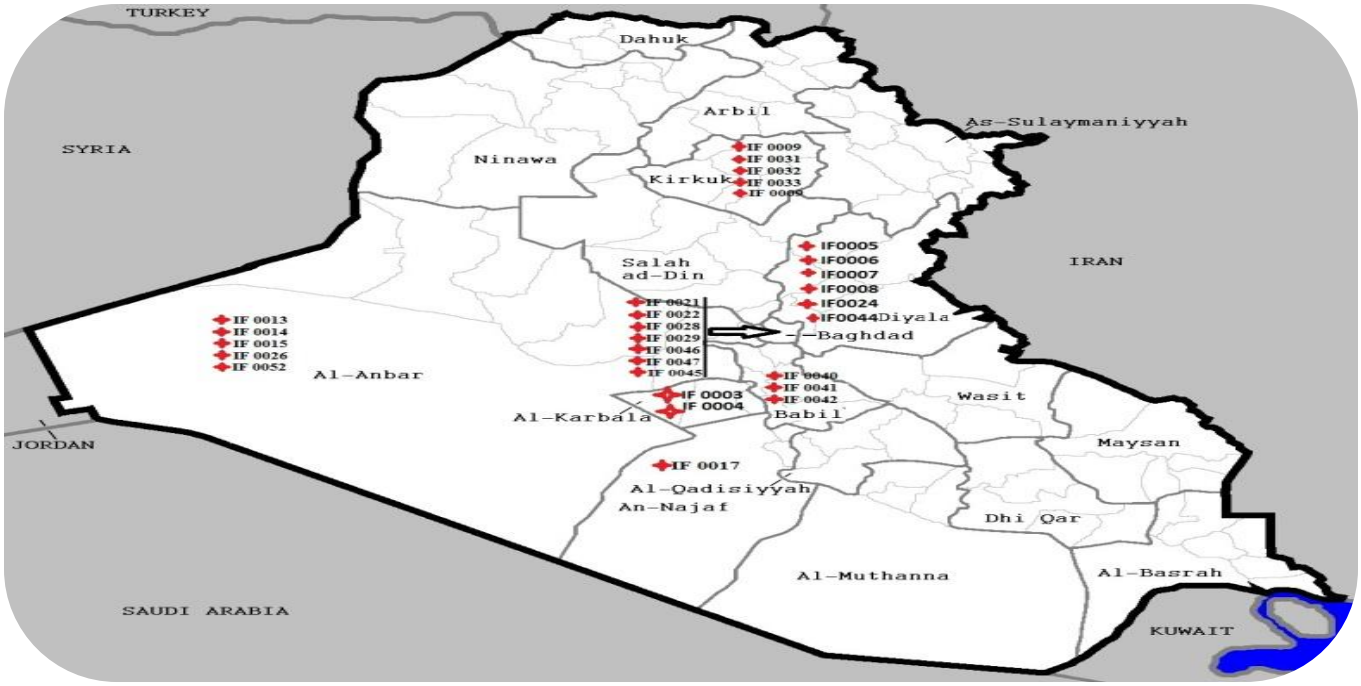

Figure 1. Map showing localities of collecting sites for *Fusarium* isolates obtained from different Iraqi wheat fields.

DNA Extraction and amplification of fungal DNA: DNA was extracted from each of the 29 morphotype isolates recovered following the standard protocol for REDExtract-N-Amp Plant Tissue Kits (Sigma-Aldrich, USA), with a minor modification. Briefly, a small portion of hyphae was removed with a sterilized needle from the growing edge of the fungal mycelium and placed into 0.2 ml centrifuge vials with 50 µl of kit extraction solution and heated to 65˚ C in the thermocycler for 10 min before following the standard kit procedure. Extracted DNAs were tested for quality and quantified using a NanoDrop 2000 (Thermo-Fisher Scientific, USA).

Amplification of *translation elongation factor 1 alpha* (TEF-1α) was carried out using the primers *EF1* and *EF2*

(O'Donnell *et al.,* 2000) following the protocol outlined previously (Matny *et al.,* 2016). Primer pairs used in the mating type study (Table 1) included *fusALPHAfor* and *fusALPHArev* (amplifying the mating-type alpha-box protein MAT1-1) and *fusHMGfor* and *fusHMGrev* (amplifying the high mobility group HMG-box protein MAT1-2). For these primers, each 20 µl PCR reaction contained 10 µl of GoTaq Master Mix (Promega, USA), 0.5 µl (10 nM) of each primer, 5 µl of DNA-free water, and 4 µl DNA template (~5–10 ng). The following parameters were used in PCR: 94˚ C for 3 min (1 cycle), followed by 35 cycles at 94˚ C for 30 s, 55˚ C for 45 s, and 72˚ C for 2min, these cycles being followed by a final extension step of 72˚ C for 5 min. Amplified products were visualized in 1% agarose gels stained with SYBR safe DNA gel stain (Invitrogen, USA) in 1X TAE.

DNA sequencing: To prepare the DNAs for sequencing, the QIAquick PCR purification kit (Qiagen, USA) was used following the standard protocol. DNA was quantified and checked for purity using a NanoDrop 2000. Sequencing was carried out commercially at ACGT, Inc. (Chicago, USA). The resultant sequences were edited manually in MEGA6 (Tamura *et al.,* 2013) after reviewing the chromatograms. To confirm species identities, TEF-1α sequences were compared with those in GenBank using BLAST (Altschul *et al.,* 1990). The consensus sequences generated for each isolate were submitted to GenBank, and the accession numbers for these are found in Table 2.

Table 1. Primers used in this study.

Primer	Target Gene	Nucleotide sequence (5´to 3´)	Product band/bp	Annealing temp. (˚C)	References
fusALPH for	*MAT-1*	CGCCCTCTKAAYGSCTTCATG	200	55	Kerényi *et al.* (2004)
fusALPHArev		GGARTARACYTTAGCAATYAGGGC			
fusHMGfor	*MAT-2*	CGACCTCCCAAYGCYTACAT	260	55	Kerényi *et al.* (2004)
fusHMGrev		TGGGCGGTACTGGTARTCRGG			

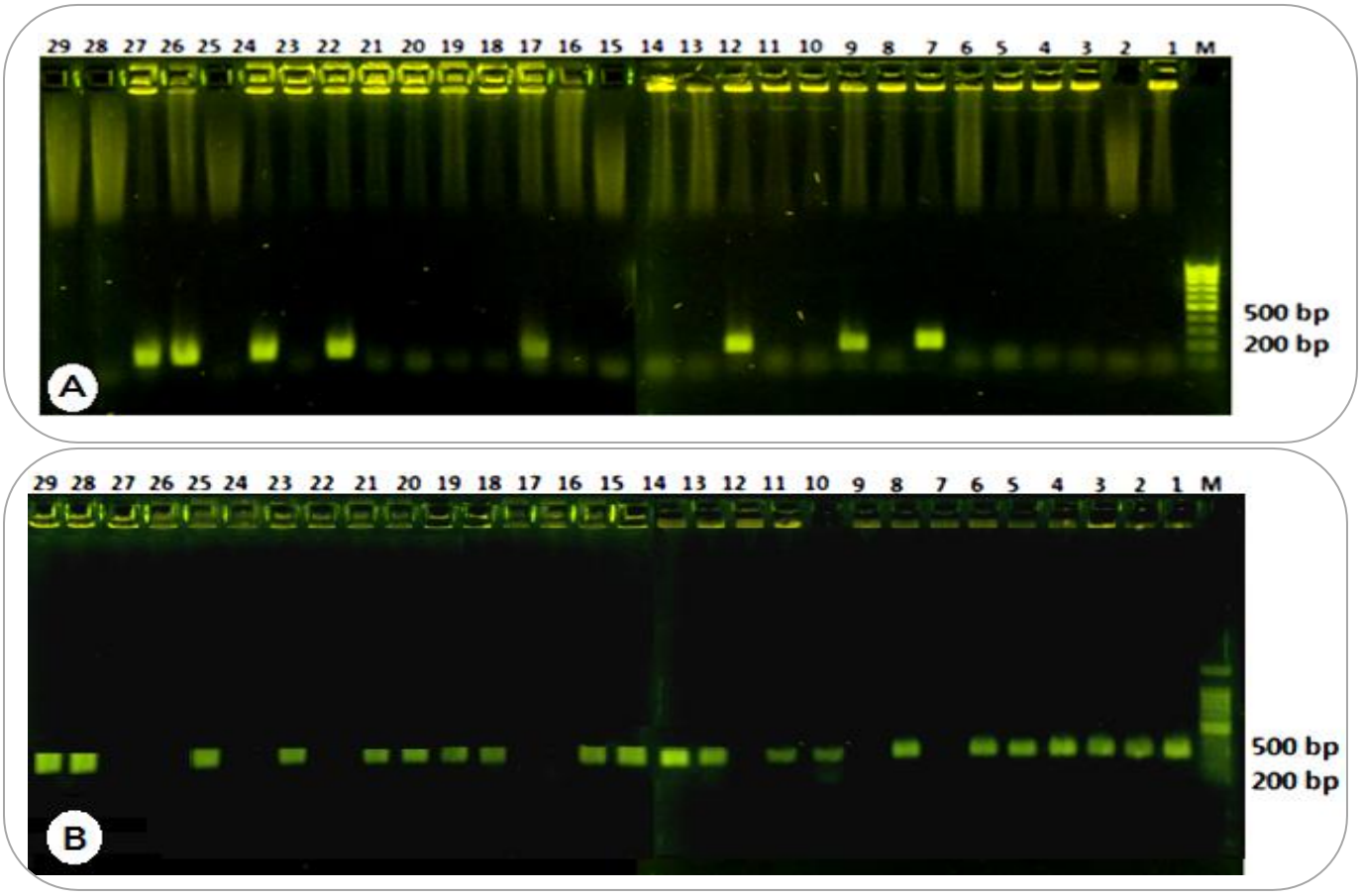

Figure 2. Mating type profiles of *F. culmorum* isolates 1-29 examined in this study. A- Amplification of ALFA box (MAT-1 type). B- Amplification of HMG box (MAT-2 type).

RESULT AND DISCUSSIONS

Sequencing of the *TEF-1α* gene confirmed the identity of our isolates to be among the *F. culmorum* species group (all identities >97% sequence similarity), which is a known CR pathogen of wheat in Iraq (Matny *et al.,* 2012). Subsequently, each *F. culmorum* isolate was subjected to PCR with the two different mating type diagnostic primers. Eight (27.6%) of the *F. culmorum* isolates showed the characteristic 200bp fragment corresponding to MAT-1 and 21 of the isolates (72.4%) showed the characteristic 260bp fragment of MAT-2 (Figure 2). The MAT-2 mating type was distributed widely across all sites in Iraq, being found among isolates from Anbar, Baghdad, Babylon, Diyala, Karbala, Kirkuk, and Najaf (Table 2). Likewise, the MAT-1 type isolates were found broadly across Iraqi sites, but in only four (Anbar, Baghdad, Babylon, and Kirkuk) of the seven sites sampled. Overall, the MAT-2 type was found more frequently across the Iraqi site and appears to be restricted to only three sites (Diyala, Karbala, and Najaf); however, two of these sites were not sampled extensively, with only one or two isolates being recovered from Najaf and Karbala, respectively. While the MAT-1 type was found more frequently among warmer, drier sites of the mid-latitude region of Iraq, it was also detected in one isolate from a site (Kirkuk) in the northern region. Thus, mating compatibility locus idiomorphs (MAT-1 or MAT-2) do not appear to be restricted to particular regions and may be generally distributed, though not evenly, within specific areas widely across the country.

Table 2. Molecular characterization of *F. culmorum* mating types for Iraqi isolates (*see Matny *et al.,* 2016 for chemotype characterization).

Sample number	Accession No.	Culture No.	Location	Species	Mating type		Chemotypes*	
					MAT-1	MAT-2	DON	NIV
1	KY205745	IF 0003	Karbala	*F. culmorum*	-	+	-	+
2	KY205746	IF 0004	Karbala	*F. culmorum*	-	+	-	+
3	KY190104	IF 0005	Diyala	*F. culmorum*	-	+	+	-
4	KY190106	IF 0006	Diyala	*F. culmorum*	-	+	+	-
5	KY190111	IF 0007	Diyala	*F. culmorum*	-	+	+	-
6	KY190107	IF 0008	Diyala	*F. culmorum*	-	+	+	-
7	KY190127	IF 0009	Kirkuk	*F. culmorum*	+	-	+	-
8	KY190118	IF 0013	Anbar	*F. culmorum*	-	+	-	+
9	KY190123	IF 0014	Anbar	*F. culmorum*	+	-	+	-
10	KY190116	IF 0015	Anbar	*F. culmorum*	-	+	+	-
11	KY190108	IF 0017	Najaf	*F. culmorum*	-	+	+	-
12	KY205747	IF 0021	Baghdad	*F. culmorum*	+	-	+	-
13	KY190121	IF 0022	Baghdad	*F. culmorum*	-	+	+	-
14	KY190126	IF 0024	Diyala	*F. culmorum*	-	+	+	-
15	KY190122	IF 0026	Anbar	*F. culmorum*	-	+	+	-
16	KY190112	IF 0028	Baghdad	*F. culmorum*	-	+	+	-
17	KY205748	IF 0029	Baghdad	*F. culmorum*	+	-	+	-
18	KY190109	IF 0030	Kirkuk	*F. culmorum*	-	+	+	-
19	KY190113	IF 0031	Kirkuk	*F. culmorum*	-	+	+	-
20	KY190114	IF 0032	Kirkuk	*F. culmorum*	-	+	+	-
21	KY190117	IF 0033	Kirkuk	*F. culmorum*	-	+	+	-
22	KY190124	IF 0040	Babylon	*F. culmorum*	+	-	+	-
23	KY190110	IF 0041	Babylon	*F. culmorum*	-	+	+	-
24	KY190119	IF 0042	Babylon	*F. culmorum*	+	-	+	-
25	KY205749	IF 0044	Diyala	*F. culmorum*	-	+	+	-
26	KY190105	IF 0045	Baghdad	*F. culmorum*	+	-	+	-
27	KY190125	IF 0046	Baghdad	*F. culmorum*	+	-	+	-
28	KY190120	IF 0047	Baghdad	*F. culmorum*	-	+	-	+
29	KY190115	IF 0052	Anbar	*F. culmorum*	-	+	-	+

The distribution of mating types had no direct correspondence to patterns observed previously in Iraq for *F. culmorum* isolate chemotypes (Matny *et al.,* 2016). The MAT-1 type isolates of this study were found only occasionally among DON chemotypes, while the MAT-2 mating type was found among both DON and NIV chemotypes (Table 2). These results are consistent with those of Laraba *et al.* (2017) who found the mating type

idiomorphs of *F. culmorum* are essentially evenly distributed, even within regions where the DON chemotypes considerably outnumber those of the NIV-type. Additionally, Rebib *et al.* (2014) reported that both MAT1-1 and MAT1-2 mating types of *F. culmorum* were found in approximately equal proportions in wheat fields in Tunisia, where all isolates were exclusively DON chemotypes (3-AcDON).

The sexual status of *F. culmorum* continues to be the subject of debate, with studies hypothesizing the occurrence of sexual reproduction within the species (Mishra *et al.,* 2003; Obanor *et al.,* 2010); however, a teleomorph has never been reported for this species. We found nearly three-fourths of our isolates were of the MAT-2 type across Iraq with no clear patterns related to geography, collection locality climate, or chemotype status. This finding is within the range of haplotype diversity documented from a number of countries (Miedaner *et al.,* 2008), but does not suggest sexual recombination to the degree that the results of Obanor *et al.,* (2010) did, which found MAT-1 and MAT-2 mating types in nearly equal proportions across a portion of three continents, with Middle Eastern isolates exhibiting a perfect 1:1 correspondence. With the absence of a known sexual state, another mechanism of recombination for *F. culmorum*, such as long-range conidia dispersal, is an area of research that needs to be addressed. However, continued monitoring of disease-associated strains of *F. culmorum* more broadly across Iraq, and other countries in the region, and with more isolates being sampled across individual sites is advisable given the potentially aggressive strains as well as the levels recombination that occur there.

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