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Phylogenetic diversity among Egyptian isolates of *Fusarium* species from sugar beet

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Genetic variation was done to differentiate between *Fusarium* species endemic in sugar beet grown fields throughout different geographic locations of Egypt. TEF 1α, β-tubulin and histone 3 genes could separate obviously between *F. oxysporum, F. solani, F. proliferatum, F. equiseti* and *F. veticillioides.* TEF 1α was shown to be the best gene in this respect. As *F.oxysporum* and *F.solani* comprisethe most common pathogens of sugar beet in Egypt as well as other countries, the phylogenetic analyses were done by TEF 1α gene to correlate between the Egyptian isolates of these two species and the GenBank sequences representing species and clades. The phylogenetic TEF 1α 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.Phylogentic 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 1α, 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 numerousproposed characters thatcombined to make strain identification and species diagnosisvery 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 includefour major groups, generally (Aoki *et al*., 2014). These are*Fusariumfujikuroi* species complex, *Fusariumgraminearum* species complex,*Fusariumoxysporum* species complex and *Fusariumsolani* species complex

F. solani species complex consisting of at least 26 phylogenetically distinct species (O'Donnell, 2000), seven of which correspond to mating populationsMPs 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 1α) 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 specialsbeta,* thecause offusarium yellows of sugar beet (*Beta vulgaris*)was first reported in Colorado in 1931(Stewart, 1931). Since that time, the disease was known and cause 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 tocharacterize the genetic diversity and evolutionary origin of *Fusarium*speciesrecovered from symptomatic sugarbeet in USA and Europe (Nitschke *et al*., 2009; Hill *et al*., 2011; Webb *et al*., 2013; Covey *et al*., 2014). These methods includerandom- 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 ofEgypt 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]	% Plant stand[b]	DI[c]
K4	Kafr el-Sheikh	*oxysporum*	74.42	52.38	LP
K26	Kafr el-Sheikh	*oxysporum*	51.16	42.86	LP
K29	Kafr el-Sheikh	*solani*	41.86	38.10	NP
K34	Kafr el-Sheikh	*solani*	67.44	38.10	LP
D40	Dakahlia	*oxysporum*	90.70	52.38	P
Dm48	Damietta	*oxysporum*	93.02	59.52	LP
Dm51	Damietta	*equiseti*	90.70	45.24	P
Dm54	Damietta	*oxysporum*	90.70	35.71	P
Dm55	Damietta	*oxysporum*	79.07	59.52	LP
Dm60	Damietta	*oxysporum*	44.19	33.33	P
D65	Dakahlia	*solani*	72.09	54.76	LP
Dm67	Damietta	*solani*	79.07	59.52	P
B72	Beni-Suef	*oxysporum*	93.02	59.52	LP
B73	Beni-Suef	*oxysporum*	93.02	69.05	P
K93	Kafr el-Sheikh	*proliferatum*	41.86	30.95	LP
K96	Kafr el-Sheikh	*oxysporum*	16.28	9.52	NP
K97	Kafr el-Sheikh	*oxysporum*	18.60	14.29	NP
K98	Kafr el-Sheikh	*oxysporum*	93.02	47.62	LP
K101	Kafr el-Sheikh	*solani*	30.23	28.57	LP
K106	Kafr el-Sheikh	*oxysporum*	90.70	80.95	LP
K112	Kafr el-Sheikh	*oxysporum*	93.02	66.67	P
D119	Dakahlia	*solani*	23.26	21.43	NP
M126	Minia	*oxysporum*	46.51	42.86	P
B134	Beni-Suef	*solani*	46.51	35.71	NP
B135	Beni-Suef	*solani*	88.37	45.24	NP
B138	Beni-Suef	*oxysporum*	76.74	71.43	LP
B139	Beni-Suef	*proliferatum*	32.56	23.81	LP
B140	Beni-Suef	*oxysporum*	55.81	54.76	P
B141	Beni-Suef	*oxysporum*	60.47	42.86	P
B148	Beni-Suef	*oxysporum*	44.19	42.86	P
B150	Beni-Suef	*oxysporum*	97.67	64.29	P
B154	Beni-Suef	*oxysporum*	97.67	64.29	P
B166	Beni-Suef	*oxysporum*	95.35	50.00	NP
B167	Beni-Suef	*proliferatum*	79.07	76.19	P
B178	Beni-Suef	*equiseti*	81.40	76.19	P
F186	Faiyum	*oxysporum*	44.19	28.57	P
K196	Kafr el-Sheikh	*equiseti*	37.21	21.43	LP
K199	Kafr el-Sheikh	*verticilloides*	0.00	0.00	LP
F203	Faiyum	*proliferatum*	37.21	33.33	P
D211	Dakahlia	*proliferatum*	23.26	19.05	LP

B218	Beni-Suef	*oxysporum*	90.70	50.00	NP
B219	Beni-Suef	*oxysporum*	79.07	69.05	LP
M221	Minia	*solani*	48.84	42.86	LP
Cont			100.00	95.45	
LSD			21.90	18.47	

[a]Emergence of sugar beet plants after 15 days of planting, [b]Plant stand after 45 days of planting, [c]DI: disease index of pathogenicity (Fahmy *et al*., 2015)

DNA extraction

The genomicDNA 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 anddried with sterile cheesecloth. Approximately 1–2 g of mycelial tissue wasground 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-1α (TEF1α) gene including coding and introns was performed using EF1 and EF2 primers (O'Donnell *et al.*, 1998). The highly conserved exon region ß-tubulin was amplified using primersdescribed by Koenraadt *et al.* (1992). The highly extensive modifiedregion Histone3was 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 amplificationwas carried out in PCR tubes consisted of 12.5 µl EmeraldAmp GT PCR Master Mix (Takara Bio Inc.), 1µl of each primer forward and reverse, 2.5 µl DNA template and the volume was completed to25 µl with sterile distilled water. Polymerase chain reaction (PCR) for the target genes,TEF 1α 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°Cfor 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 (Koenraadt *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× TBE buffer. DNA was photographed with ethidium bromide under UV by a transilluminator. PCR products were purified and sequenced by Macrogen (Macrogen, Inc.).

Phylogenetic analysis

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

Table (2): Primer sequences and corresponding amplification targets

Target gene	Primer	Primer DNA Sequence (5'- 3')	Target size	Reference
TEF- 1α	EF1	ATGGGTAAGGA(A/G)GACAAGAC	700 bp	O'Donnell *et. al.*, 1998
	EF2	GGA(G/A)GTACCAGT(G/C)ATCATGTT		
β – tubulin	βt C	GAGGAATTCCCAGACCGTATGATG	436 bp	Koenraadt *et. al.*, 1992
	βt D	GCTGGATCCTATTCTTTGGGTCGAACAT		
Histone 3	H3-1a	ACTAAGCAGACCGCCCGCAGG	560 bp	Glass and Donaldson 1995
	H3-1b	GCGGGCGAGCTGGATGTCCTT		

Table (3): TEF 1α 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.
F. oxysporum species complex			
F17[a]	*F. oxysporum* f. sp. *betae*	Oregon	JX978492
F19[a]	*F. oxysporum* f. sp. *betae*	Oregon	JX978478
F38[a]	*F. oxysporum* f. sp. *betae*	Oregon	JX978475
Fob13[a]	*F. oxysporum* f. sp. *betae*	Oregon	JX978494
Fob216[a]	*F. oxysporum* f. sp. *betae*	Colorado	JX978503
F28[a]	*F. oxysporum* f. sp. *betae*	Colorado	JX978480
Fo37[a]	*F. oxysporum* f. sp. *betae*	Minnesota	JX978498
F174[a]	*F. oxysporum* f. sp. *betae*	California	JX978486
FOB220a[a]	*F. oxysporum* f. sp. *betae*	Colorado	JX978487
FOB257a[a]	*F. oxysporum* f. sp. *betae*	Colorado	JX978500
H7[a]	*F. oxysporum* f. sp. *betae*	Montana	JX978477
H8[a]	*F. oxysporum* f. sp. *betae*	Montana	JX978499
Fo17[a]	*F. oxysporum* f. sp. *betae*	Minnesota	JX978501
FUS001[a]	*F. oxysporum* f. sp. *spinaciae*	Washington	JX978505

FUS003 [a]	*F. oxysporum* f. sp. *spinaciae*	Washington	JX978484
FUS004 [a]	*F. oxysporum* f. sp. *spinaciae*	Washington	JX978473
F. solani species complex			
NRRL31779[b]	*F. brasiliense*	Brazil	AY320150
NRRL31949[b]	*F. cuneirostrum*	Brazil	AY320161
NRRL22090[c]	*F. illudens*	New Zealand	AF178326
NRRL31156[c]	*F. phaseoli*	USA	AY220187
NRRL22402[c]	*F. solani* f.sp. *batatas* MP- II	USA	AF178344
NRRL22142[c]	*F. solani* f. sp. *cucurbitae* Race-2 MP-V	USA	AF178347
NRRL22153[c]	*F. solani* f.sp. *cucurbitae* Race-1Mp- I	--	AF178346
NRRL22157[c]	*F. solani* f.sp. *mori* MP- III	Japan	AF178359
NRRL22820[c]	*F. solani* f.sp. *pisi* MP- VI	USA	AF178355
NRRL22586[c]	*F. solani* f.sp. *robiniae* MP- VII	USA	AF178353
NRRL22277[c]	*F. solani* f.sp. *xanthoxyli* MP- IV	Japan	AF178336
NRRL22098[c]	*F. solani* f.sp. *cucurbitae* Race-1MP- I	--	AF178327
NRRL22823[c]	*F. virguliforme*	USA	AF395647
NRRL22632[c]	*Nectria plagianthi*	New Zealand	AF178354

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

Also TEF 1α sequences representing species and clades (Clade 1, 2 and 3)of *F. solani* species complex previously described by O'Donnell (2000) andAoki *et al.* (2005) (Table 3) were downloaded from GenBank.Multiplesequence alignments from all sequences ofeach genewere performed using Clustal W version2.0 (Larkin*et al.*,2007) with default settings. Phylogenetic analyseswere constructed by the Maximum likelihood (ML) method withmaximum parsimony (MP) using MEGA version 6 (Tamura *et al.*, 2013). The bootstrapvalues illustrated on the phylogenetic trees weregenerated 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 1α, 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 sequenceswith high percentage of homology.

Parsimony bootstrap analyses of individual datasets (TEF 1α, β-tubulin and histone 3) were made and showed progressively increasing resolution. TEF 1α showed the highly resolution comparable to β- tubulin and histone 3(Figs. 1,2&3). Resultsof alignment of TEF 1α dataset showed597 characterwere 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

wereparsimony uninformative, whereas, 41 out of them wereparsimony informative. As regards to the histone 3alignment, 92/ 408 wereparsimony informative compared with the 199/ 409 were parsimony uninformative. The phylogenetic trees generated from analyses of TEF 1α,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.veticillioides.*The highly resolution TEF 1α 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 histone3 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.verticilloiedes* 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 1α sequencesfor 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 phylogenetictree (Fig.4) separated *F. oxysporum* into three groups. These groups corresponded with the three clades (A, B and C) previously described by Webb

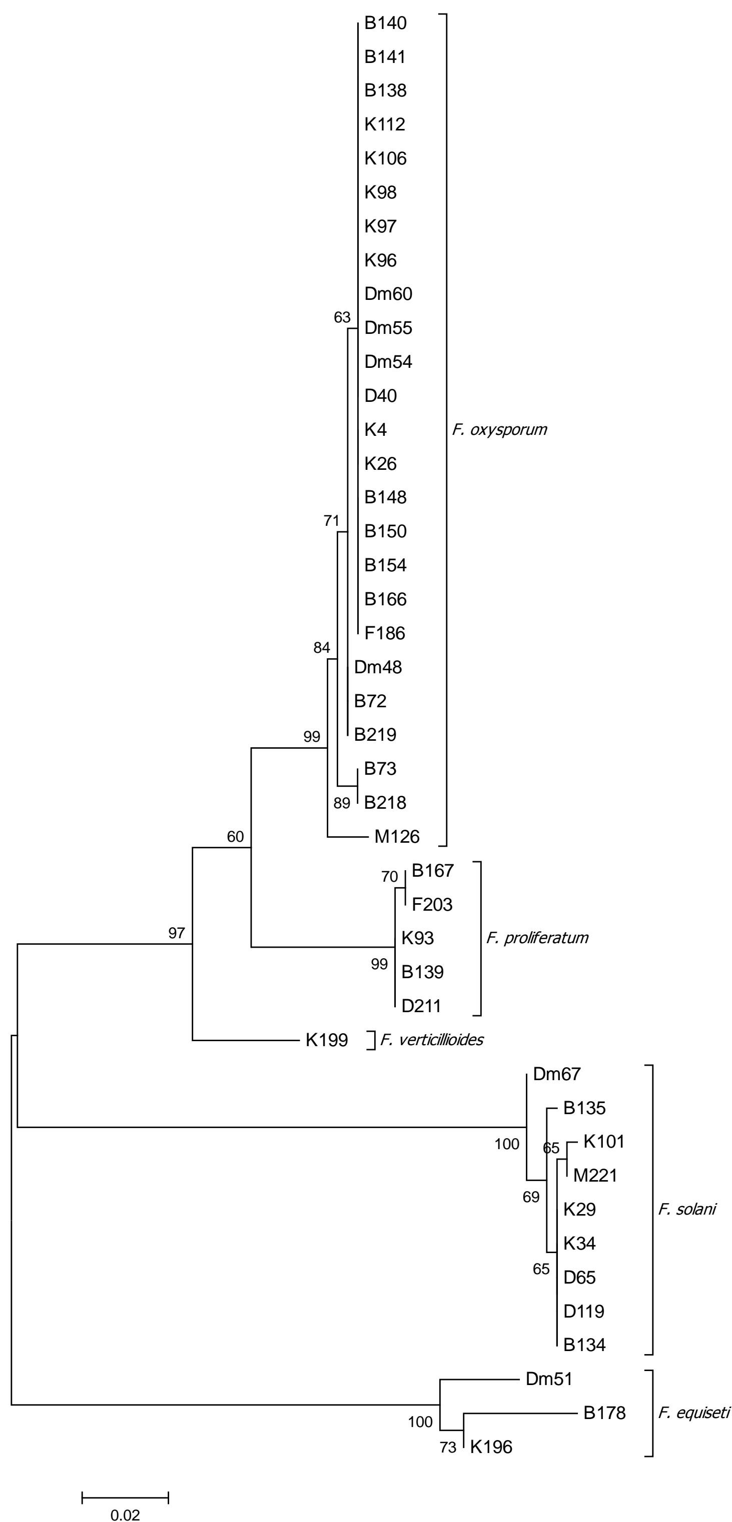
B140
B141
B138
K112
K106
K98
K97
K96
Dm60
Dm55
Dm54
D40
K4
K26
B148
B150
B154
B166
F186
Dm48
B72
B219
B73
B218
M126
F. oxysporum
63
71
84
99
89
60
B167
F203
K93
B139
D211
F. proliferatum
70
99
97
K199
F. verticillioides
Dm67
B135
K101
M221
K29
K34
D65
D119
B134
F. solani
100
65
69
65
Dm51
B178
K196
F. equiseti
100
73
0.02

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

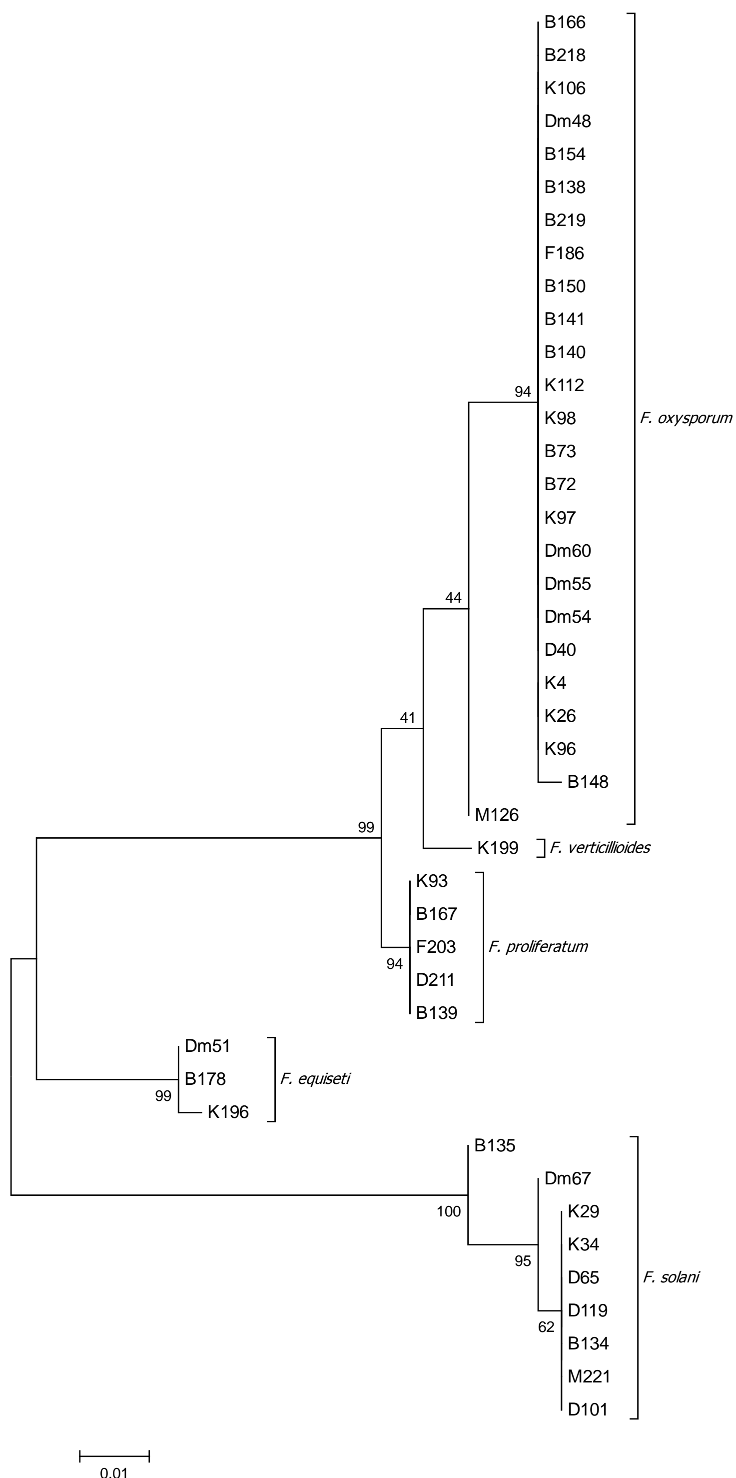
B166
B218
K106
Dm48
B154
B138
B219
F186
B150
B141
B140
94
K112
K98
F. oxysporum
B73
B72
K97
Dm60
Dm55
44
Dm54
D40
K4
41
K26
K96
B148
M126
99
K199
F. verticillioides
K93
B167
F203
F. proliferatum
94
D211
B139
Dm51
B178
F. equiseti
99
K196
B135
Dm67
100
K29
K34
95
D65
F. solani
D119
62
B134
M221
D101
0.01

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

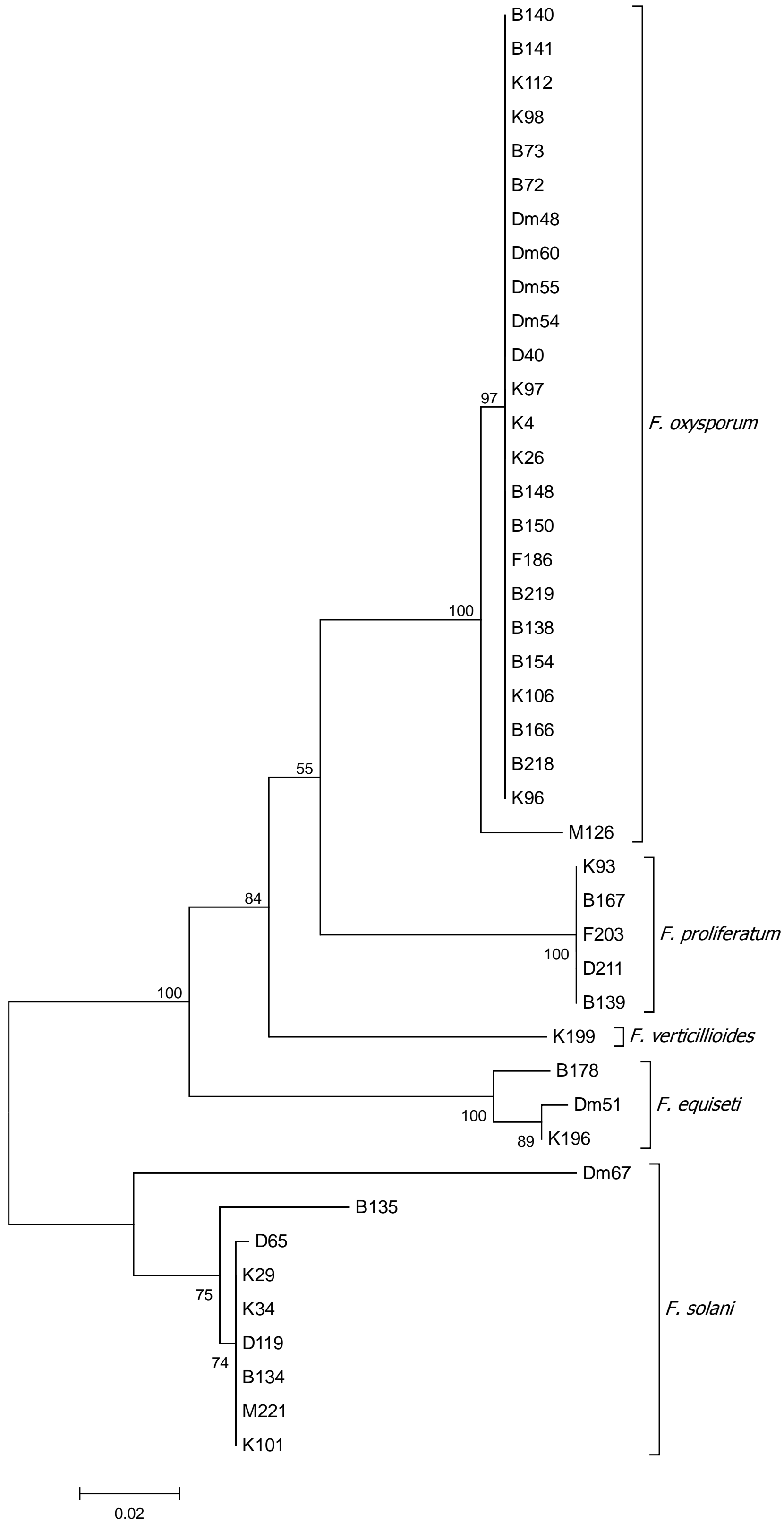
B140
B141
K112
K98
B73
B72
Dm48
Dm60
Dm55
Dm54
D40
K97
K4
K26
B148
B150
F186
B219
B138
B154
K106
B166
B218
K96
M126
F. oxysporum
97
100
55
84
100
K93
B167
F203
D211
B139
F. proliferatum
100
K199
F. verticillioides
B178
Dm51
K196
F. equiseti
100
89
Dm67
B135
D65
K29
K34
D119
B134
M221
K101
F. solani
75
74
0.02

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

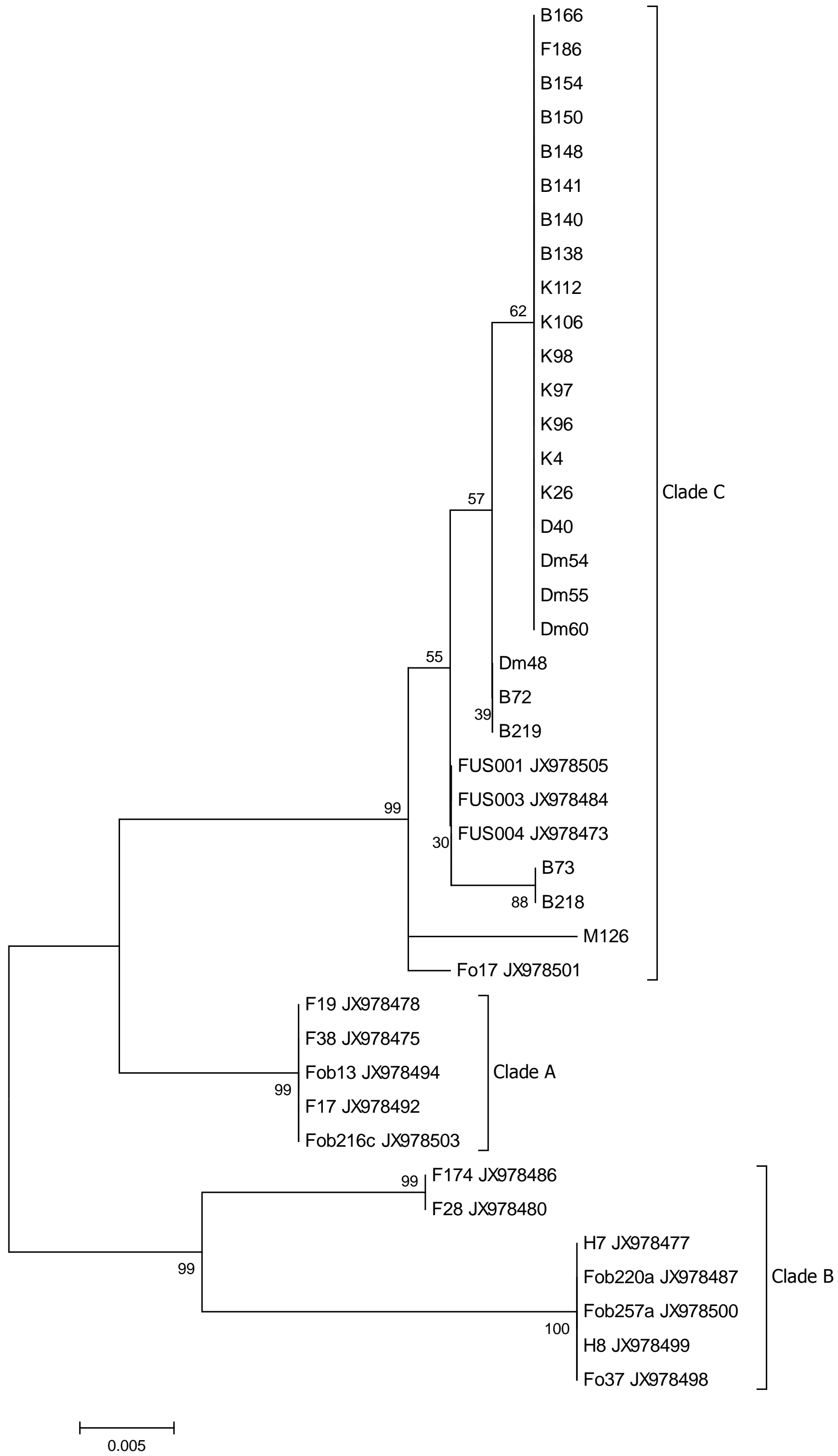
B166
F186
B154
B150
B148
B141
B140
B138
K112
K106
K98
K97
K96
K4
K26
D40
Dm54
Dm55
Dm60
Dm48
B72
B219
FUS001 JX978505
FUS003 JX978484
FUS004 JX978473
B73
B218
M126
Fo17 JX978501
Clade C
62
57
55
39
99
30
88
F19 JX978478
F38 JX978475
Fob13 JX978494
F17 JX978492
Fob216c JX978503
Clade A
99
F174 JX978486
F28 JX978480
99
H7 JX978477
Fob220a JX978487
Fob257a JX978500
H8 JX978499
Fo37 JX978498
Clade B
99
100
0.005

Fig. 4: Unrooted phylogenetic tree showing relation between TEF 1α 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.

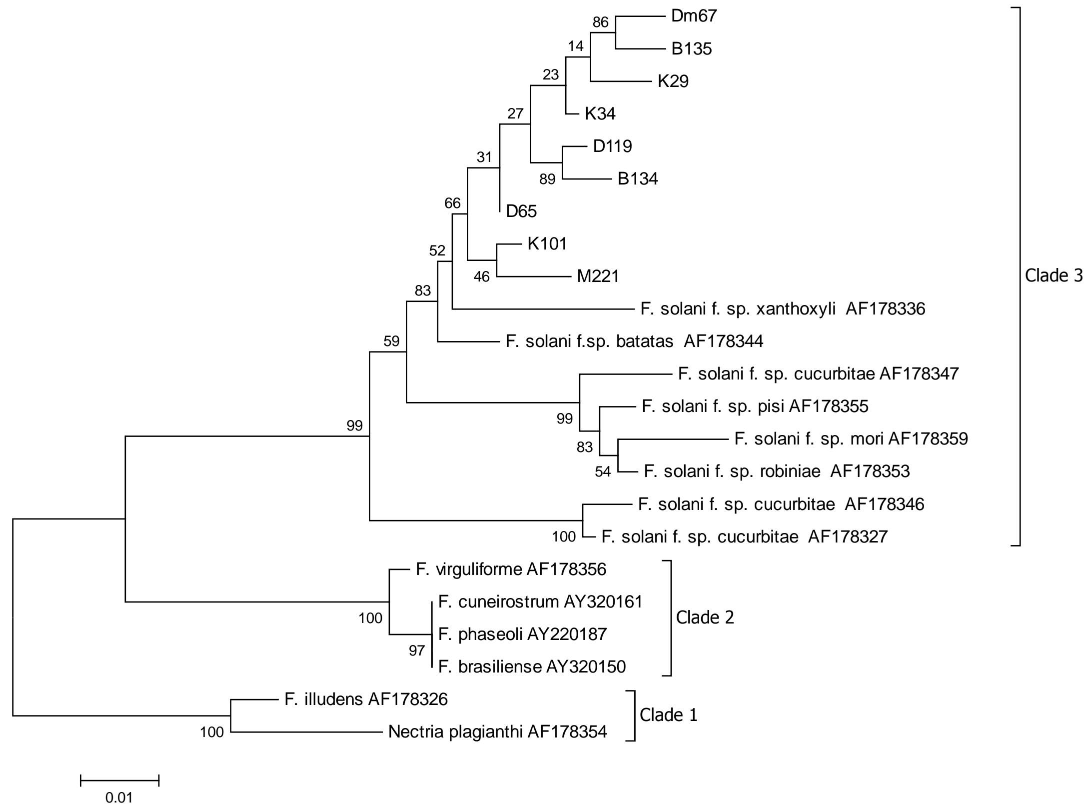

Fig. 5: Unrooted phylogenetic tree showing relation between TEF 1α of the Egyptian *F.solani* sequences as well as 14 GenBank sequences representing previously described species and clades of *F. solani*species complex.

et al. (2012) and Covey *et al*. (2014).The Egyptian strains of *F.oxysporum* obtained from roots of sugar beet were clearly 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* isolateswere polyphyletic.

Concerning the aligned TEF 1α 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 informativewere 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 1α, β- tubulin and Histone 3 genes of *Fusarium* speciesisolated 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 datasetTEF 1α showed progressively increasing the resolution comparable with β tubulin and histone 3. It is worthy to note that TEF 1α gene are coding regions of the genomehas the highly resolution assisting in differentiating within and among species in comparison withtheintron-rich portionsof protein-coding genes such as β- tubulin and histone 3 (Geiser *et al.*, 2004; Barik and Tayung, 2012).

As was found that TEF 1α separated*F.oxysporum* under study into four groups, they were compared with the GenBank sequences representing the three clades of the pathogenic species reported byHill *et al.* (2011); Webb *et al.* (2012) and Covey *et al.*(2014). Accordingly, all theEgyptian 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 1α, β-tubulin & 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 isolatesdivided into three clades.Clade 1 comprised two knownspecies (*Fusarium illudens* and *Nectria plagianthi*). Members of clade 2 included a number of importantpathogens 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 affinitiesamong members of clade 3. This group comprised many species that are important in agricultural crops andmedicine (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.

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