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Field treatment of three wheat varieties with *Trichoderma harzianum* bioagent to control *Anguina tritici*

Nawres Abdulelah Sadeq Alkuwaiti*, Ammar Amjad Aish, Saad Tareq Abdulmalak, Tariq A. Kareem, Mohammed Mahmood Sulaiman

Plant Protection Department, College of Agricultural Engineering Sciences, University of Baghdad, Baghdad, Iraq.

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ABSTRACT

A field experiment was performed to assess the susceptibility of *Triticum aestivum* L. var. Cham 6, Aras, and Baraka wheat local varieties, treated with *Trichoderma harzianum* Rifai bioagent, against seed gall infection. *Anguina tritici* identification was confirmed based on PCR amplification and sequence comparison of 18S small subunit ribosomal DNA. Sequence analysis showed *A. tritici* isolated from Waset and Kirkuk provinces shared 100% maximum nucleotide sequence identity with the equivalent GenBank sequences from Mexico (AF363107) and India (JF826516), respectively, suggesting their common origin. Field experiment revealed that *T. aestivum* L. var. Aras was less susceptible to seed call infection when scored 28.9% infectivity percent followed by Baraka and Cham 6 varieties which scored 29.6 and 33.1% infectivity, respectively. *T. harzianum* root watering treatment could decrease the disease incidence when scored 15.4, 17.5, and 19.7% compared to spraying treatment which scored 19.3, 20.8, and 22.4% infectivity, for Aras, Baraka, and Cham 6 varieties, respectively. Besides, root watering treatment reduced seed gall disease up to 46.71% compared to 33.22% for foliar spraying treatment. Thus, *T. harzianum* bioagent can be used as an ecofriendly nematicide alternative to control seed gall disease in Iraq.

1. INTRODUCTION

Wheat *Triticum aestivum* L. is a major grain crop grown worldwide within the family Poaceae [1]. It is thought this plant has originated from southeastern parts of Turkey 10,000 years BC [2]. Whereas in Iraq, bread wheat has been domesticated 9500 years BC [3]. Wheat is grown in Iraq in winter season. Based on 2020 statistics, the estimated Iraqi production and growing area of wheat were 6,238,392 tons and 2,143,421 ha, respectively [4]. About 46 different wheat varieties have been reported to be grown in Iraq [5]. Wheat is attacked by several pests including nematodes [1]. At least, four nematode groups infect wheat including cereal cyst, root knot, root lesion, and seed gall nematodes [5]. *Anguina tritici* (Steinbuch, 1799) Chitwood, 1935, or ear cockle nematode is a major plant pathogenic nematode infecting wheat causing serious losses worldwide [6]. It has been described for the 1st time by Needham since 1743 [1]. Seed gall disease has been reported in Iraq for the 1st time since 1921 [7] but the causal agent *A. tritici* was recently confirmed by molecular approach [8]. This disease can cause yield losses ranged 10.76–75% in wheat [9,10]. Limited studies conducted in Iraq were investigated *A. tritici* control which were based on cultural, chemical, and biological methods [11-13]. Due to the limited molecular studies regarding seed gall disease in Iraq, this study, therefore, was initiated to identify the nematode causing seed gall disease in Wasit and Kirkuk provinces and its phylogenetic relatedness and to investigate the efficacy of the bioagent *Trichoderma harzianum* against seed gall disease on three wheat in the study area.

2. MATERIALS AND METHODS

2.1. Pathogenic Nematode Isolation and Inoculum Preparation

In 2021 growing season, gall samples were obtained from wheat fields located at Kirkuk and Waset provinces in Iraq. Ten galls were socked in 500 ml sterilized distilled water for 24 h at ambient temperature to release the second stage juveniles (J2). The number of J2 nematode was estimated using microscopic examination at 40X [14].

2.2. Molecular Conformation of the Pathogenic Nematode

Two seed galls from each location were crushed with liquid nitrogen and DNA extraction was performed using commercial DNA extraction kit (Bioneer, South Korea), following the manufacturer's instructions. PCR amplification was performed following [15], using AccuPower PCR PreMix commercial kit (Bioneer, South Korea) and ITS rRNA universal primers (rDNA2: TTGATTACGTCCCTGCCCTTT and rDNA1: ACGAGCCGAGTGATCCACCG) [16,17]. DNA

*Corresponding Author:
Nawres Abdulelah Sadeq Alkuwaiti,
Plant Protection Department, College of Agricultural Engineering Sciences, University of Baghdad, Baghdad, Iraq.
E-mail: n.alkuwaiti @ coagri.uobaghdad.edu.iq

fragment amplified was visualized by ethidium bromide agarose gel electrophoresis [18]. PCR products were directly sequenced (Macrogen, South Korea). Sequences were analyzed using MEGA11 [19] and Sequence Demarcation Tool Version 1.2 (SDT v 1.2) [20] software packages.

2.3. Field Experiment

Local wheat varieties *T. aestivum* L. vars. Cham 6, Baraka, and Aras were tested against seed gall nematode infection. The experimental field was plotted into 1 m^2 areas with three replicates of each treatment.

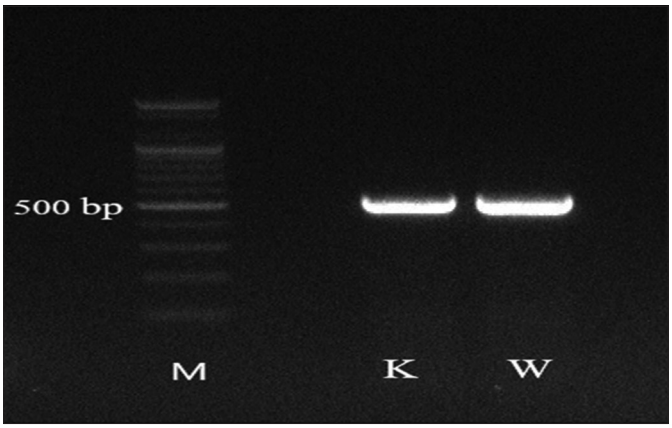

Figure 1: Ethidium bromide stained gel pattern shows ~ 500 bp amplified by rDNA2/rDNA1 primer set from seed galls collected. K: Kirkuk sample, W: Wasit sample, and M: 100 bp DNA marker (Bioneer, S. Korea).

T. harzianum suspension (in the form of trichozone biopesticide, Al-Joud Company for Industry and Modern Agriculture, Iraq), was applied by foliar spraying or root watering at concentration 2 g/L. Nematode inoculum, adjusted to 10,000 J2 individuals' concentration, was applied at 1000 mL/m^2 rate, 25 days of seed sowing. All agricultural practices necessary for plant growth were followed, during the experiment. Infectivity percent and disease reduction (DR) were calculated using the following equation:

$$\text{Infectivity\%} = \left(\frac{\text{Number of infected seeds}}{\text{Total number of seeds}}\right) \times 100$$

$$\text{DR \%} = \left(\frac{\text{Number of seed galls in infected control-Number of seed galls in treatment}}{\text{Number of seeds galls in infected control}}\right) \times 100$$

Microsoft Excel 2010 was used to analyze data calculated.

3. RESULTS AND DISCUSSION

Sequence comparison confirmed DNA fragments amplified [Figure 1] were identical to18S small subunit (SSU) ribosomal DNA genomic region of *A. tritici* retrieved from the NCBI [Figure 2]. *A. tritici* isolated from Wasit and Kirkuk scored 100% maximum nucleotide (nt) sequence identities to equivalent GenBank sequences from Mexico (AF363107) and India (JF826516), respectively, suggesting their common origin [Figure 2]. Neighbor-joining (NJ) phylogenic tree, based on SSU nt sequences, confirmed the relatedness when grouped Wasit and Kirkuk sequences to relevant isolates from Mexico and India [Figure 2]. Despite the high identity, Wasit and Kirkuk isolates showed

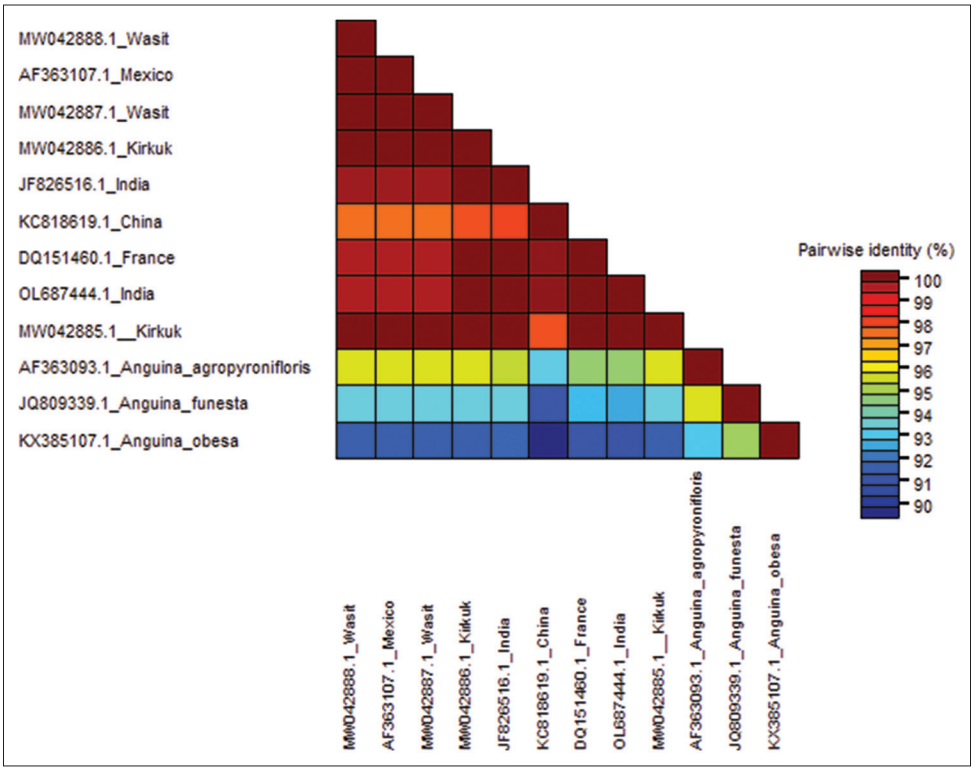

Figure 2: Three color mode matrixes showing *A. tritici* identities, constructed from partial 18S SSU ribosomal DNA sequences of Kirkuk and Wasit and equivalent sequences from the GenBank. *A. agropyronifloris* Norton, 1965, *A. funesta* Price, Fisher and Kerr, 1979, and *A. obesa* Mobasseri, Pedram, Pourjam and Bertozzi, 2017, were used as out-group comparisons. Data were analyzed using Muscle algorithm. This matrix was generated using Sequence Demarcation Tool Version 1.2 (SDTv1.2) software package [20].

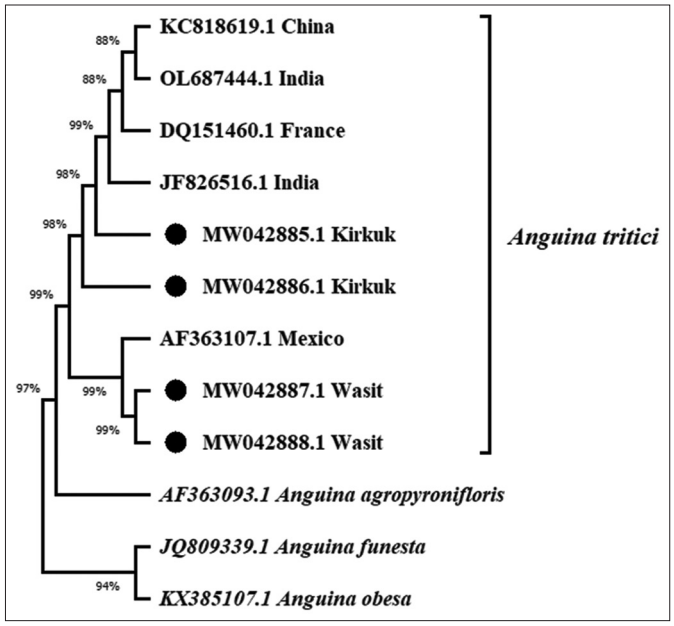

Figure 3: Neighbor-joining phylogenetic tree constructed from partial 18S SSU rDNA nucleotide sequences of *Anguina tritici* from Iraq (marked with ●) and equivalent sequences from the GenBank. *A. agropyronifloris* Norton, 1965, *A. funesta* Price, Fisher and Kerr, 1979, and *A. obesa* Mobasseri, Pedram, Pourjam and Bertozzi, 2017, were used as out-group comparisons. Data were analyzed using Maximum Composite Likelihood method. This tree was constructed by MEGA 11 software [19].

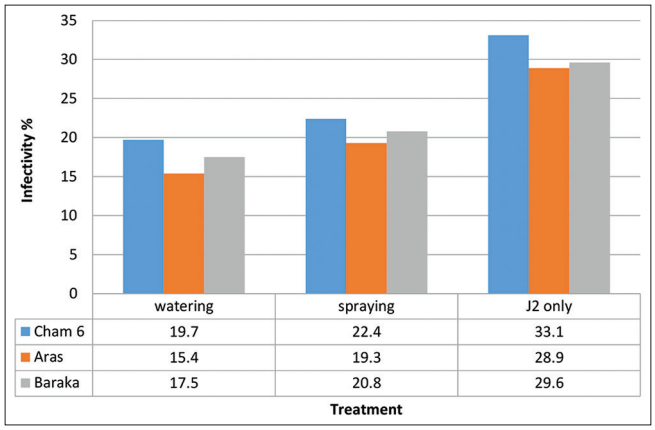

Figure 4: *T. harzianum* field treatments of three wheat varieties (Cham 6, Aras, and Baraka) against *A. tritici* using foliar spraying and root watering applications. Root watering treatments show the lowest infectivity percentages. J2: Represents untreated infarcted control. This bar chart was generated using Microsoft Excel 2010.

that they could be two variants when N-J phylogenetic tree separated them into two clades within *A. tritici* branch [Figure 3]. Thus, SSU could be useful molecular loci for identification, differentiation, and phylogeny reconstruction of *A. tritici* in Iraq [8,21].

This variation may be related to the selective pressure of many constraints favoring certain pathotypes over others in wheat growing area including varieties grown, alternative hosts available, and limited movements of nematode-infected plant materials, nationwide [22,23].

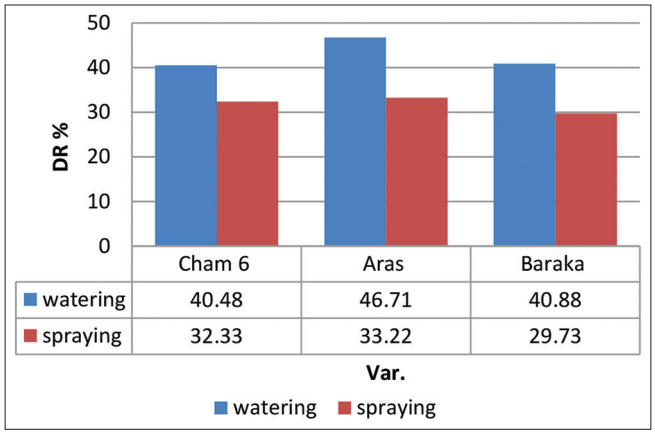

Figure 5: Disease reduction (DR) percentages resulted from *T. harzianum* field treatments of three wheat varieties (Cham 6, Aras, and Baraka) against *A. tritici* using foliar spraying and root watering applications. Root watering treatments show the lowest infectivity percentages. J2 only: Untreated infarcted control. This bar chart was generated using Microsoft Excel 2010.

Field experiment revealed that *T. harzianum* treatments could decrease infectivity percent and reduce seed gall disease in all three varieties compared to J2 only treatment. Root watering treatment showed high efficiency to control seed gall disease scoring 15.4% lowest infectivity [Figure 4] and 46.71% highest DR [Figure 5] in Aras variety, compared to foliar spraying. Root watering treatment with *T. harzianum* might minimize *A. tritici* infection much effectively than foliar spraying through direct interaction with J2 individuals, systemic induced resistance, and/or promoting or inducing plant growth [24,25]. This study confirmed the incidence of *A. tritici* in two Iraqi provinces, Kirkuk and Wasit. Based on phylogenetic relatedness and high identity percentage, these two geographical variants may have been introduced into Iraq through imported contaminated wheat seeds in the near past [26]. Except Saber-Beg, all wheat varieties are seed gall sensitive [27-29]. Aras, Baraka, and Cham6 wheat varieties showed to be sensitive to seed gall disease infection. Field experiment revealed that Aras wheat variety was less susceptible to seed call infection when scored 28.9% infectivity percent followed by Baraka and Cham 6 which scored 29.6 and 33.1% infectivity, respectively [Figure 4]. These three varieties are cultivated in Iraq due to their high yield quality and quantity compared to Saber-Beg. Bioagent treatment of desirable varieties can be the most efficient among others controlling methods as it offers a sustainable, eco-friendly alternative nematicide, to manage seed gall disease in Iraq [30]. Besides, it can enhance wheat production through promoting plant growth [24,25].

4. CONCLUSIONS

This study confirmed the detection of *A. tritici* collected from two Iraqi provinces, Kirkuk and Wasit. Phylogenetic relatedness showed high identity percentage of the Iraqi seed gall nematode to the equivalent GenBank isolates from Mexico and India. Root treatment with *T. harzianum* was much effective than foliar treatment, against *A. tritici* infection, when decrease the infectivity percent of seed galls in all treatments. *T. aestivum* L. var. Aras was less susceptible to seed gall infection compared to Baraka and Cham 6 varieties. Bioagent treatment of desirable varieties can be the most efficient among others controlling methods as it offers a sustainable, eco-friendly alternative nematicide, to manage seed gall disease in Iraq.

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6. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors requirements/ guidelines.

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8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

The authors confirmed that all the relevant data were included in the article.

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