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ABSTRACT

The identification of wheat endophytes that are present in locally grown wheat is a necessary step in developing the potential of endophytes in order to enhance wheat production in Jordan. The main objectives of current research were to (i) isolate and identify cultivable fungal wheat endophytes from healthy wheat plants grown in different regions in Jordan and to (ii) verify the endophytic characteristics through conducting *invitro* test on seeds and testing the germination success of seeds with and without endophyte. Wheat plants were collected from different wheat growing regions in northern, middle and southern Jordan. Fungal endophytes were isolated from wheat roots and aerial organs, including leaves, stems, and spikes using the cultivable dependent approach. A total of 83 representatives of the most dominant cultivable endophytes were sequenced using the ITS4/5 gene region. All the identified isolates were belonging to phylum Ascomyctoa, sub-phylum Pezizomycotina. In Pezizomycotina, five classes, seven orders, and 12 families were recognized. Sordariomycetes accounted for the highest frequency followed by Dothidiomycetes. Fungi were most abundant in roots as compared to the other plant parts from which they were isolated. A total of 22 genera and 44 species were identified from different wheat plant parts. *Chaetomium* sp. was the most recovered fungus followed by *Fusarium* sp. and *Alternaria* sp. Different genera were identified from the same organ and some were identified from all plant parts. Some of the isolated fungi had been reported in previous studies as pathogenic to wheat such but according to the pathogenicity *in vitro* experiment, all the tested isolates except one isolate were non-pathogenic and were not significantly different in all the parameters from the control. Seven genera and two unknown fungal species are new reports as fungal endophytes in wheat. The distribution of the different fungal endophytes among the different governates showed diversity and richness for some genera and in certain locations. For example *Chaetomium* was found in all governates suggesting the adaptability of this fungus to wheat regardless of the location. Future work is in progress to study the effect of these endophytes on wheat agronomic traits, as biological control agents against wheat major diseases, and as aphid repellents.

KEYWORDS:
Fungi, Endophytes, Identification, Distribution, Wheat, Jordan

INTRODUCTION

The word endophyte means “in the plant” (endon Gr. = within, phyton = plant). Endophyte describes microorganisms (bacteria and fungi) that can be detected within the tissue of healthy plant host at a particular moment [1]. Most endophytes are facultative that are able to colonize different plant tissues [2, 3]. The potential fungal pathogens for some hosts may be asymptomatic for others [4].

Endophytes represent a large component of microbial biodiversity and have been isolated from almost all plants [5, 6]. Many factors such as host species, genotypes, geographic location, and plant organs can influence endophyte colonization [6].

Wheat (*Triticum aestivum* L.) is an important staple and strategic cereal crop for the majority of world’s populations. The domestication, selection and breeding are methods used throughout the history to improve yield of wheat [7]. Wheat is infected by many economical fungal pathogens [8] that cause high yield reduction and in some of them such as *Fusarium* spp. can produce mycotoxins that are very toxic to plants and animals [9]. Fungicides along with cultural control methods are used to control these diseases. There are no fully resistant wheat cultivar exists. The use of endophytic symbionts is a promising alternative approach for wheat improvement and can enhance seed germination [10]. Additionally, wheat endophyte can protect from biotic and abiotic stresses [11, 12, 13], and can reduce the need for irrigation.

Fungi were found to be a dominant endophyte of wheat [14, 15, 16, 17, 18, 19]. However, most of the previous studies and surveys relate only to Claviceptaceae endophytes [20, 21, 22]. On the other hand, bacterial endophytes were a major focus in wheat especially Actinobacteria, [23, 24, 25, 26].

The wheat crop may be cultivated in many areas of Jordan that have abiotic stresses such as salinity and drought. The identification of wheat endophytes that are present in locally grown wheat is a necessary step in developing the potential of endophytes in order to enhance wheat production in Jordan. Moreover, endophytes in general and in wheat in particular, is not well explored and needs to be investigated in Jordan. The main objectives of current research were to (i) isolate and identify cultivable fungal wheat endophytes from healthy wheat plants grown in different regions in Jordan and to (ii) verify the endophytic characteristics through conducting *invitro* test on seeds and testing the germination success of seeds with and without endophyte.

MATERIALS AND METHODS

Sampling. Wheat plants were collected from different wheat growing regions in northern, middle and southern Jordan (Figure 1). Five healthy plants were randomly collected per field. The total number of fields was 88 fields. Longitude, latitude and altitude information were recorded for each field. The stage of the wheat plants was the heading stage. Plants were collected in the period of March to May 2017. The samples were kept in large paper bags and brought to the laboratory for further analysis.

Isolation and Purification of Fungal Endophytes. The roots and aerial organs, including leaves, stems, and spikes were excised, separated, and surface-sterilized by washing with tap water, dipping in 70% Ethanol for 2 min, in 0.5% NaOCL for two minutes, in 70% EtOH for 1 min and rinsed briefly in sterile distilled water [14]. The plant parts were placed on sterile 9 – cm Whatman filter papers and air - dried under laminar flow hood. Randomly, 24 pieces (1.5-2.0 cm long) representing the different plant parts from the same field were excised. Pieces for each part were placed on two potato dextrose agar PDA (HIMEDIA Inc) amended with Ampicillin antibiotic. The PDA plates were incubated at 25±2 °C for ten days. Emergent fungal colonies were purified on PDA fresh media for isolation into pure cultures by hyphal tipping.

Identification. Morphological identification of isolates. Cultures of all isolates recovered from the different fungi were first separated based on their color on the PDA plates. Thereafter, each group was further separated to sub-groups based on growth pattern on the media. Slides from representatives were prepared and examined under the microscope to view spores and mycelium shape and color. The most frequent recovered fungal genera were further considered for many testing either in this study or in future studies. Fungi that were existing in low frequency were not considered in this study but will be separately studied.

Molecular Identification. DNA extraction. Total DNA was extracted from 14 days grown fungal cultures using the CTAB method [27] with some modifications. Two hundred milligrams of fungal mycelia were scraped from the petri, mixed with 1000 µl of extraction buffer (2% CTAB powder, 100 mM Tris-HCl 8, 25 mM EDTA, 1.5 M NaCl), 2 µl mercaptoethanol and 20 SDS), and grounded to fine size using plastic pistol inside 1.5 ml Eppendorf tube. Then, the samples were vortexed and the mixture was incubated in water bath at 65±2 ºC for 60 min and inverted every 10 minutes. After incubation, the mixture was spun for 10 min at 13000 rpm (Heraeus - Biofuge fresco centrifuge, Germany). The aqueous phase (upper layer) was transferred into a new 1.5 ml tube. After that, 750-800 µl (one volume) of 24:1 chloroform: Isoamyl alcohol was added under fume hood, mixed well by gently inverting and spun for 10 min at 13000 rpm. The aqueous phase (upper layer) was transferred into a new 1.5 ml tube and then 3 µl of 10 mg/ml RNase was added and incubated at 37 ºC for 30 min (or overnight in the refrigerator). Thereafter, one volume of 24:1 chloroform: Isoamyl alcohol was added under fume hood, mixed well by gently inverting and spun for 10 min at 13000 rpm. The aqueous phase (upper layer) was transferred into a new 2 ml tube, then 0.6x volume of isopropanol or Ice cold 100% ethanol was added and inverted several times to mix, and then it was spun for 10 min at 13000 rpm. Finally, isopropanol was removed and the pellet was washed by adding 500 µl of 70- 95% ethanol, then it was spun for 10 min at 13000 rpm in a 4 ºC-centrifuge. Ethanol was poured and the tubes turned upside down to dry under laminar hood, DNA pellet was re-suspended with 50-100 µl of DNA elution buffer or nuclease free-water. DNA quality and quantity was measured using the Nanodrop ND-1000 spectrophotometer.

Polymerase chain reaction and identification. Polymerase chain reaction (PCR) was performed using the Internal transcribed Spacer (ITS) gene region (Forward: ITS5, Reverse: ITS4) [28]. Each reaction was composed of 12.5 µl of OneTaq® Quick-loading® 2X MM w/ Std buffer # M0486S (BioLabs, England), 1 µl of each of forward and reverse primers (10 µM), 2 µl of DNA (20-100 ng/µl) and the volume was completed to 25 µl with nuclease free water (8.5 ml Water). PCR program used was as follow: initial denaturation at 95 ºC for 5 min, followed by 35 cycles contain denaturation at 95 ºC for 30 sec, annealing at 53 ºC for 30 sec, extension at 72 ºC for one min and a separate final extension cycle at 72 ºC for 10 min. PCR products were tested using 1.5% agarose gel

(CSL-AG500, Cleaver Scientific Ltd. United Kingdome). Easy Stain III (A4205, Biomatik, Canada) (4-5µl / 50 ml gel) was added. Gels were loaded with samples and ran at 80 V for 30 min. Gels were observed on gel documentation system (Alpha Innotech Corp. USA).

PCR products were sent to Macrogen Inc, South Korea for purification and sequencing following the company protocol. Sequences were received as FASTA files, edited and consensus sequences were created using BioEdit V.7.0.5 software [29]. Edited sequences were blasted in NCBI nucleotide database (http://blast.ncbi.nlm.nih.gov) for similar reference sequences to identify species. Sequences were deposited in the GenBank database under accession numbers from MN534768 to MN534850.

Pathogenicity Test. Wheat seeds (cv. Hourani) were heat-sterilized at 55 ºC for 15 minutes in an oven to ensure no external microbes could be found. Primary study for heat sterilization was conducted at different time periods and 55 ºC was chosen based on the germination percentage. Thereafter, seeds were cooled, surface-sterilized by soaking in 70% alcohol for one minute, hypochlorite (1%) for five minutes and washed three times in sterile distilled water. Ten wheat seeds were placed in sterile 11-cm glass plate lined with autoclaved Whatman filter papers. Three replicates per isolate were used. Seeds were treated by soaking in a spore suspension of a 10- days-old culture for 30 minutes. Thereafter, treated seeds were incubated in darkness at 25 ± 2 ºC for five days. The negative controls were seeds soaked in sterile distilled water for 30 minutes and positive controls were seeds soaked in spore suspension of *F. culmorum* isolate (accession number MH001550) [30]. Many measurements were considered after five days: germination percentage, coleoptile length, radicle length, longest seminal root length, number of seminal roots, first leaf length, and sum of above parts length (coleoptile + first leaf).

An index (1-3) was established for the pathogenicity *in vitro* tests to determine whether the fungus is slight pathogen, weak pathogen, or nonpathogenic. The index was based mainly on germination percentage, coleoptile length, radicle length, and longest seminal root. Radicle and longest seminal root were given a value of 0.5 each, while coleoptile and germination were given a value of 1; that is a total of 3. The value for each measurement was compared with 50% of the value for the different parameters in the control. The value/fungus for each measurement was color-coded by red if it is less the 50% of the control value and with green color if it is higher than the controls value (see Table 3). Germination was the starting point for the index. If the germination was less and significant from the control then the fungus would be considered pathogenic to the seeds. If the value was not significantly different from the control then the scale from 1-3 was followed. For example, if the values for germination percentage, coleoptile length, radicle length, and longest seminal root for the control treatment were 80%, 4.00 cm, 6.00 cm, and 8.00 cm, respectively; then the 50% value for all the measurements will be 40%, 2 cm, 3 cm, and 4 cm, respectively. If a fungus X scored 3 (0.5 for radicle length, 0.5 for longest seminal root, 1 for the coleoptile length, and 1 for the germination percentage), in this case fungus X is considered weak pathogen. If the fungus had a score of 2 then it is considered slight pathogen, while if the value was 1, it is considered as non-pathogenic.

Analysis of Data. Analysis of data including ANOVA, means, standard errors and Tukey mean separation test were conducted using Minitab 18 Software [31].

RESULTS

Taxonomy of Endophytic Fungal Community. A total of 1290 fungal isolates were isolated and purified from 88 locations. All sampled plants harbored fungi in their inner tissues. Overall 22 genera and 42 species have been isolated in this study. Information about the taxonomy of the identified fungi was searched at Mycobank database (mycobank.org). The fungal genera were belonging to phylum Ascomycota, sub-phylum Pezizomycotina. In Pezizomycotina, five classes, seven orders, and 12 families were recognized (Table 1). Classes of the identified fungi were Dothideomyctes, Eurotiomycetes, Leotiomyctes, Sordariomycetes, and Pezizomycetes. Fungal orders included Pleosporales, Eurotiales, Heliotiales, Hypocreales, Sordariales, Xylariales, and Pezizales. The families of the identified fungi included Pleosporaceae, Aspergillaceae, Trichocomaceae, Sclerotinaceae, Nectriaceae, Hypocreaceae, Chaetomiaceae, Lasiophariaceae, Sordariaceae, Apiosporaceae, Microdochiaceae, and Ascobolaceae. One fungal species *Monosporoascus* sp. family name was termed Incertae sedis because of the uncertainty of the identity of this fungus family. The taxonomy of Ascomycetes fungal sp. was not included due to the lack of information of the similar ncbi accession.

According to classes, Sordariomycetes accounted for the highest frequency (68.32%) followed by Dothidiomycetes (22%). On the other hand, according to orders, Sordariales had the highest frequency followed by Pleosporeales, and Hypocreales with 46.74%, 22.75%, and 19.80%, respectively. According to family level, the highest frequency percent was for Chaetomiaceae with 43.26% followed by Pleosporaceae (22.75%) and Nectriaceae (19.80%) (Table 1).

TABLE 1
Overview of the diversity of cultivated endophytes from wheat plants.

Phyllum	Ascomycota						
Sup-Phyllum	Pezizomycotina						
	Genus	**Class**	**%**	**Order**	**%**	**Family**	**%**
1	*Ulocladium sp.*	Dothideomycetes	22.75	Pleosporales	22.75	Pleosporaceae	22.75
2	*Alternaria sp.*	Dothideomycetes		Pleosporales		Pleosporaceae	
3	*Bipolaris sp.*	Dothideomycetes		Pleosporales		Pleosporaceae	
4	*Lewia*	Dothideomycetes		Pleosporales		Pleosporaceae	
5	*Pyrenophora sp.*	Dothideomycetes		Pleosporales		Pleosporaceae	
6	*Stemphylium sp.*	Dothideomycetes		Pleosporales		Pleosporaceae	
7	*Penicillium sp.*	Eurotiomycetes	7.45	Eurotiales	7.45	Aspergillaceae	6.99
8	*Paecilomyces sp.*	Eurotiomycetes		Eurotiales		Trichocomaceae	0.47
9	*Stromatinia sp.*	Leotiomycetes	0.16	Helotiales	0.16	Sclerotiniaceae	0.16
10	*Fusarium sp*	Sordariomycetes	68.32	Hypocreales	19.80	Nectriaceae	19.61
11	*Trichoderma sp.*	Sordariomycetes		Hypocreales		Hypocreaceae	0.16
12	*Monosporascus sp.*	Sordariomycetes		Sordariales	46.74	Incertaesedis	0.54
13	*Chaetomium sp.*	Sordariomycetes		Sordariales		Chaetomiaceae	43.26
14	*Taifanglania sp.*	Sordariomycetes		Sordariales		Chaetomiaceae	
15	*Schizothecium sp.*	Sordariomycetes		Sordariales		Lasiosphaeriaceae	1.01
16	*Asordaria sp.*	Sordariomycetes		Sordariales		Sordariaceae	1.86
17	*Neurospora sp.*	Sordariomycetes		Sordariales		Sordariaceae	
18	*Sordaria sp.*	Sordariomycetes		Sordariales		Sordariaceae	
19	*Nigrospora sp.*	Sordariomycetes		Xylariales	1.78	Apiosporaceae	0.54
20	*Microdochium*	Sordariomycetes		Xylariales		Microdochiaceae	1.24
21	Ascobolaceaesp	Pezizomycetes	0.16	Pezizales	0.16	Ascobolaceae	0.16
22	Ascomycetes fungal sp.	-	1.16	-	1.16	-	1.16

TABLE 2
Plant organs from which the different fungi were isolated[1].

#	Genus	Plant organ				
		Roots	Leaves	Spikes	Stems	Percent
1	*Alternaria*	√	√	√	√	14.96
2	*Ascobolaceae*	√	-	-	-	0.16
3	*Ascomycetes*	√	√	√	√	1.16
4	*Asordaria*	√	-	-	-	0.23
5	*Bipolaris*	√	√	√	√	2.79
6	*Chaetomium*	√	√	√	√	42.79
7	*Fusarium*	√	√	√	√	19.61
8	*Lewia*	√	√	√	√	0.78
9	*Microdochium*	√	√	√	√	1.24
10	*Monosporascus*	-	-	√	√	0.54
11	*Neurospora*	√	√	√	-	1.01
12	*Nigrospora*	-	-	√	√	0.54
13	*Paecilomyces*	-	-	√	√	0.47
14	*Penicillium.*	√	√	√	√	6.98
15	*Pyrenophora*	√	√	√	√	3.02
16	*Schizothecium*	-	√	√	√	1.01
17	*Sordaria*	√	√	-	-	0.62
18	*Stemphylium*	√	-	√	√	0.31
19	*Stromatinia*	√	-	-	-	0.16
20	*Taifanglania*	-	-	√	√	0.47
21	*Trichoderma*	-	√	-	-	0.16
22	*Ulocladium*	√	√	√	√	1.01
	Percent	35.04	21.24	23.48	20.23	100

[1]A collection of 1290 isolates were purified and 83 representative isolates were sequenced and studied.

Composition of Endophytic Fungal Community. Fungi were most abundant in roots (35%) as compared to the other plant parts from which they were isolated. Leaves, spikes, and stems were almost equal in their abundance (around 20%) (Table 2). A total of 22 genera and 44 species were identified from different wheat plant parts. To our knowledge, these fungi are all new records on wheat in Jordan. According to fungal genus, *Chaetomium* sp. was the most recovered fungus with 42.79% followed by *Fusarium* sp. (19.61%) and *Alternaria* sp. (14.96%). Different genera were identified from the same organ. Some species such as *Alternaria*, *Bipolaris*, *Chaetomium*, *Fusarium*, *Lewia*, and *Microdochium*, *Penicillium*, *Pyrenophora*, and *Ulocladium* were identified from all plant parts (Table 2). Other genera such as *Trichoderma*, Ascobolaceae, *Asordaria* and *Stemotinis* were only identified from one organ.

Distribution of Endophytic Fungal Community Among Jordan Governates. The number of wheat growing fields from which the wheat plants were collected ranged from 1 (Jerash, Mafraq, Tafilah) to 52 (Irbid) fields (Figure 1). The characterized representatives of the different fungal species (n=83) were distributed all over the wheat growing regions in Jordan. Many genera were recovered from multiple fields such as in Irbid, Madaba, Mafraq, and Karak (Figure 2). Fields varied in their fungal diversity. For example in Mafraq, only one fungal genus was recovered from the field; *Fusarium*. On the other hand, fields such as those found in Tafilah and Jerash had high diversity of fungi although samples were collected from one field each (Figure 2). Irbid and Madaba governates were the most diverse in fungal species with 21 and 17 genera, respectively (Figure 2).

Pathogenicity Test. According to Table 3, 71.08% of the fungal isolates were non-pathogenic (score 0 and 1), 16.87% slight pathogen (score 2), 10.84 weak pathogen (score 3), and one isolate 1.20% was pathogenic. The pathogenic isolate was isolate # 39 which was belonging to *F. equesiti*. This isolate had less and significant germination percentage than the negative control (*F. culmorum*). Many isolates had higher values for at least two parameters than the control. Moreover, some isolates were higher in their values in all parameters compared to the control such as isolates 8, 10, 36, 51, 67, 88, 121, and 122 (Table 3).

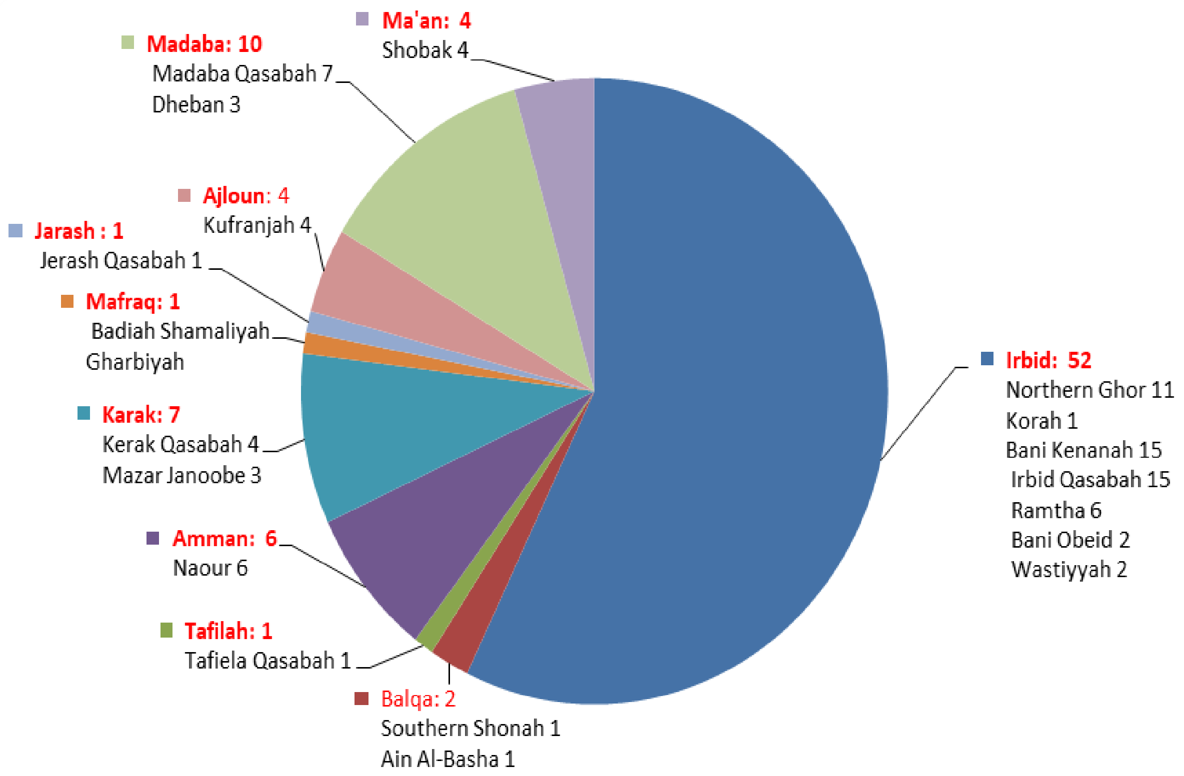

FIGURE 1
Number of fields (88) in each governate from which wheat plants were collected.
The red color font represents the governate with the total number of fields, and the black font below each governate represents the districts and the total number of fields/district.

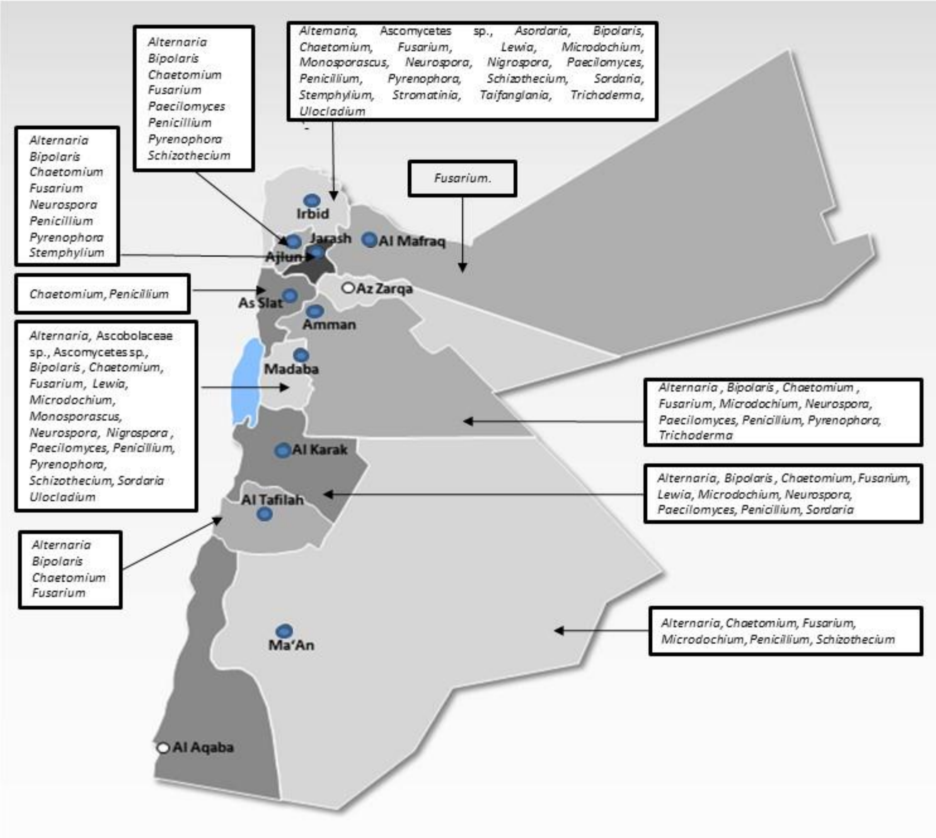

FIGURE 2
Jordan map showing the governates from which the healthy wheat plants were collected.

TABLE 3
Descriptive statistics and scoring of pathogenicity for wheat seeds *invitro* based on many seed parameters.

Isolate	Species	First leaf length		Coleoptile length		Number of seminal roots		Radicle length		Longest seminal root		Sum of above parts		Germination percent		Score	Status
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE		
1	*Fusarium equiseti*	2.23	4.52	1.15	0.43	0.73	0.28	2.68	1.07	3.73	1.41	3.38	1.25	20.00	0.00	1	Non-pathogenic
3	*Fusarium acuminatum*	1.42	2.72	2.13	0.56	1.17	0.33	2.96	0.86	3.22	0.94	3.55	0.98	33.33	8.82	1	Non-pathogenic
5	*Ascobolaceae sp*	1.90	3.20	1.50	0.43	1.20	0.34	1.85	0.56	2.32	0.67	3.41	1.00	30.00	15.30	2	Slight pathogen
6	*Fusarium equiseti*	2.07	3.20	1.50	0.40	1.03	0.29	2.89	1.00	3.57	1.06	3.57	0.97	33.33	3.33	1	Non-pathogenic
7	*Stromatinia narcissi*	0.54	1.40	4.28	0.48	3.10	0.38	2.78	0.45	2.64	0.45	4.82	0.61	80.00	10.00	0	Non-pathogenic
8	*Sordaria fimicola*	3.55	2.68	4.68	0.33	3.80	0.26	11.63	1.01	9.79	0.88	8.23	0.71	90.00	10.00	0	Non-pathogenic
9	*Alternaria chlamydosporigena*	2.62	3.71	5.76	2.48	2.00	0.36	4.82	1.56	6.66	2.23	8.39	2.86	60.00	10.00	0	Non-pathogenic
10	*Alternaria mouchaccae*	3.84	2.46	5.04	0.29	3.70	0.22	9.99	0.88	10.57	0.74	8.89	0.62	93.33	3.33	0	Non-pathogenic
11	*Fusarium equiseti*	2.31	3.55	1.70	0.42	1.50	0.37	2.62	0.81	3.48	1.09	4.01	1.05	36.67	6.67	1	Non-pathogenic
12	*Fusarium equiseti*	1.80	3.20	1.41	0.40	1.13	0.32	2.15	0.74	3.16	0.97	3.21	0.96	30.00	0.00	2	Slight pathogen
13	*Fusarium equiseti*	1.34	3.09	0.99	0.37	0.73	0.28	2.61	1.04	2.33	0.88	2.33	0.90	20.00	5.77	2	Slight pathogen
15	*Fusarium incarnatum*	2.88	3.52	2.52	0.46	2.13	0.38	5.13	1.18	10.29	5.11	5.40	1.07	53.30	14.50	0	Non-pathogenic
17	*Fusarium equiseti*	1.05	2.89	0.90	0.38	0.57	0.24	1.61	0.83	2.38	1.03	1.95	0.86	16.67	6.67	3	Weak pathogen
18	*Chaetomium elatum*	1.67	2.25	4.33	0.47	2.63	0.32	5.17	0.70	5.20	0.76	5.99	0.73	80.00	11.50	0	Non-pathogenic
23	*Fusarium redolens*	0.32	0.84	2.11	0.56	1.03	0.30	3.08	0.97	2.22	0.68	2.43	0.66	30.00	5.77	2	Slight pathogen
24	*Fusarium verticillioides*	0.71	1.82	1.14	0.39	0.77	0.27	2.56	0.95	1.95	0.75	1.86	0.68	23.30	14.50	3	Weak pathogen
25	*Fusarium oxysporum*	1.24	2.40	1.51	0.40	1.13	0.31	2.64	0.77	3.11	0.94	2.75	0.79	33.33	8.82	2	Slight pathogen
26	*Fusarium avenaceum*	1.52	3.20	1.73	0.48	1.27	0.34	3.49	0.99	2.48	0.70	3.25	0.98	33.33	8.82	2	Slight pathogen
27	*Fusarium equisiti)*	1.63	3.08	1.46	0.42	1.10	0.33	2.88	0.95	2.84	0.95	3.09	0.94	30.00	11.50	2	Slight pathogen
28	*Microdochium nivale*	1.61	2.69	1.86	0.41	1.30	0.32	3.27	0.97	4.21	1.07	3.47	0.85	36.67	3.33	1	Non-pathogenic
29	*Alternaria sorghi*	3.23	3.52	4.35	0.50	2.73	0.32	7.84	1.02	8.05	1.03	7.58	0.98	76.67	8.82	0	Non-pathogenic
31	*Fusarium equiseti*	2.06	2.71	2.12	0.48	1.63	0.35	4.46	1.03	5.50	1.28	4.18	0.94	43.33	8.82	0	Non-pathogenic
32	*Fusarium equiseti*	1.70	3.16	1.30	0.40	1.00	0.31	3.43	1.21	2.96	0.97	3.00	0.96	26.67	3.33	2	Slight pathogen
33	*Fusarium avenaceum*	0.98	2.39	2.07	0.59	1.30	0.35	3.58	1.05	3.09	0.91	3.05	0.90	33.30	14.50	2	Slight pathogen
34	*Fusarium equiseti*	1.68	3.53	1.04	0.39	0.73	0.28	2.14	0.93	1.98	0.85	2.72	1.02	20.00	0.00	3	Weak pathogen
36	*Taifanglania parvispora*	3.52	2.78	4.66	0.43	3.37	0.32	7.97	0.88	8.34	0.84	8.18	0.85	83.30	12.00	0	Non-pathogenic
37	*Fusarium sacchari*	1.12	2.86	0.91	0.34	0.67	0.28	1.99	0.96	1.82	0.80	2.03	0.83	16.67	6.67	3	Non-pathogenic
38	*Fusarium acuminatum*	5.16	19.06	1.79	0.44	1.17	0.31	8.98	5.10	3.83	1.01	6.95	3.68	36.70	12.00	1	Non-pathogenic
39	*Fusarium equiseti*	0.76	2.16	0.68	0.32	0.53	0.25	1.98	0.95	0.97	0.46	1.44	0.70	13.33	6.67	3	Pathogenic
40	*Paecilomyces variotii*	3.60	2.77	4.51	0.41	3.23	0.31	8.79	1.02	8.86	0.95	7.96	0.85	83.33	6.67	0	Non-pathogenic
43	*Nigrospora oryzae*	1.93	3.27	2.63	0.56	1.63	0.37	3.92	0.98	3.96	0.97	4.56	1.06	46.67	8.82	0	Non-pathogenic
44	*Ascomycetes Fungal sp.*	2.64	3.33	4.62	0.53	2.93	0.33	7.40	0.97	6.34	0.93	7.25	0.99	80.00	10.00	0	Non-pathogenic
46	*Chaetomium globosum*	1.45	2.27	1.43	0.34	2.03	0.47	0.93	0.25	1.22	0.30	2.88	0.72	45.00	2.89	2	Slight pathogen
47	*Chaetomium globosum*	1.37	2.86	1.97	0.48	1.10	0.30	3.32	0.99	3.24	0.97	3.34	0.93	36.70	12.00	1	Non-pathogenic
48	*Chaetomium globosum*	2.22	2.58	5.26	0.42	2.97	0.30	5.15	0.66	5.67	0.62	7.48	0.68	86.67	8.82	0	Non-pathogenic
49	*Bipolaris sorokiniana*	0.02	0.11	0.73	0.21	0.33	0.15	0.47	0.16	0.33	0.15	0.75	0.22	40.00	5.77	2	Slight pathogen
50	*Chaetomium elatum*	1.90	3.20	1.52	0.43	1.17	0.34	4.06	1.21	3.10	0.93	3.42	1.00	30.00	10.00	1	Non-pathogenic
51	*Chaetomium elatum*	3.81	2.79	5.07	0.32	3.83	0.23	8.63	0.83	9.89	0.71	8.88	0.71	93.33	3.33	0	Non-pathogenic
52	*Chaetomium elatum*	1.30	2.59	3.00	1.40	1.53	0.36	3.43	0.89	2.79	0.86	4.29	1.53	43.33	6.67	0	Non-pathogenic
53	*Chaetomium globosum*	1.34	2.41	2.15	0.43	2.00	0.37	5.96	1.29	4.72	1.08	3.49	0.79	53.33	8.82	0	Non-pathogenic
55	*Chaetomium elatum*	1.25	2.42	1.85	0.45	1.37	0.37	2.76	0.89	5.14	2.81	3.11	0.82	40.00	11.50	1	Non-pathogenic
56	*Chaetomium elatum*	1.13	1.91	3.38	0.63	2.03	0.38	4.06	0.86	5.46	1.06	4.51	0.88	53.30	12.00	0	Non-pathogenic
57	*Chaetomium rectangulare*	0.32	1.39	1.52	0.50	0.97	0.33	1.73	0.63	1.51	0.55	1.84	0.64	23.33	3.33	3	Weak pathogen

Isolate	Species	First leaf length		Coleoptile length		Number of seminal roots		Radicle length		Longest seminal root		Sum of above parts		Germination percent		Score	Status
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE		
58	*Chaetomium madrasense*	1.52	1.96	4.61	0.54	2.83	0.33	5.49	0.92	5.48	0.81	6.14	0.77	73.33	8.82	0	Non-pathogenic
60	*Chaetomium globosum*	1.21	2.33	2.38	0.57	1.43	0.36	2.71	0.79	2.88	0.82	3.59	0.89	40.00	0.00	0	Non-pathogenic
61	*Chaetomium elatum*	1.50	2.61	3.42	0.60	1.70	0.32	4.41	0.98	4.60	0.91	4.92	0.95	53.30	13.30	0	Non-pathogenic
62	*Chaetomium rectangulare*	4.32	13.75	1.48	0.43	1.27	0.36	2.02	0.67	2.38	0.70	5.80	2.77	30.00	5.77	2	Slight pathogen
63	*Chaetomium elatum*	2.43	3.45	3.42	0.60	1.67	0.33	5.43	1.06	6.19	1.29	5.85	1.11	53.30	20.30	0	Non-pathogenic
64	*Chaetomium elatum*	2.19	4.00	1.51	0.43	1.27	0.36	4.33	1.31	3.88	1.21	3.70	1.12	30.00	0.00	1	Non-pathogenic
65	*Chaetomium elatum*	0.61	1.88	1.33	0.38	0.83	0.27	1.40	0.53	1.82	0.65	1.94	0.65	30.00	0.00	3	Weak pathogen
67	*Chaetomium globosum*	3.03	2.58	4.81	0.36	3.70	0.28	8.43	0.88	8.96	0.83	7.84	0.72	86.67	6.67	0	Non-pathogenic
68	*Chaetomium cochliodes*	1.04	1.85	7.09	2.36	2.57	0.30	6.10	0.74	5.10	0.68	8.12	2.40	80.00	5.77	0	Non-pathogenic
70	*Chaetomium globosum*	0.69	1.70	4.50	0.58	2.27	0.35	5.98	0.93	5.12	0.86	5.20	0.72	66.67	8.82	0	Non-pathogenic
71	*Chaetomium elatum*	3.68	3.96	3.01	0.43	2.90	0.31	7.26	1.25	6.24	0.95	6.69	1.11	86.67	6.67	0	Non-pathogenic
75	*Chaetomium globosum*	1.85	3.17	3.65	0.57	2.07	0.33	5.42	0.96	7.27	1.20	5.49	0.97	63.33	8.82	0	Non-pathogenic
76	*Chaetomium elatum*	1.35	2.93	5.52	1.48	2.27	0.35	7.36	1.39	7.29	1.24	6.87	1.69	60.00	10.00	0	Non-pathogenic
77	*Alternaria sp.*	1.86	2.09	5.52	0.12	4.20	0.09	8.01	0.62	9.05	0.42	7.38	0.42	100.00	0.00	0	Non-pathogenic
78	*Alternaria alternata*	1.00	2.63	1.57	0.48	0.83	0.26	2.81	1.05	2.44	0.85	2.57	0.87	30.00	0.00	2	Slight pathogen
79	*Alternaria tellustris*	2.08	3.13	1.92	0.40	1.57	0.33	3.32	0.91	4.43	1.08	4.00	0.94	48.33	9.28	0	Non-pathogenic
81	*Ulocladium sp.*	2.88	4.98	2.75	0.49	2.27	0.37	6.22	1.31	7.12	1.35	5.64	1.26	60.00	15.30	0	Non-pathogenic
82	*Neurospora crassa*	2.03	2.77	3.91	0.59	2.37	0.38	7.21	1.24	6.81	1.15	5.94	1.00	60.00	10.00	0	Non-pathogenic
83	*Bipolaris sorokiniana*	0.01	0.07	0.70	0.19	0.47	0.15	0.90	0.32	0.75	0.32	0.72	0.20	36.67	3.33	3	Weak pathogen
84	*Alternaria sorghi*	2.86	3.11	3.65	0.44	2.90	0.34	8.42	1.18	9.39	1.20	6.51	0.91	73.30	12.00	0	Non-pathogenic
85	*Microdochium bolleyi*	2.26	3.23	4.20	1.91	2.07	0.38	2.66	0.55	3.24	0.72	6.46	2.27	60.00	5.77	0	Non-pathogenic
86	*Alternaria malorum*	2.73	3.31	2.08	0.42	1.93	0.37	5.49	1.18	6.83	1.40	4.81	1.01	50.00	10.00	0	Non-pathogenic
87	*Alternaria chlamydosporigena*	2.00	2.44	4.32	0.61	2.63	0.37	4.83	0.87	4.16	0.73	6.32	0.98	66.67	3.33	0	Non-pathogenic
88	*Alternaria alternata*	3.49	2.16	4.85	0.37	3.70	0.29	8.99	0.91	8.86	0.82	8.34	0.69	86.67	6.67	0	Non-pathogenic
89	*Bipolaris sorokiniana*	1.47	3.07	1.33	0.42	0.97	0.30	1.67	0.56	1.84	0.58	2.80	0.92	26.67	8.82	3	Weak pathogen
90	*Alternaria infectoria*	1.30	3.10	1.05	0.38	0.87	0.30	2.56	0.98	2.48	0.95	2.36	0.92	23.30	14.50	3	Weak pathogen
91	*Alternaria infectoria*	1.99	3.60	1.63	0.46	1.23	0.35	2.63	0.81	3.03	0.90	3.62	1.08	31.70	10.90	1	Non-pathogenic
95	*Lewia infectoria*	4.68	10.46	2.12	0.48	1.57	0.36	5.59	2.76	3.26	0.77	6.80	2.22	40.00	10.00	0	Non-pathogenic
96	*Fusarium tricinctum*	3.79	2.31	5.18	0.30	3.60	0.22	9.94	0.90	10.56	0.82	8.97	0.64	93.33	3.33	0	Non-pathogenic
101	*Monosporascus ibericus*	2.05	2.53	4.45	0.51	3.10	0.32	6.37	0.84	5.20	0.74	6.50	0.80	86.67	3.33	0	Non-pathogenic
102	*Fusarium redolens*	1.59	2.89	2.95	0.58	1.80	0.34	3.52	0.78	3.97	0.80	4.54	0.94	50.00	10.00	0	Non-pathogenic
107	*Asordaria arctica*	3.16	3.58	2.96	0.53	1.97	0.35	6.30	1.18	5.82	1.09	6.12	1.09	56.67	8.82	0	Non-pathogenic
110	*Trichoderma citrinoviride*	0.55	1.33	1.30	0.40	0.53	0.22	1.08	0.52	0.87	0.39	1.85	0.57	26.67	8.82	3	Weak pathogen
112	*Bipolaris sorokiniana*	0.10	0.57	1.24	0.36	0.80	0.21	0.92	0.28	1.47	0.40	1.34	0.39	46.67	6.67	2	Slight pathogen
113	*Alternaria infectoria*	2.88	4.12	3.95	0.52	2.70	0.34	4.84	0.75	4.87	0.79	6.83	1.10	80.00	5.77	0	Non-pathogenic
114	*Pyrenophora teres*	4.43	2.27	5.36	0.21	3.90	0.16	10.96	0.86	11.84	0.54	9.79	0.51	96.67	3.33	0	Non-pathogenic
116	*Penicillium chrysogenum*	1.79	3.11	2.23	0.51	1.23	0.32	2.54	0.71	2.51	0.68	4.02	1.01	40.00	15.30	1	Non-pathogenic
118	*Schizothecium inaequale*	2.20	3.05	2.63	0.53	1.53	0.34	3.59	0.82	3.62	0.82	4.84	1.03	46.70	12.00	0	Non-pathogenic
121	*Stemphylium vesicarium*	4.52	2.83	4.86	0.38	3.60	0.27	9.86	0.92	10.34	0.88	9.38	0.80	90.00	10.00	0	Non-pathogenic
122	*Ulocladium dauci*	3.84	3.20	4.50	0.43	3.00	0.31	8.13	0.86	7.15	0.83	8.34	0.90	83.33	3.33	0	Non-pathogenic
500	Sterile distilled water (negative control)	2.93	1.11	3.61	0.26	2.80	0.21	5.72	0.49	6.15	0.64	6.54	0.43	75.33	2.40	0	Non-pathogenic
501	*Fusarium culmorum* (positive control)	0.02	0.09	0.29	0.07	0.34	0.08	0.48	0.18	0.38	0.09	0.31	0.08	18.33	3.33	3	Pathogenic

DISCUSSION

Endophytes presence and association with wheat plant and wild *Triticum* has been demonstrated in the literature [16, 17, 18, 21, 32, 33]. In this study, we explored the cultivable endogenous fungi of wheat plants from different wheat growing locations in Jordan, and tested their effect on wheat seeds in vitro in order to better characterize them. We choose the dominant cultivable fungal endophytes rather than the uncultivable because we have further studies for evaluating these fungi on agronomic traits, as biological control agents against major wheat fungal diseases, increase tolerance against abiotic factor (salinity), and to study their interaction with aphid.

Endophytic fungi mainly consist of members of the Ascomycota, some taxa of the Basidiomycota, Zygomycota and Oomycota [34, 35, 36]. All the isolated fungal endophytes in this study were belonging to phylum Ascomycota. The reason behind that could be due to culture-dependent method, in which some uncultivable fungi cannot be detected. Such endophytes can only be detected and identified through molecular approaches utilizing extracted nucleic acids. Although the culture-dependent methods are routinely employed in endophyte diversity studies, yet they do not reflect the true number of endophytes in plant tissues [37, 38]. In this study the less frequent isolated fungi were not included; other phyla could be detected among these fungi.

In this study, Sordariomycetes was the major class and accounted for the highest frequency followed by Dothidiomycetes and Eurotiomycets. Previous study conducted by [33]. on fungal endophytes of recent and ancient wheat ancestors found that Dothidiomycetes was found with high frequent followed by Sordariomyctes and Eurotiomycetes. In tropical and temperate plants, the major class of endophytes was Sordariomycetes, followed by Dothideomycetes and Leotiomycetes [39, 40]. Fungal endophytes in sub-phylum Pezizomycotina is very common among the Ascomycota and represent at least five classes and dozens of families [39, 41, 42].

According to our findings, fungal endophytes were most abundant in roots, while the remained parts had almost equal abundance of endophytes. In previous studies, endophytes were mostly isolated from wheat leaves [16] or stems [33] compared to seeds. Vegetative upper parts contain higher number and diverse community of endophytes due to the restricted ability of endophytes movement within the plant [33]. Microbial communities in general are highly structured by the host organ and may have temporal variation [14].

In our study, 22 genera were identified using the ITS gene region. This region is known as the barcode for fungal identification [43]. The endophytes were very diverse within the plants regardless of the organ from which the fungus was isolated. Each genera was isolated at least from two organs except for *Trichoderma* which was isolated from leaves, while Ascobolaceae, *Asordaria*, and *Stemotinis* from roots. *In planta* environment is suggested to be very suitable for organisms co-existing [44].

Chaetomium, *Fusarium* and *Alternaria* were the most isolated fungal endophytes. Some of the isolated fungi had been reported in previous studies as pathogenic to wheat such as *Bipolaris*, *Microdochium*, and *Fusarium*. According to the pathogenicity *in vitro* experiment, all the tested isolates except one isolate were non-pathogenic and were not significantly different in all the parameters from the control (distilled water). As all fungi were isolated from healthy wheat tissues, it is not surprising to be non-pathogenic. The recorded wheat pathogens such as *Bipolaris* that were isolated in our study, could be avirulent or hypovirulent strains. Some of the isolated fungi from wheat may be beneficial to the host either as growth promoting organisms or as biocontrol agents: e.g. *Chaetomium*, *Alternaria*, and *Penicillium*. These fungi will be further evaluated on wheat seedlings in green house experiments and in antagonistic in vitro experiments. Seven genera and two unknown fungal species are new reports as fungal endophytes in wheat, those are: Ascobolaceae, Asocmycetes fungal sp., *Asordaria*, *Monosporoascus*, *Neurospora*, *Schizothecium*, *Sordaria*, *Stromatinia*, and *Taifanglanica*. These new reports will also be further evaluated as growth promoters and as biological control agents.

The distribution of the different fungal endophytes among the different governates showed diversity and richness for some genera and in certain locations. For example *Chaetomium* was found in all governates suggesting the adaptability of this fungus to wheat regardless of the location. Irbid, Madaba and Mafraq were found to be very diverse in endophytes. These locations are major wheat growing regions and diversity is expected. Diversity in these locations was reported on major wheat fungal diseases such as rusts as reported by Alananbeh et al. [45]. Further analysis regarding wheat fungal endophytes diversity and structure will be presented and discussed in a separate ecological study.

CONCLUSION

This is the first study that deals with isolating fungal endophytes from wheat plants from different wheat growing locations in Jordan. This study reveals an important diversity of fungi inside wheat plant and among regions. Some of the isolated genera in our study were previously recorded as wheat endophytes or as pathogens, however, the in vitro pathogenicity test proved that all the isolates including the pathogenic genera are non-virulent to seeds. Seven genera in our study are considered as new endophyte records on wheat. The advantage of this study is the establishment of cultivable fungal endophyte collection for future screening as growth promoters, biological control agents against major wheat fungal diseases and pests, and as agents that overcome abiotic stresses such as salinity and drought.

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