
Phylogenetic Diversity among Egyptian Isolates of *Fusarium* Species from Sugar Beet

Taha, E. M.¹, Rabie, W.¹, Mousa, A. S. M.², Yasser, M. M.² and Fahmy, Z. M.¹

¹Plant Pathology Research Institute, Agricultural Research Center ²Botany and Microbiology Department, Faculty of Science, Beni-Suef University, Egypt.

Taha, E. M., Rabie, W., Mousa, A. S. M., Yasser, M. M. and Fahmy, Z. M. (2016). Phylogenetic diversity among Egyptian isolates of *Fusarium* species from sugar beet. International Journal of Agricultural Technology 12(2):365-381.

Abstract Genetic variation was done to differentiate between *Fusarium* species endemic in sugar beet grown fields throughout different geographic locations of Egypt. TEF 1 α , β -tubulin and histone 3 genes could separate obviously between *F. oxysporum*, *F. solani*, *F. proliferatum*, *F. equiseti* and *F. verticillioides*. TEF 1 α was shown to be the best gene in this respect. As *F. oxysporum* and *F. solani* comprise the most common pathogens of sugar beet in Egypt as well as other countries, the phylogenetic analyses were done by TEF 1 α gene to correlate between the Egyptian isolates of these two species and the GenBank sequences representing species and clades. The phylogenetic TEF 1 α tree separated the *F. oxysporum* into three clades and the Egyptian candidates placed in clade C, whereas, our isolates of *F. solani* aggregated into one group corresponding to clade 3. Phylogenetic analysis could not reveal correlation among the geographic origin or the pathogenicity of *Fusarium* isolates on sugar beet.

Keywords: *Fusarium*, *Beta vulgaris*, genetic diversity, TEF 1 α , β -tubulin, histone 3

Introduction

Fusarium species are diverse and widespread pathogens (Smith, 2007) causing several diseases in economically important crops such as head blights, dry rot, wilting and decline, ear rot, resulted in yield and quality reduction (Desjardins *et al.*, 2002; Schmale and Bergstrom, 2003; Saremi *et al.*, 2011).

Fusarium genus including sections, species, subspecies and numerous proposed characters that combined to make strain identification and species diagnosis very complicated, thus making phylogenetic identification useful for this purpose (O'Donnell *et al.*, 1998; Kistler, 2001). In the time being, *Fusarium* comprises at least 300 genealogically exclusive phylogenetic species. The most important plant pathogens include four major groups, generally (Aoki *et al.*, 2014). These are

*Corresponding author: Taha, E. M.; Email: -

Fusarium fujikuroi species complex, *Fusarium graminearum* species complex, *Fusarium oxysporum* species complex and *Fusarium solani* species complex.

F. solani species complex consisting of at least 26 phylogenetically distinct species (O'Donnell, 2000), seven of which correspond to mating populations MPs I–VII (O'Donnell, 2000; Aoki *et al.*, 2005). They are divided into three large clades; 1, 2 and 3, according to phylogenetic analysis by 28S ribosomal DNA, internal transcribed spacer (ITS) regions, and the translation elongation factor (TEF 1 α) gene-coding region (O'Donnell, 2000; O'Donnell *et al.*, 2008; Nalim *et al.*, 2011).

The *F. oxysporum* species complex is a cosmopolitan and morphologically indistinguishable. It comprised both pathogenic and non-pathogenic strains (Lievens *et al.*, 2008). The pathogenic isolates are subdivided into *formae speciales* based on host specificity; there are more than 70 described species (Armstrong and Armstrong, 1981). The *formae specialis beta*, the cause of fusarium yellows of sugar beet (*Beta vulgaris*) was first reported in Colorado in 1931 (Stewart, 1931). Since that time, the disease was known and caused problems in many places (Windels *et al.*, 2005; Stojšin *et al.*, 2006; Burlakoti, 2007; Fahmy *et al.*, 2015) resulted in severe losses in root yield, sugar percentage and juice purity (Hanson and Jacobsen, 2009; Campbell *et al.*, 2011).

Fusarium yellows of sugar beet characterized by interveinal yellowing, wilting, necrosis of leaves and gray to reddish or red-brown vascular discoloration in root (Khan *et al.*, 2003). Some other *Fusarium* species were recorded as affecting pathogens to sugar beet. These are *F. solani*, *F. equiseti*, *F. proliferatum*, *F. verticillioides*, *F. acuminatum*, *F. avenaceum*, *F. culmorum*, and *F. graminearum* (Hanson and Hill, 2004; Burlakoti *et al.*, 2012; Fahmy *et al.*, 2015).

Many studies and methods have been used to characterize the genetic diversity and evolutionary origin of *Fusarium* species recovered from symptomatic sugar beet in USA and Europe (Nitschke *et al.*, 2009; Hill *et al.*, 2011; Webb *et al.*, 2013; Covey *et al.*, 2014). These methods include random-amplified polymorphic DNA markers (RAPDs) (Cramer *et al.*, 2003), vegetative compatibility grouping (VCG) (Harveson and Rush, 1997; Webb *et al.*, 2013), restriction fragment length polymorphism (RFLP) (Nitschke *et al.*, 2009), and comparisons of DNA sequences from conserved genomic regions (Hill *et al.*, 2011; Webb *et al.*, 2012; Covey *et al.*, 2014). While, many of these technologies succeeded in distinguishing *Fusarium* spp., but, identifying *formae speciales* still hard to be done. Besides, distinguishing between the pathogenic and the non-pathogenic isolates of the pathogen is hard so far.

Up till now, little attempts have been done to identify *Fusarium* populations in sugar beet growing regions of Egypt. Current study was done to determine the phylogenetic relationships between *Fusarium* species

recovered from symptomatic sugar beet from different locations of Egypt and to study the genetic variations among isolates of *Fusarium oxysporum* species complex and *Fusarium solani* species complex.

Material and methods

Source of Fusarium isolates

The different *Fusarium* isolates obtained from infected sugar beet roots collected from different locations of lower and middle Egypt (Fahmy *et al.*, 2015) were used in this study (Table 1).

Table 1. The *Fusarium* species used in the present investigation along with their geographic origins and pathogenicity as previously stated (Fahmy *et al.*, 2015)

Isolate code	Geographic origin	Species	% Emergence ^a	% stand ^b	Plant	DI ^c
K4	Kafr el-Sheikh	<i>oxysporum</i>	74.42	52.38		LP
K26	Kafr el-Sheikh	<i>oxysporum</i>	51.16	42.86		LP
K29	Kafr el-Sheikh	<i>solani</i>	41.86	38.10		NP
K34	Kafr el-Sheikh	<i>solani</i>	67.44	38.10		LP
D40	Dakahlia	<i>oxysporum</i>	90.70	52.38		P
Dm48	Damietta	<i>oxysporum</i>	93.02	59.52		LP
Dm51	Damietta	<i>equiseti</i>	90.70	45.24		P
Dm54	Damietta	<i>oxysporum</i>	90.70	35.71		P
Dm55	Damietta	<i>oxysporum</i>	79.07	59.52		LP
Dm60	Damietta	<i>oxysporum</i>	44.19	33.33		P
D65	Dakahlia	<i>solani</i>	72.09	54.76		LP
Dm67	Damietta	<i>solani</i>	79.07	59.52		P
B72	Beni-Suef	<i>oxysporum</i>	93.02	59.52		LP
B73	Beni-Suef	<i>oxysporum</i>	93.02	69.05		P
K93	Kafr el-Sheikh	<i>proliferatum</i>	41.86	30.95		LP
K96	Kafr el-Sheikh	<i>oxysporum</i>	16.28	9.52		NP
K97	Kafr el-Sheikh	<i>oxysporum</i>	18.60	14.29		NP
K98	Kafr el-Sheikh	<i>oxysporum</i>	93.02	47.62		LP
K101	Kafr el-Sheikh	<i>solani</i>	30.23	28.57		LP
K106	Kafr el-Sheikh	<i>oxysporum</i>	90.70	80.95		LP
K112	Kafr el-Sheikh	<i>oxysporum</i>	93.02	66.67		P
D119	Dakahlia	<i>solani</i>	23.26	21.43		NP
M126	Minia	<i>oxysporum</i>	46.51	42.86		P
B134	Beni-Suef	<i>solani</i>	46.51	35.71		NP
B135	Beni-Suef	<i>solani</i>	88.37	45.24		NP
B138	Beni-Suef	<i>oxysporum</i>	76.74	71.43		LP
B139	Beni-Suef	<i>proliferatum</i>	32.56	23.81		LP

Table 1. (Con.)

Isolate code	Geographic origin	Species	% Emergence ^a	% stand ^b	Plant	DI ^c
B140	Beni-Suef	<i>oxysporum</i>	55.81	54.76	P	
B141	Beni-Suef	<i>oxysporum</i>	60.47	42.86	P	
B148	Beni-Suef	<i>oxysporum</i>	44.19	42.86	P	
B150	Beni-Suef	<i>oxysporum</i>	97.67	64.29	P	
B154	Beni-Suef	<i>oxysporum</i>	97.67	64.29	P	
B166	Beni-Suef	<i>oxysporum</i>	95.35	50.00	NP	
B167	Beni-Suef	<i>proliferatum</i>	79.07	76.19	P	
B178	Beni-Suef	<i>equiseti</i>	81.40	76.19	P	
F186	Faiyum	<i>oxysporum</i>	44.19	28.57	P	
K196	Kafr el-Sheikh	<i>equiseti</i>	37.21	21.43	LP	
K199	Kafr el-Sheikh	<i>verticilloides</i>	0.00	0.00	LP	
F203	Faiyum	<i>proliferatum</i>	37.21	33.33	P	
D211	Dakahlia	<i>proliferatum</i>	23.26	19.05	LP	
B218	Beni-Suef	<i>oxysporum</i>	90.70	50.00	NP	
B219	Beni-Suef	<i>oxysporum</i>	79.07	69.05	LP	
M221	Minia	<i>solani</i>	48.84	42.86	LP	
Cont			100.00	95.45		
LSD			21.90	18.47		

^aEmergence of sugar beet plants after 15 days of planting, ^bPlant stand after 45 days of planting, ^cDI: disease index of pathogenicity (Fahmy *et al.*, 2015)

DNA extraction

The genomic DNA was extracted from each isolate by transferring 7 mm diameter of fresh mycelium into flasks containing 50 ml of potato dextrose broth (PDB). Cultures were grown for 5 days at 25°C on a rotary shaker at 100 rpm. Fungal tissues were filtered through sterile cheesecloth, rinsed with sterile water and dried with sterile cheesecloth. Approximately 1–2 g of mycelial tissue was ground in liquid nitrogen. DNA was extracted using the GeneJET plant genomic DNA purification kit (Thermo Scientific) according to the manufacturer's instructions.

PCR amplification and DNA sequencing

PCR amplification of translation elongation factor-1 α (TEF1 α) gene including coding and introns was performed using EF1 and EF2 primers (O'Donnell *et al.*, 1998). The highly conserved exon region β -tubulin was amplified using primers described by Koenraadt *et al.* (1992). The highly extensive modified region Histone3 was amplified using H3-1a and H3-1b primers described by Glass and Donaldson (1995). Table (2) shows the primer sequence along with the corresponding amplification targets. DNA amplification was carried out in PCR tubes consisted of 12.5 μ l

EmeraldAmp GT PCR Master Mix (Takara Bio Inc.), 1 µl of each primer forward and reverse, 2.5 µl DNA template and the volume was completed to 25 µl with sterile distilled water. Polymerase chain reaction (PCR) for the target genes, TEF 1 α and histone 3, were based upon the protocol of O'Donnell *et al.* (1998) with some modifications. Initial denaturation was done at 95°C for 2 min. followed by 35 cycles at 95°C for 30 sec, 56°C for 40 sec, and an extension cycle of 72°C for 1 min, then, a final extension cycle of 72°C for 5 min and held at 4°C. The PCR reaction for β - tubulin was run at 94°C for 2 min followed by 35 cycles of 94°C for 40 sec, 58°C for 45 sec, 72°C for 1 min, followed by a final extension cycle of 72°C for 5 min and then held at 4°C (Koenraad *et al.*, 1992). PCR reactions were performed with a 2720 thermal cycler, Applied Biosystems. Amplified DNA products were run on 1% agarose gel and separated by electrophoresis in 1 \times TBE buffer. DNA was photographed with ethidium bromide under UV by a trans illuminator. PCR products were purified and sequenced by Macrogen (Macrogen, Inc.).

Phylogenetic analysis

All sequences were compared with sequences of *Fusarium* species available in the FUSARIUM-ID v. 1.0 database (Geiser *et al.*, 2004) and GenBank database using BLAST search network services for similarities present in both the FUSARIUM-ID database and the NCBI database (National Center for Biotechnology Information). TEF 1 α sequences of *F. oxysporum* species complex representing clades (Clades A, B and C) previously described by Covey *et al.*, (2014) (Table 3) were obtained from GenBank for inclusion in the present study.

Table 2. Primer sequences and corresponding amplification targets

Target gene	Primer	Primer DNA Sequence (5'- 3')	Target size	Reference
TEF-1 α	EF1	ATGGGTAAGGA(A/G)GACAAGAC	700 bp	O'Donnell <i>et al.</i> ,
	EF2	GGA(G/A)GTACCAGT(G/C)ATCATGTT		
β - tubulin	β t C	GAGGAATTCCCAGACCGTATGATG	436 bp	Koenraad <i>et al.</i> ,
	β t D	GCTGGATCCTATTCTTTGGGTCGAACAT		
Histone 3	H3-1a	ACTAAGCAGACCGCCCGCAGG	560 bp	Glass and Donaldson
	H3-1b	GCGGGCGAGCTGGATGTCCTT		

Table 3. TEF 1 α strains obtained from GenBank and included in this study to differentiate between species and clades of *F. oxysporum* and *F. solani* species complexes.

Isolate name	Species	Origin	GenBank Acc. No.
<i>F. oxysporum</i> species complex			
F17 ^a	<i>F. oxysporum</i> f. sp. <i>betae</i>	Oregon	JX978492
F19 ^a	<i>F. oxysporum</i> f. sp. <i>betae</i>	Oregon	JX978478
F38 ^a	<i>F. oxysporum</i> f. sp. <i>betae</i>	Oregon	JX978475
Fob13 ^a	<i>F. oxysporum</i> f. sp. <i>betae</i>	Oregon	JX978494
Fob216 ^a	<i>F. oxysporum</i> f. sp. <i>betae</i>	Colorado	JX978503
F28 ^a	<i>F. oxysporum</i> f. sp. <i>betae</i>	Colorado	JX978480
Fo37 ^a	<i>F. oxysporum</i> f. sp. <i>betae</i>	Minnesota	JX978498
F174 ^a	<i>F. oxysporum</i> f. sp. <i>betae</i>	California	JX978486
FOB220a ^a	<i>F. oxysporum</i> f. sp. <i>betae</i>	Colorado	JX978487
FOB257a ^a	<i>F. oxysporum</i> f. sp. <i>betae</i>	Colorado	JX978500
H7 ^a	<i>F. oxysporum</i> f. sp. <i>betae</i>	Montana	JX978477
H8 ^a	<i>F. oxysporum</i> f. sp. <i>betae</i>	Montana	JX978499
Fo17 ^a	<i>F. oxysporum</i> f. sp. <i>betae</i>	Minnesota	JX978501
FUS001 ^a	<i>F. oxysporum</i> f. sp. <i>spinaciae</i>	Washington	JX978505
FUS003 ^a	<i>F. oxysporum</i> f. sp. <i>spinaciae</i>	Washington	JX978484
FUS004 ^a	<i>F. oxysporum</i> f. sp. <i>spinaciae</i>	Washington	JX978473
<i>F. solani</i> species complex			
NRRL31779 ^b	<i>F. brasiliense</i>	Brazil	AY320150
NRRL31949 ^b	<i>F. cuneirostrum</i>	Brazil	AY320161
NRRL22090 ^c	<i>F. illudens</i>	New Zealand	AF178326
NRRL31156 ^c	<i>F. phaseoli</i>	USA	AY220187
NRRL22402 ^c	<i>F. solani</i> f.sp. <i>batatas</i> MP- II	USA	AF178344
NRRL22142 ^c	<i>F. solani</i> f. sp. <i>cucurbitae</i> Race-2 MP-V	USA	AF178347
NRRL22153 ^c	<i>F. solani</i> f.sp. <i>cucurbitae</i> Race-1Mp- I	--	AF178346
NRRL22157 ^c	<i>F. solani</i> f.sp. <i>mori</i> MP- III	Japan	AF178359
NRRL22820 ^c	<i>F. solani</i> f.sp. <i>pisi</i> MP- VI	USA	AF178355
NRRL22586 ^c	<i>F. solani</i> f.sp. <i>robiniae</i> MP- VII	USA	AF178353
NRRL22277 ^c	<i>F. solani</i> f.sp. <i>xanthoxyli</i> MP- IV	Japan	AF178336
NRRL22098 ^c	<i>F. solani</i> f.sp. <i>cucurbitae</i> Race-1MP- I	--	AF178327
NRRL22823 ^c	<i>F. virguliforme</i>	USA	AF395647
NRRL22632 ^c	<i>Nectria plagianthi</i>	New Zealand	AF178354

^a(Covey *et al.* 2014), ^b(Aoki *et al.*,2005), ^c(O'Donnell 2000)

Also TEF 1 α sequences representing species and clades (Clade 1, 2 and 3) of *F. solani* species complex previously described by O'Donnell (2000) and Aoki *et al.* (2005) (Table 3) were downloaded from GenBank. Multiple sequence alignments from all sequences of each gene were performed using Clustal W version2.0 (Larkin *et al.*, 2007) with default settings. Phylogenetic analyses were constructed by the Maximum likelihood (ML) method with maximum parsimony (MP) using MEGA

version 6 (Tamura *et al.*, 2013). The bootstrap values illustrated on the phylogenetic trees were generated with 1000 replicate heuristic searches.

Results

Initially, a total of 43 *Fusarium* isolates recovered from roots of symptomatic sugar beet plants collected from different localities of Egypt from the previous study (Fahmy *et al.*, 2015) were used in the present investigation (Table 1). The TEF 1 α , β - tubulin and histone 3 sequences of all isolates were subjected to the FUSARIUM-ID database and GenBank database using BLAST search and the results were recorded as the most closely related sequences with high percentage of homology.

Parsimony bootstrap analyses of individual datasets (TEF 1 α , β - tubulin and histone 3) were made and showed progressively increasing resolution. TEF 1 α showed the highly resolution comparable to β - tubulin and histone 3 (Figs. 1,2 and 3). Results of alignment of TEF 1 α dataset showed 597 character were found to be constant with 409 parsimony uninformative, whereas, 188 with parsimony informative. But, aligned β - tubulin dataset showed 303 constant characters consisted of 262 were parsimony uninformative, whereas, 41 out of them were parsimony informative. As regards to the histone 3 alignment, 92/ 408 were parsimony informative compared with the 199/ 409 were parsimony uninformative. The phylogenetic trees generated from analyses of TEF 1 α , β - tubulin and histone 3 (Fig.1,2,3) demonstrated that isolates recovered from different locations of Egypt with similar named species clustered together forming well distinct five clades of *Fusarium*, *i.e.* *F. oxysporum*, *F. solani*, *F. proliferatum*, *F. equiseti* and *F. verticillioides*. The highly resolution TEF 1 α dataset (Fig. 1) separated *F. oxysporum* into four groups, *F. solani* into four groups, *F. proliferatum* and *F. equiseti* into two groups. While β - tubulin and histone 3 datasets (Figs. 2, 3) put *F. oxysporum* in one group except M126, *F. proliferatum* and *F. equiseti* in one group and separated *F. solani* into three groups. It was observed, also from the phylogenetic trees (Figs. 1, 2) that *F. verticillioides* and *F. proliferatum* have the same ancestor with *F. oxysporum* (MP >95 %). *F. solani* and *F. equiseti* were found to be at the base of the three trees.

Phylogenetic analysis was done using TEF 1 α sequences for the 25 candidates of *F. oxysporum* under study along with 16 GenBank sequences (Table 3). The alignment showed that 422/460 were found to be parsimony uninformative, but, 38/460 were parsimony informative. Also, the phylogenetic tree (Fig.4) separated *F. oxysporum* into three groups. These groups corresponded with the three clades (A, B and C) previously described by Webb *et al.* (2012) and Covey *et al.* (2014). The Egyptian strains of *F. oxysporum* obtained from roots of sugar beet were clearly

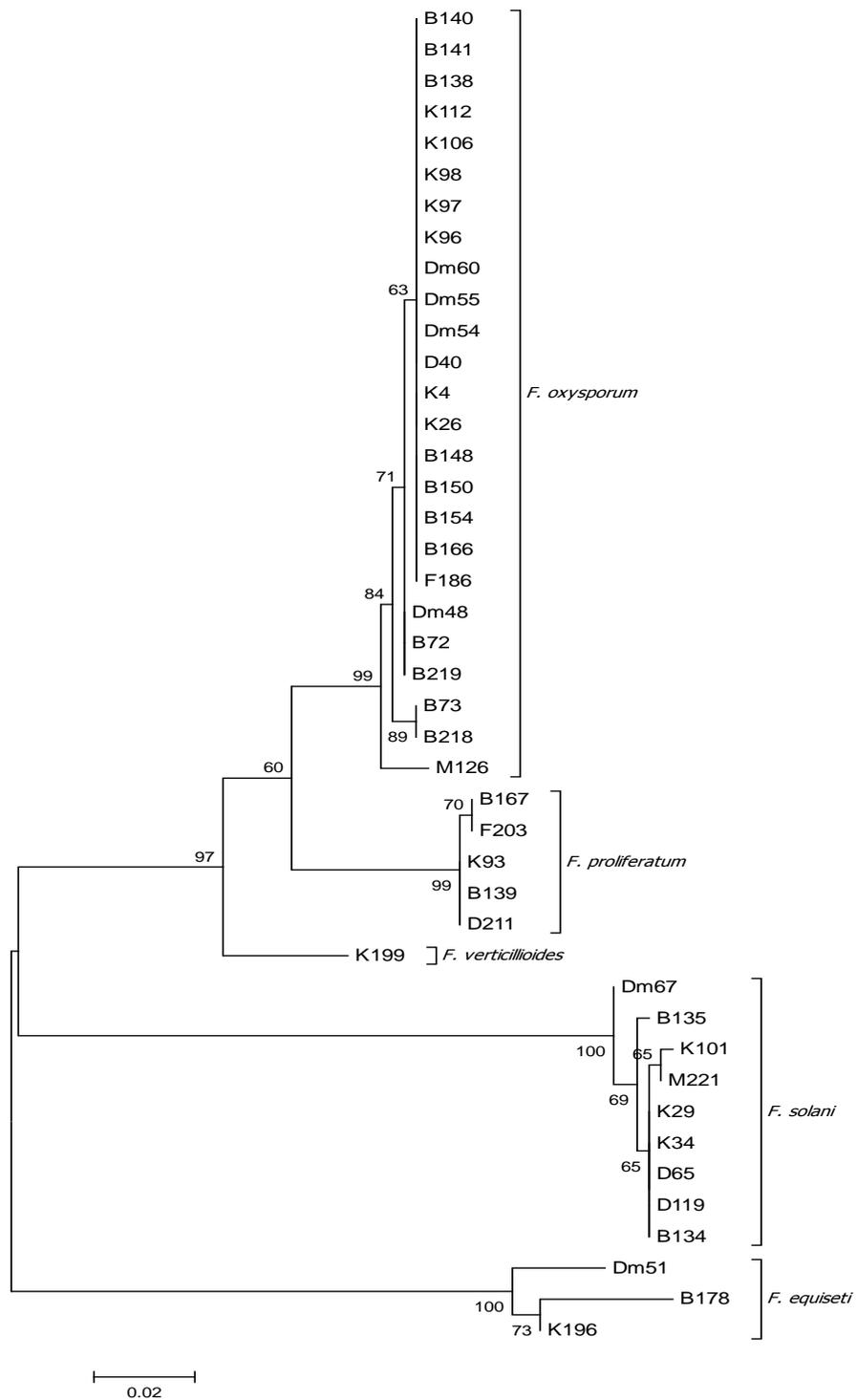


Figure 1. Unrooted phylogenetic tree inferred from translation elongation factor 1 α (TEF 1 α) sequences of all *Fusarium* species using Maximum Likelihood method with 1000 bootstrap replications.

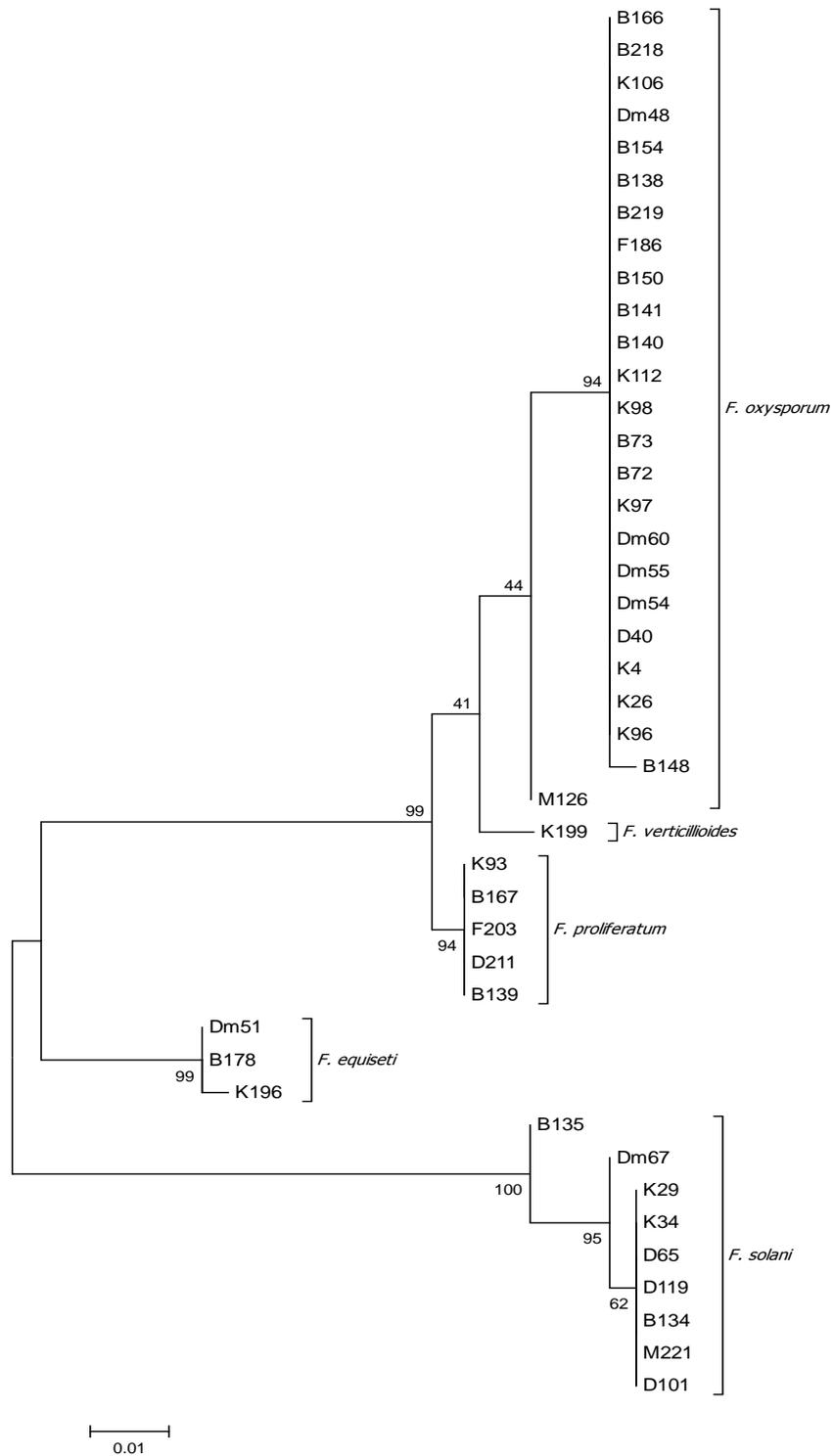


Figure 2. Phylogenetic tree for β - tubulin gene sequences of all *Fusarium* species using Maximum Likelihood method with 1000 bootstrap replications.

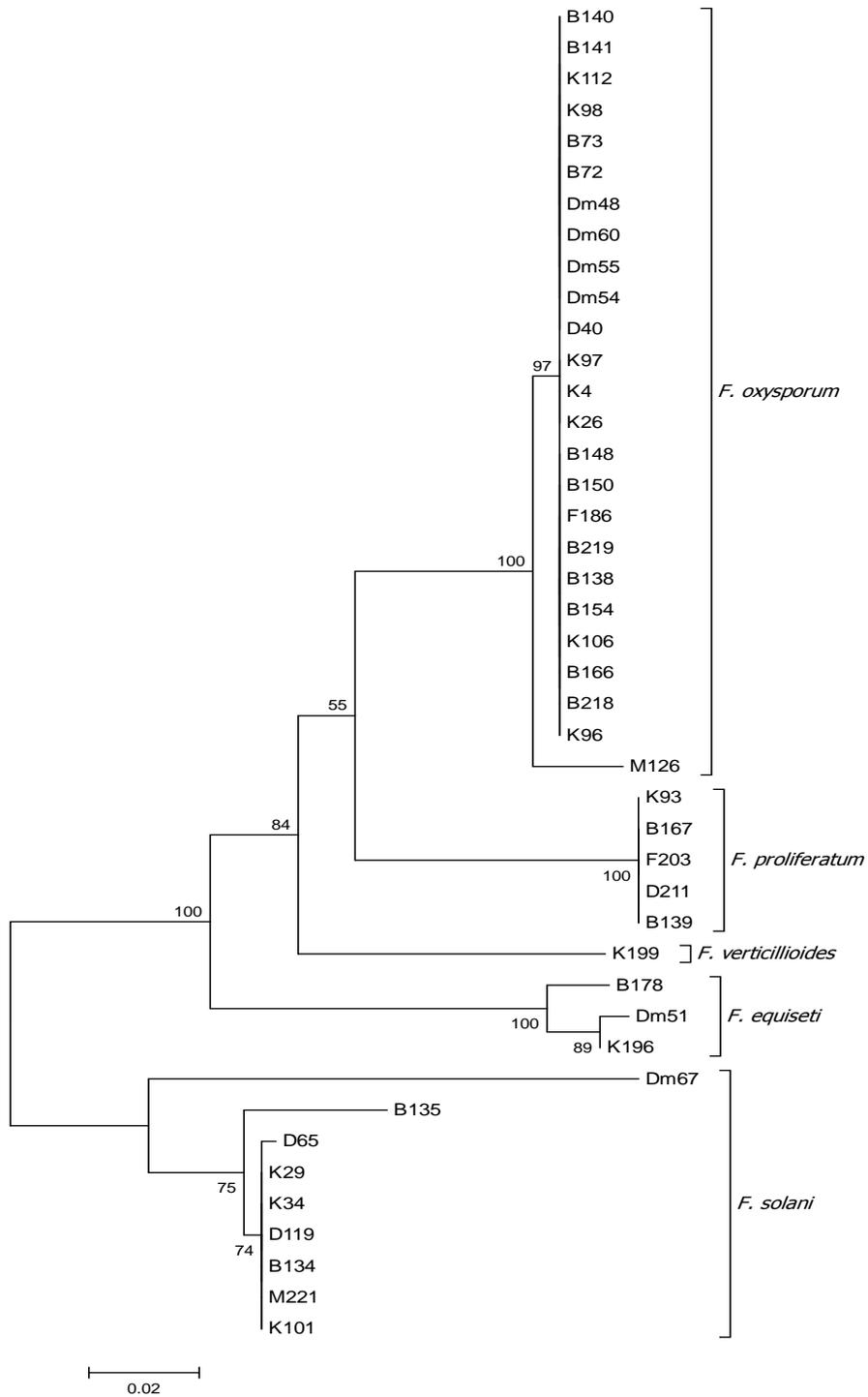


Figure 3. Unrooted phylogenetic tree generated from histone 3 sequences of all *Fusarium* species using Maximum Likelihood method with 1000 bootstrap replications.

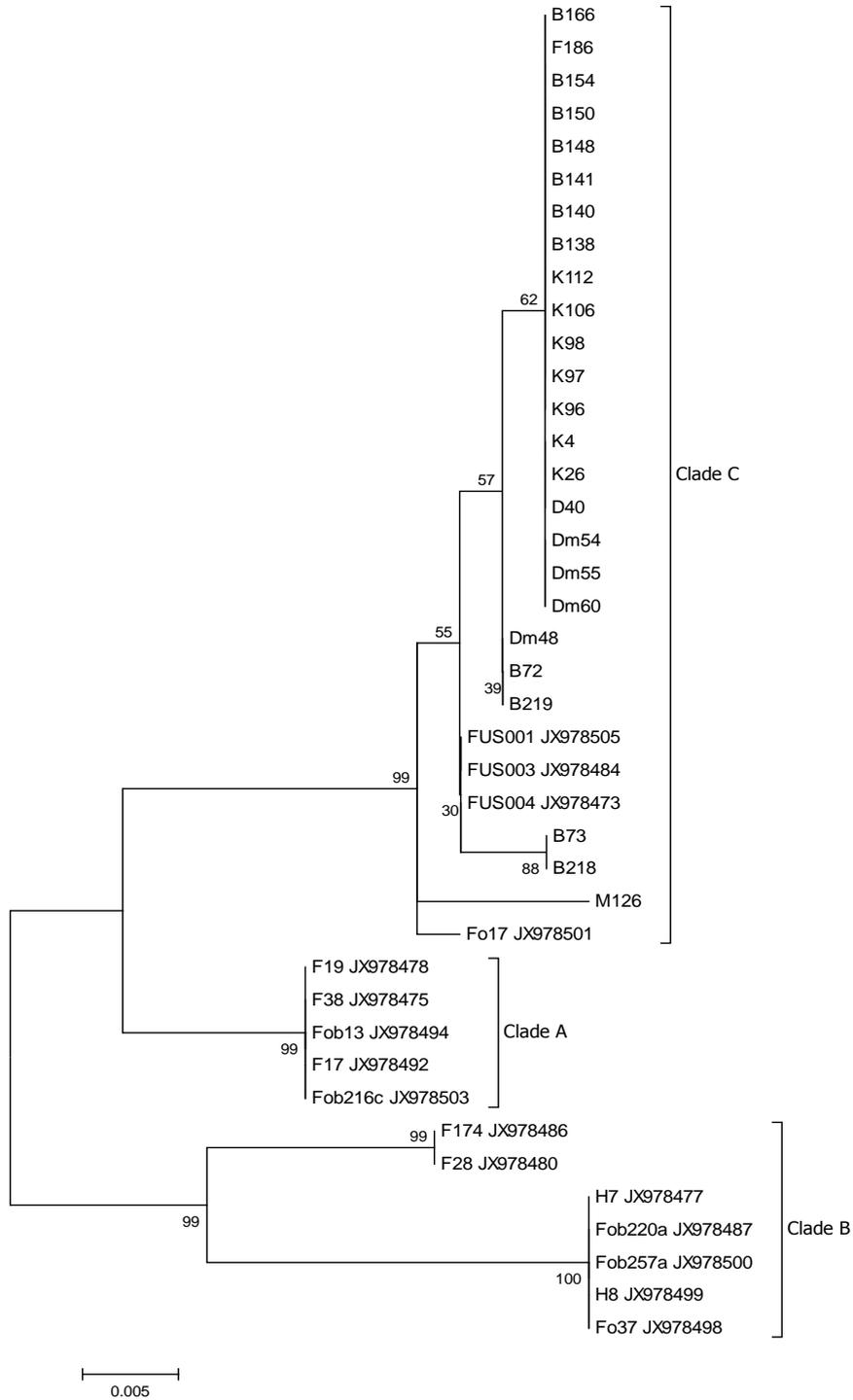


Figure 4. Unrooted phylogenetic tree showing relation between TEF 1 α of the Egyptian *F. oxysporum* sequences along with 16 GenBank sequences of *F.oxysporum* f. sp. *betae* and *spinaceae* using Maximum Likelihood method with 1000 bootstrap replications.

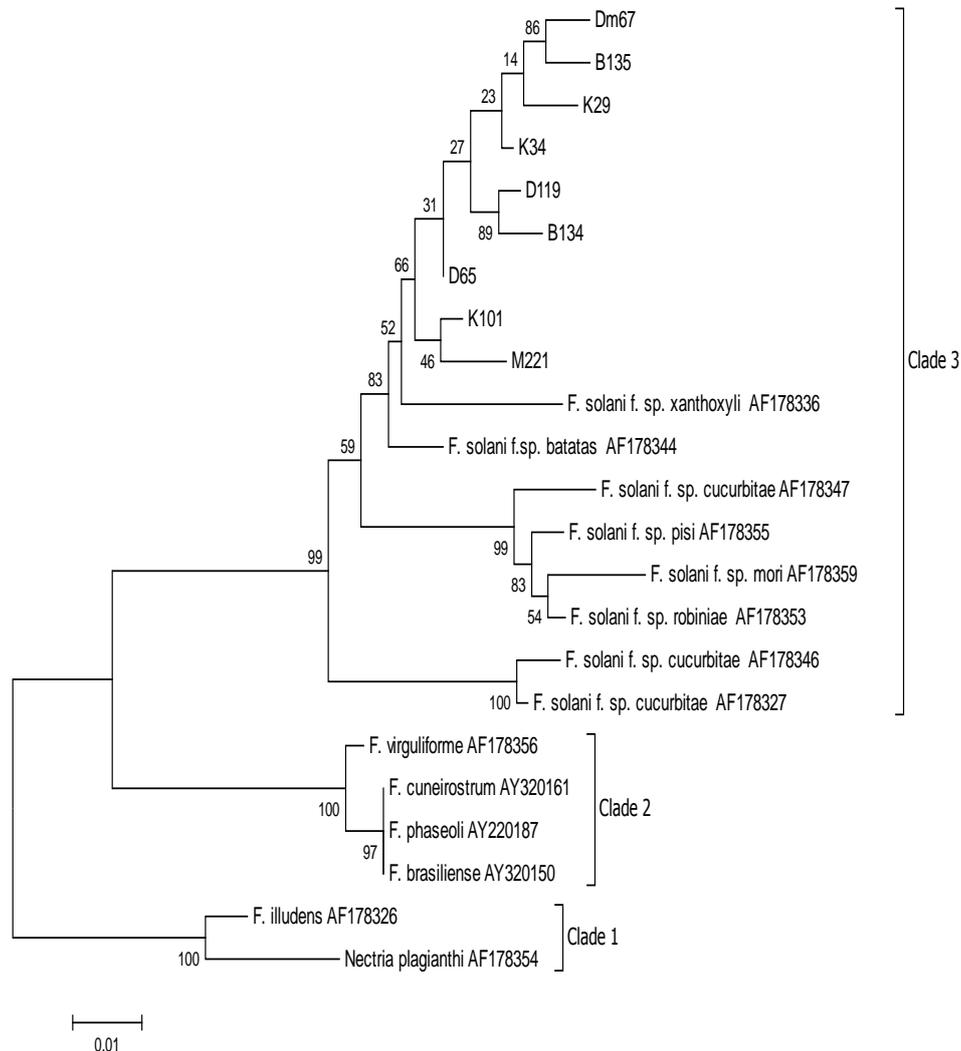


Figure 5. Unrooted phylogenetic tree showing relation between TEF 1 α of the Egyptian *F. solani* sequences as well as 14 GenBank sequences representing previously described species and clades of *F. solani* species complex.

related to clade C with strong bootstrap support (99%) as compared with the GenBank sequences of *F. oxysporum* f.sp. *betae* and *F. oxysporum* f.sp. *spinaciae*. Results, (Fig.4) indicate, also, that *F. oxysporum* isolates were polyphyletic.

Concerning the aligned TEF 1 α dataset of *F. solani* species complex of 9 *F. solani* from the current study and 14 GenBank sequences (Table 3), the parsimony uninformative were 583/697 and parsimony informative were 114/697. As a result of the maximum likelihood parsimony with 1000 bootstrap replications, the phylogenetic tree (Fig. 5) separated into three

groups representing species and clades (Clades 1,2 and3) previously characterized for the *F. solani* species complex (O'Donnell, 2000; Aoki *et al.*, 2005). All the Egyptian strains of *F. solani* isolated from roots of sugar beet were closely related to clade 3 with strong bootstrap support (99%).

Discussion

Phylogenetic analyses of TEF 1 α , β - tubulin and Histone 3 genes of *Fusarium* species isolated from roots of sugar beet plants from different beet grown fields of lower and middle Egypt was done throughout the present study. The three genes could separate the recovered isolates to five species (clades). These are *F. oxysporum*, *F. solani*, *F. proliferatum*, *F. equiseti* and *F. veticillioides*. The obtained results confirmed and supported the previous work about the main pathogens that attack sugar beet roots in most of beet grown locations all over the world (Hanson and Hill, 2004; Stojšin *et al.*, 2006; Nitschke *et al.*, 2009). Also, these findings are consistent with those obtained by other investigators who differentiate between *Fusarium* species. As has been found from the present study, Nitschke *et al.*, (2009) demonstrated that TEF 1 α revealed sufficient variability to differentiate between the *Fusarium* spp. resulting in species-dependent separation of the isolates. Phylogenetic analysis of individual dataset TEF 1 α showed progressively increasing the resolution comparable with β tubulin and histone 3. It is worthy to note that TEF 1 α gene are coding regions of the genome has the highly resolution assisting in differentiating within and among species in comparison with the intron-rich portions of protein-coding genes such as β - tubulin and histone 3 (Geiser *et al.*, 2004; Barik and Tayung, 2012).

As was found that TEF 1 α separated *F. oxysporum* under study into four groups, they were compared with the GenBank sequences representing the three clades of the pathogenic species reported by Hill *et al.*, (2011); Webb *et al.* (2012) and Covey *et al.* (2014). Accordingly, all the Egyptian isolates of *F. oxysporum* placed in clade C. Current work was shown to be in consistence with findings of Hill *et al.*, (2011) and Webb *et al.*, (2012). The three genes (TEF 1 α , β -tubulin and histone 3) were used by Webb *et al.* (2012) in order to find out the genetic variability among isolates of *F. oxysporum* from sugar beet and found that no clades based on the geographic origin or a single clade consisting of pathogens. Phylogenetic tree (Fig. 4) indicates that all isolates of *F. oxysporum* obtained from different origins under investigation of the country were polyphyletic.

Hill *et al.*, (2011) and Webb *et al.*, (2012) could not found relation between the pathogenicity and the genetic variation of *F. oxysporum* they worked on. They did this study on the pathogenic and non-pathogenic isolates to root at adult stage. Whereas, the current study dealt with the isolates, whether pathogenic to seedlings or roots of adults plants. The

genetic variation in our isolates could not correlate either with their virulence or the geographic origin. The findings obtained from the present work are consistent with those obtained by previous investigators who found no link with pathogenicity to sugar beet or geographic region.

As regards to *F. solani*, the phylogenetic tree of the GenBank sequences of the *F. solani* species complex (O'Donnell, 2000; Aoki *et al.*, 2005) and Egyptian isolates divided into three clades. Clade 1 comprised two known species (*Fusarium illudens* and *Nectria plagianthi*). Members of clade 2 included a number of important pathogens that cause sudden death syndrome (SDS) of soybean (Aoki *et al.*, 2005) and their molecular phylogeny show that they are paraphyletic (Nalim *et al.*, 2011). Molecular phylogeny showed diverse phylogenetic affinities among members of clade 3. This group comprised many species that are important in agricultural crops and medicine (O'Donnell, 2000; O'Donnell *et al.*, 2008; Chehri *et al.*, 2015). The Egyptian isolates placed in clade 3. This clade was stated by various investigators as a pathogenic clade to different hosts (O'Donnell, 2000; Bogale *et al.*, 2009; Tomioka *et al.*, 2011). Isolates of *F. solani* under study were found in previous study to be pathogenic to beet plants, causing seedling damping-off (Fahmy *et al.*, 2015). This pathogen ranked second pathogen on sugar beet after *F. oxysporum*. So, this pathogen affects seriously the plant stand in the beet growing area of country. This study threw the light on the importance of *F. solani* as a serious pathogen that attacks the crop in Egypt.

This study was planned to correlate between the phylogenetic diversity of *Fusarium* species recovered from sugar beet in relation to the geographic distribution and their virulence on plants as well. Although no link was observed to support this hypothesis, it could classify the common species of *Fusarium* in sugar beet production area of Egypt into groups (clades) that can be considered as a base for broaden the information about the population of *Fusarium* species to the benefit the agriculture in the country.

References

- Aoki, T., O'Donnell, K. and Geiser, D. M. (2014). Systematics of key phytopathogenic *Fusarium* species: current status and future challenges. *Journal of General Plant Pathology* 80:189-201.
- Aoki, T., O'Donnell, K. and Scandiani, M. M. (2005). Sudden death syndrome of soybean in South America is caused by four species of *Fusarium*: *Fusarium brasiliense* sp. nov., *F. cuneirostrum* sp. nov., *F. tucumaniae*, and *F. virguliforme*. *Mycoscience* 46:162-183.
- Armstrong, G. M. and Armstrong, J. K. (1981). Formae speciales and races of *Fusarium oxysporum* causing wilt diseases. p. 391-399. In P.E. Nelson, T.A. Toussoun, and R.J. Cook (ed.) *Fusarium: Diseases, Biology, and Taxonomy*. The Pennsylvania State University Press, University Park.

- Barik, B. P. and Tayung, K. (2012). Molecular differentiation of *Fusarium* spp. with varied lifestyles based on TEF 1 alpha gene sequence analysis. *Interdisciplinary Sciences: Computational Life Sciences* 4:201-208.
- Bogale, M., Steenkamp, E. T., Wingfield, M. J. and Wingfield, B. D. (2009). Diverse *Fusarium solani* isolates colonise agricultural environments in Ethiopia. *European Journal of Plant Pathology* 124:369-378.
- Burlakoti, P. (2007). *Fusarium* species associated with sugarbeet grown in the Red River Valley: Pathogenicity, cultivar response, and baseline sensitivity to fungicides. MS North Dakota State University.
- Burlakoti, P., Rivera, V., Secor, G. A., Qi, A., Del Rio-Mendoza, L. E. and Khan, M. F. R. (2012). Comparative pathogenicity and virulence of *Fusarium* species on sugar beet. *Plant Disease* 96:1291-1296.
- Campbell, L. G., Fugate, K. K. and Niehaus, W. S. (2011). Fusarium yellows affects postharvest respiration rate, sucrose concentration and invert sugar in sugar beet. *The Journal of Sugar Beet Research* 48:17-39.
- Chehri, K., Salleh, B. and Zakaria, L. (2015). Morphological and Phylogenetic analysis of *Fusarium solani* Species Complex in Malaysia. *Microbial Ecology* 69:457-471.
- Covey, P. A., Kuwitzky, B., Hanson, M. and Webb, K. M. (2014). Multilocus analysis using putative fungal effectors to describe a population of *Fusariumoxysporum* from sugar beet. *Phytopathology* 104:886-896.
- Cramer, R. A., Byrne, P. F., Brick, M. A. Panella, L., Wickliffe, E. and Schwartz, H. F. (2003). Characterization of *Fusariumoxysporum* isolates from common bean and sugar beet using pathogenicity assays and Random-amplified Polymorphic DNA markers. *Journal of Phytopathol* 151:352-360.
- Desjardins, A. E., Munkvold, G. P., Plattner, R. D. and Proctor, R. H. (2002). FUM1 – a gene required for fumonisin biosynthesis but not for maize ear rot and ear infection by *Gibberella moniliformis* in field tests. *Molecular Plant-Microbe Interactions Journal* 15:1157-1164.
- Fahmy, Z. M., Yasser M. M., Mousa, A. S. M., Taha, E. M. and Rabie, W. (2015). *Fusarium* species infecting sugar beet in Egypt. *Journal of Applied Sciences* 30:346-356.
- Geiser, D. M., Jimenez-Gasco, M. M., Kang, S., Makalowska, I., Veeraraghavan, N., Ward, T. J., Zhang, N., Kuldau, G. A. and O'Donnell, K. (2004). Fusarium-id v. 1.0: A DNA sequence database for identifying *Fusarium*. *European Journal of Plant Pathology* 110:473-479.
- Glass, N. L. and Donaldson, G. C. (1995). Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Applied and Environmental Microbiology* 61:1323-1330.
- Hanson, L. E. and Jacobsen, B. J. (2009). Fusarium yellows. Pages 28-29 in: *Compendium of Beet Diseases and Pests*. R. M. Harveson, L. E. Hanson, and G. L. Hein, eds. American Phytopathological Society Press, St. Paul, MN.
- Hanson, L. E. and Hill, A. L. (2004). *Fusarium* species causing Fusarium yellows of sugarbeet. *The Journal of Sugar Beet Research* 41:163-178.
- Harveson, R. M. and Rush, C. M. (1997). Genetic variation among *Fusariumoxysporum* isolates from sugar beet as determined by vegetative compatibility. *Plant Disease* 81:85-88.
- Hill, A. L., Reeves, P. A., Larson, R. L., Fenwick, A. L., Hanson, L. E. and Panella, L. (2011). Genetic variability among isolates of *Fusarium oxysporum* from sugar beet. *Plant Pathology* 60:496-505.
- Khan, M., Bradley, C. A. and Windels, C. E. (2003). Fusarium yellows of sugar beet. Univ. Minn. Ext. Serv. and North Dakota State University. 1247 pp.

- Kistler, H. C. (2001). Evolution of host specificity in *Fusarium oxysporum*, p. 70-96. In B. A. Summerell, J. F. Leslie, D. Backhouse, W. L. Bryden, and L. W. Burgess (eds.), *Fusarium: Paul E. Nelson Memorial Symposium*. APS Press, St. Paul, Minnesota.
- Koenraadt, H., Somerville, S. C. and Jones, A. L. (1992). Characterization of mutations in the Beta-tubulin gene of benomyl-resistant field strains of *Venturia inaequalis* and other plant pathogenic fungi. *Phytopathology* 82:1348-1354.
- Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A., Lopez, R., Thompson, J. D., Gibson, T. J. and Higgins, D. G. (2007). Clustal W and Clustal X version 2.0. *Bioinformatics* 23: 2947-2948.
- Lievens, B., Rep, M. and Thomma, B. P. (2008). Recent developments in the molecular discrimination of formae speciales of *Fusarium oxysporum*. *Pest Management Science* 64:781-788.
- Nalim, F. A., Samuels, G. J., Wijesundera, R. L. and Geiser, D. M. (2011). New species from the *Fusarium solani* species complex derived from perithecia and soil in the Old World tropics. *Mycologia* 103:1302-1330.
- Nitschke, E., Nihlgard, M. and Varrelmann, M. (2009). Differentiation of eleven *Fusarium* spp. isolated from sugar beet, using restriction fragment analysis of a polymerase chain reaction amplified translation elongation factor 1a gene fragment. *Phytopathology* 99:921-929.
- O'Donnell, K. (2000). Molecular phylogeny of the *Nectria haematococca-Fusariumsolani* species complex. *Mycologia* 92: 919-938.
- O'Donnell, K., Kistler, H. C., Cigelnik, E. and Ploetz, R. C. (1998). Multiple evolutionary origins of the fungus causing Panama disease of banana: Concordant evidence from nuclear and mitochondrial gene genealogies. *Proceedings of the National Academy of Sciences (USA)* 95:2044-2049.
- O'Donnell, K., Sutton, D. A., Fothergill, A., McCarthy, D., Rinaldi, M. G., Brandt, M. E., Zhang, N. and Geiser, D. M. (2008). Molecular phylogenetic diversity, multilocus haplotype nomenclature, and in vitro antifungal resistance within the *Fusariumsolani* species complex. *Journal of Clinical Microbiology* 46:2477-2490.
- Saremi, H., Okhovvat, S. M. and Ashrafi, S. J., (2011). *Fusarium* diseases as the main soil borne fungal pathogen on plants and their control management with soil solarization in Iran. *African Journal of Biotechnology* 10:18391-18398.
- Schmale, D. G. and Bergstrom, G. C. (2003). *Fusarium* head blight in wheat. *The Plant Health Instructor*. DOI:10.1094/PHI-I-2003-0612-01.
- Smith, S. N. (2007). An Overview of Ecological and Habitat Aspects in the Genus *Fusarium* with Special Emphasis on the SoilBorne Pathogenic Forms. *Journal of Plant Pathology* 16: 97-120.
- Stewart, D. (1931). Sugar-beet yellows caused by *Fusarium conglutinans* var. *betae*. *Phytopathology* 21:59-70.
- Stojšin, V. B., Maric, A. A., Jasnic, S. M., Bagi, F. F. and Marinkovic, B. J. (2006). Root rot of sugar beet in the Vojvodina Province [Serbia (Serbia and Montenegro)]. *Matica Srpska Proceedings for Natural Sciences* 110:65-74.
- Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution* 30: 2725-2729.
- Tomioka, K., Hirooka, Y., Takezaki, A., Aoki, T. and Sato, T. (2011) *Fusarium* root rot of prairie gentian caused by a species belonging to the *Fusarium solani* species complex. *Journal of General Plant Pathology* 77:132-135.
- Webb, K. M., Case, A. J., Brick, M. A., Otto, K. and Schwartz, H. F. (2013). Cross pathogenicity and vegetative compatibility of *Fusarium oxysporum* isolated from sugar beet. *Plant Disease* 97:1200-1206.

- Webb, K. M., Covey, P. A. and Hanson, L. E. (2012). Pathogenic and phylogenetic analysis of *Fusarium oxysporum* from sugar beet in Michigan and Minnesota. *The Journal of Sugar Beet Research* 49:38-56.
- Windels, C. E., Brantner, J. R., Bradley, C. A. and Khan, M. F. R. (2005). First report of *Fusarium oxysporum* causing yellows on sugar beet in the Red River Valley of Minnesota and North Dakota. *Plant Disease* 89:341.

(Received: 20 February 2016, accepted: 28 February 2016)