ANKARA UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

Ph.D. THESIS

DETERMINATION OF *FUSARIUM* SPECIES ASSOCIATED WITH CROWN ROT OF WHEAT IN TURKEY AND ASSESSMENT OF RESISTANCE STATUS OF SOME WHEAT GENOTYPES TO *FUSARIUM CULMORUM*

Elfinesh Shikur GEBREMARIAM

DEPARTMENT OF PLANT PROTECTION

ANKARA
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THESIS APPROVAL

The jury members found the research entitled **DETERMINATION OF *FUSARIUM* SPECIES ASSOCIATED WITH CROWN ROT OF WHEAT IN TURKEY AND ASSESSMENT OF RESISTANCE STATUS OF SOME WHEAT GENOTYPES TO *FUSARIUM CULMORUM*** carried out by Elfinesh Shikur GEBREMARIAM satisfactory and recommended that it be accepted as a dissertation **for DOCTOR OF PHILOSOPHY (Ph.D.**) degree in Plant Pathology.

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ETHICS

I declare that this thesis is prepared according to the thesis writing guidelines of Ankara University, Graduate School of Natural and Applied Sciences. All the information provided in this thesis that I prepared is true and accurate. I adhered to Scientific ethics during conducting the research and collecting information. I cited all the references that are used in this thesis.

27/10/2015

Elfinesh Shikur GEBREMARIAM

ÖZET

Doktora Tezi

TÜRKİYE'DE BUĞDAYDA KÖK BOĞAZI ÇÜRÜKLÜĞÜ İLE İLİŞKİLİ *FUSARİUM* TÜRLERİNİN TESPİTİ VE BAZI BUĞDAY GENOTİPLERİNİN *FUSARİUM CULMORUM*'A DAYANIKLILIK DURUMLARININ BELİRLENMESİ

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Buğday (*Triticum* spp.) insan beslenmesinde önemli bir role sahip olup dünya nüfusunun % 40'ı için temel besindir (Bockus vd. 2010). Türkiye'de buğday beslenmedeki protein ve kalorilerin yarısından fazlasını sağlamaktadır (Hanson vd. 1982). Türkiye buğday üretiminde dünyada onuncu sırada olup 2013 yılında 7.77 ha alanda 22.1 milyon ton buğday üretimi gerçekleştirilmiştir (Anonymous 2014). Türkiye'de ortalama buğday verimi hektara 2.8 tondur (Anonymous 2014). Buğday bitkisinde görülen kök boğazı hastalıklarından dolayı %30'dan fazla ürün kaybı rapor edilmiştir (Cook 1968, 1992, Mishra 1973, Klein vd. 1991, Burgess vd. 2001, Hekimhan vd. 2004). Türkiye'de yetiştirilen ekmeklik buğdaylarda kökboğazı çürüklükleri tarafından %24 ile %43 arasında değişen oranlarda ürün kayıpları rapor edilmiştir (Nicol vd. 2001, Hekimhan vd. 2004). Her ne kadar Türkiye'de buğdaylarda kök boğazı çürüklüğü ile ilgili olarak bazı çalışmalar yapılmış olsa da bunların çoğunluğu belli coğrafik alanlarda yapılmış olup 5 yıldan daha eski tarihlerde yapılmış çalışmalardır (Aktaş vd. 1996, Mamluk vd. 1997, Aktaş vd. 1999, Aktaş vd. 2000, Demirci ve Dane 2003, Bentley vd. 2006a, Akgül ve Erkılıç 2007, Tunali vd. 2008, Arıcı ve Koç, 2010). Bu çalışmada Türkiye'nin ekolojik olarak değişik zirai

bölgelerinde kök boğazı çürüklüğü belirtileri gösteren buğday bitkileri ile ilişkili *Fusarium* türleri ve patojenisiteleri ortaya konulmuştur. Ek olarak, tanımlanan en virulent *Fusarium culmorum* izolatına karşı bazı buğday hatlarının dayanıklılık durumları ortaya konulmuştur.

Türkiye'nin buğday yetiştirilen ana bölgelerinden surveyler yapılarak hastalıklı buğday örnekleri toplanmıştır. 2013 yılının Mayıs, Haziran ve Temmuz aylarında buğday örnekleri Ege, Akdeniz, Karadeniz, Orta Anadolu, Güneydoğu Anadolu ve Doğu Anadolu bölgelerinden toplanmıştır. Örnekler 200 tarladan toplanmış ve funguslar belirti gösteren kök boğazı dokularından izole edilmişlerdir. İzolasyonları antibiyotik katılmış (100'er mg/l ampisilin ve streptomisin sülfat) Peptone PCNB ortamı (15 g peptone, 1 g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$, 0.75 g PCNB, and 20 g agar per 1 l distilled H_2O,) (Nash ve Synder 1962, Booth 1971, Burgess vd. 1994, Leslie ve Summerell 2006) kullanılarak yapılmış ve kültürler 5-7 gün 25°C gündüz/20°C gecesıcaklıklarında 12 saatlik fotoperiyot altında geliştirilmişlerdir. Peptone PCNB agardan elde edilen kültürler spor gelişmesini sağlamak üzere SNA ortamına (1 g KH2PO4, 1 g KNO3, 0.5 g MgSO4.7H2O, 0.5 g KCL, 0.2 g glukoz, 0.2 g sukroz, 20 g agar, bir litre distile su) aktarılmışlar ve 7-10 gün yukarıda belirtilen inkubasyon şartlarında muhafaza edilmişlerdir (Burgess vd. 1994, Leslie ve Summerell 2006). SNA ortamında geliştirilen fungusun steril saf su içinde konidi süspansiyonu hazırlanmıştır. Spor süspansiyonu su agarına (20 g agar, 1 l saf H_2O) (Burgess vd. 1994, Leslie ve Summerell 2006) dökülmüş ve fazla su ortamdan uzaklaştırılmıştır. Su agarı ve konidiler içeren Petri kutuları 30-40 derecelik açıda karanlıkta 25°C de 18-20 saat tutulmuşlardır. Su agarında çimlenen tek konidiler alınarak SNA ortamına aktarılmıştır. Monosporik *Fusarium* kültürleri %15' lik gliserolde -80 ºC de tutulmuşlardır.

Fusarium izolatları morfolojik ve moleküler yöntemler kullanılarak tür düzeyinde teşhis edilmişlerdir. *Fusarium* türlerinin morfolojik teşhis çalışmalarında makro- ve mikro konidilerin morfolojisi, fiyalid yapısı, miselyum yapıları, agarda oluşturulan pigmentler ve büyüme hızı değerlendirilmiş ve teşhis anahtarları kullanılmıştır (Booth 1971, 1977, Burgess vd. 1994, Summerell vd. 2003, Leslie ve Summerell 2006). Moleküler teşhis

çalışmaları için Patates Dekstroz Broth sıvı kültüründe 7 gün yetiştirilen fungus miselyumundan FastDNA kiti kullanılarak üretici firmanın tavsiyelerine gore fungal DNA ekstrakte edilmiştir (MP Biomedicals, Santa Ana, CA, USA). Fusarium izolatları translation elongation factor-1 alpha (TEF-1α) gen bölgesi ef1 (5'-ATGGGTAAGGARGACAAGAC-3') ve ef2 (5'-GGARGTACCAGTSATCATG-3') primerleri (O'Donell vd. 1998) kullanılarak standart bir PCR prosedürü ve 53°C bağlanma sıcaklığı kullanılarak çoğaltılmıştır (Geiser vd. 2004). TEF1–α gene sekansları manuel olarak ChromasLite software V.2.1 (Technelysium Pty Ltd, South Brisbane, Australia) kullanılarak gözden geçirilmiştir. Kontrol edilen diziler (yaklaşık 650 bp) daha sonra NCBI BLAST programı veritabanındaki *Fusarium* türlerinin referans dizileri ile karşılaştırılmış (http://blast.ncbi.nlm.nih.gov) ve *Fusarium* türleri belirlenmiştir.

Fusarium türleri teşhis edildikten sonra 17 *Fusarium* türünü temsil eden 342 izolat agar disk yöntemi ile hassas makarnalık buğday çeşidi Kızıltan-91 kullanılarak patojenisite testleri yapılmıştır. Inokulasyondan sonra bitkiler 16 saat ışık, 25/15 (+5) ^{o}C gündüz/gece sıcaklığı ve % 60/80 (+10) nisbi nem içeren büyütme odasında muhafaza edilmiştir (Mitter vd. 2006). Bitkiler gerektikçe sulanmıştır. Deneme sonuçları teyit etmek için tekrarlanmıştır. İnokulasyondan 9 hafta sonra bitkilerin toprakları yıkanmış ve yaprak kını kısımları çıkarılmıştır. Hastalık Wildermuth ve McNamara (1994) ıskalasının değiştirilmiş şekli (Nicol vd. 2001) kullanılarak kökboğazı ve ana kök üzerindeki kahverengileşmeler göz önüne alınarak değerlendirilmiştir (1-5 ıskalası: 1: % 1-9, 2: % 10-29, 3: % 30-69, 4: % 70-89, 5: %90-99).

Patojenisite testini müteakip 46 *F. culmorum* izolatı genotip tarama testlerinde kullanılacak en virulent izolatı tespit etmek için hassas makarnalık buğday çeşidi Kızıltan-91 kullanılarak kök boğazı inokulasyon yöntemi kullanılarak test edilmişlerdir. Bu çalışmada $1x10^{6}$ spor/ml spor yoğunluğu kullanılmıştır. Kontrol uygulamalarında aynı miktarda steril saf su kullanılmıştır. İnokulasyondan sonra bitkiler plastik örtü ile kaplanarak 24 saat yüksek nemde ve karanlıkta yukarıda açıklanan iklim odası

şartlarında tutulmuşlardır. Hastalık şiddetinin değerlendirilmesi inokulasyondan 21 gün sonra yukarıda açıklanan 1-5 ıskalasına göre yapılmıştır.

En virulent *Fusarium culmorum* izolatı olan Fc2 izolat 165 yazlık buğday genotipinin tepkilerini test etmek için kullanılmıştır. Ekimden 1 hafta sonra (tohumları çimlendirmeden 10-11 gün sonra) 165 yazlık buğday hattı $1x10^6$spor içeren ve % 0.1 v/v Tween 20 eklenmiş 1 ml spor süspansiyonu kök boğazına (toprak seviyesinden yaklaşık 0.5 cm yukarıya) steril bir pipet yardımı ile inokule edilmiştir (Mitter vd. 2006). Kontrol çeşitleri aynı miktar spor yoğunluğu kullanılarak inokule edilmiştir. Deneme 5 tekerrürlü olarak kurulmuştur. Uygulamalar tesadüf blokları deneme desenine göre gerçekleştirilmiş ve bitkiler yüksek nem sağlanması amacı ile 48 saat yüksek nemde ve karanlıkta tutulmuşlardır (Mitter vd. 2006). Bitkiler daha sonra yukarıda belirtilen şartlarda iklim odasında muhafaza edilmişler ve gerektikçe sulanmışlardır. Deneme sonuçların teyit edimesi amacı ile tekrar edilmiştir. İnokulasyondan 9 hafta sonra yukarıda açıklanan 1-5 ıskalası kullanılarak hastalık değerlendirmeleri yapılmıştır.

Bu çalışmada elde edilen 342 izolattan 17 değişik *Fusarium* türü tanımlanmıştır. Bu türler *F. culmorum, F. pseudograminearum, F. graminearum, F. equiseti, F. acuminatum, F. brachygibbosum, F. hostae, F. redolens, F. avenaceum, F. oxysporum, F. torulosum, F. proliferatum, F. flocciferum, F. solani, F. incarnatum, F. tricinctum* ve *F. reticulatum*'dur. *Fusarium equiseti* en yaygın izole edilen tür olmuş ve izole edilen *Fusarium* türlerinin %35.55'unu oluşturmuştur. Patojen türler arasında *F. culmorum* survey yapılan tarlaların %13.29'undan izole edilmiş ve en yaygın tür olarak bulunurken *F. pseudograminearum* ve *F. graminearum* survey yapılan tarlaların yalnızca %0.87 ve % 0.29'undan izole edilmişlerdir**.**

Patojenisite testi yapılan 17 *Fusarium* türünden altısı değişik oranlarda hastalık oluşturmuştur. *Fusarium culmorum*, *F. pseudograminearum* ve *F. graminearum*makarnalık buğday çeşidi Kızıltan-91'deyüksek derecede hastalık oluşturmuştur. *Fusarium avenaceum* ve *F. hostae* orta derecede patojen olarak

bulunmuştur. *Fusarium redolens* zayıf patojen olarak bulunmuştur. *Fusarium acuminatum'* un bazı izolatları zayıf patojen olarak bulunmuştur. *Fusarium oxysporum*, *F. equiseti*, *F. solani*, *F. incarnatum*, *F. reticulatum, F. flocciferum, F. tricinctum, F. brachygibbosum, F. torulosum* ve *F. proliferatum* türleri ise patojen olarak bulunmamışlardır.

Hassas makarnalık buğday çeşidi Kızıltan-91 kullanılarak yapılan virülenslık çalışmalarında *Fusarium culmorum* izolatlarının virülenslık bakımından farklılıklar gösterdiği bulunmuştur. Hastalık şiddeti skorları 1.2-4.4 arasında değişmiş olup ortalama 3.0 olmuştur. İzmir'den elde edilen *Fusarium culmorum* izolatı (Fc2) en virulent izolat olarak bulunmuştur (ortalama skor 4.4).

Test edilen 165 hat içinde iki hat (147 ve 158) dayanıklı reaksiyon göstermiş olup 1.4 skor değeri almışlardır. 20 hat (5, 100, 143, 163, 32, 138, 86, 89, 104, 123,153, 161, 8, 34, 142, 9, 15, 47, 116, 146) orta derecede dayanıklı reaksiyon göstermiş olup 1.6 ile 2.4 arasında değişen skor değerleri almışlardır. Dayanıklı ve orta derecede dayanıklı reaksiyon gösteren hatlar orta derecede dayanıklı kontrol çeşitleri Suntop (1.6), Carisma (1.8) ve Altay-2000 (2.4) çeşitlerinden önemli derecede farklılık göstermemişlerdir. Hatların % 63'ü orta derecede hassas reaksiyon vermiştir. Orta derecede hassas reaksiyon gösteren hatların skor değerleri 2.6 ile 3.4 arasında değişmiş olup orta derecede hassas kontrol çeşitleri Adana-99 (ıskala değeri: 2.6), Janz (ıskala değeri: 2.6) ve Emu Rock (ıskala değeri: 2.6) çeşitlerinden önemli derecede farklılık göstermemişlerdir. Test edilen 165 hattan 39 tanesi hassas reaksiyon vermişlerdir. Hassas reaksiyon gösteren hatların skor değerleri 3.6 ile 4.4 arasında değişmiş olup hassas kontrol çeşitleri Süzen-97 (ıskala değeri: 3.6) ve Kutluk-94 (ıskala değeri: 4.0) çeşitlerinden önemli derecede farklılık göstermemişlerdir.

Türkiye'nin önemli buğday yetiştiriciliği yapılan bölgelerinde kök boğazı çürüklüğü ile ilişkili *Fusarium* türlerinin çok sayıda olduğu ve türlerin bölgelere gore dağılımında farklılıkların olduğu ortaya konulmuştur. *Fusarium equiseti* Türkiye'de survey yapılan bölgelerde en yaygın tür olarak bulunmuştur. Patojen *Fusarium* türleri arasında *F.*

culmorum buğday yetiştirilen bölgelerin çoğunda göreceli olarak yüksek oranlarda bulunmuştur. Makarnalık buğday çeşidi Kızıltan-91 ile yapılan fide dönemi patojenisite çalışmalarında *Fusarium culmorum*, *F. graminearum* ve *F. pseudograminearum*'un en önemli patojen türler olduğu görülmüştür. *Fusarium graminearum* ve *F. pseudograminearum* çok düşük oranlarda bulunmuş olup Türkiye'de buğday üretimini sınırlamayacağı düşünülmektedir. Bu çalışmada bir çok diğer *Fusarium* türleri de izole edilmiştir. Bunların kök boğazı patojeni olarak önemlerinin sınırlı olduğu düşünülmektedir.

Test edilen buğday hatlarının %13'ü dayanıklı/orta derecede dayanıklı reaksiyon vermiştir. Bu hatlar Fusarium kök çürüklüğü hastalığına karşı ıslah çalışmalarında dayanıklılık kaynağı olarak kullanılabilir. Türkiye'de buğday bitkisinde kök boğazı çürüklüğü hastalığına dayanıklılık çalışmalarında *Fusarium culmorum* gözönüne alınmalıdır. *Fusarium culmorum* patojeninin yaygın olduğu alanlarda en az 2 yıllık tahıl olmayan bitkilerle münavebe, hastalığa belli ölçüde dayanıklılık gösteren buğday çeşitlerinin ekilmesi, uygun azot gübreleme ve sulama programlarının oluşturulması gerekmektedir.

Ekim 2015, 132 sayfa

Anahtar Kelimeler: Kök boğazı çürüklüğü, *Fusarium,* translation elongation factor, buğday, patojenisite, virülens, genotip, dayanıklılık

ABSTRACT

Ph.D. Thesis

DETERMINATION OF *FUSARIUM* SPECIES ASSOCIATED WITH CROWN ROT OF WHEAT IN TURKEY AND ASSESSMENT OF RESISTANCE STATUS OF SOME WHEAT GENOTYPES TO *FUSARIUM CULMORUM*

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Supervisor: Prof. Dr. Aziz KARAKAYA

This research was carried out with the aim of studying the diversity and pathogenicity of *Fusarium* species associated with crown rot of wheat in Turkey and screening wheat lines for their reaction to *Fusarium culmorum.* During summer 2013, samples were collected from 200 fields in the different agro-ecological regions of Turkey. Fungi were isolated from symptomatic crown/stem base tissues. The isolates were identified to species level using morphological and molecular methods. Morphological identification of *Fusarium* species was based on macro and microconidial morphology, phialide structure, mycelial characteristics, pigmentation on agar and growth rate using keys in Fusarium identification manuals. Molecular identification was carried out by sequencing the translation elongation factor-1 alpha (TEF-1α) gene region using primers ef1 and ef2. A total of 342 isolates representing 17 *Fusarium* species were isolated. The isolates were identified as *F. culmorum, F. pseudograminearum, F. graminearum, F. equiseti, F. acuminatum, F. brachygibbosum, F. hostae, F. redolens, F. avenaceum, F. oxysporum, F. torulosum, F. proliferatum, F. flocciferum, F. solani, F. incarnatum, F. tricinctum,* and *F. reticulatum. Fusarium equiseti* was the most commonly isolated species, accounting for 35.55% of the total *Fusarium* species isolated. Among the damaging species, *F. culmorum* was the most predominant species being isolated from 13.29% of sites surveyed while *F. pseudograminearum* and *F. graminearum* were isolated only from 0.87% and 0.29% of surveyed sites, respectively. All the 342 isolates belonging to the 17 *Fusarium* species were tested for pathogenicity on susceptible durum wheat cultivar Kızıltan-91 using agar disc inoculation method. Seven out of the 17 *Fusarium* species tested for their pathogenicity caused crown rot in different levels of severity. *Fusarium culmorum*, *F. pseudograminearum* and *F. graminearum* caused severe crown rot disease on durum wheat cultivar Kızıltan-91.

Fusarium avenaceum and *F. hostae* were moderately pathogenic. *F. acuminatum* and *F. redolens* were weakly pathogenic. On the other hand, *F. oxysporum*, *F. equiseti*, *F. solani*, *F. incarnatum*, *F. reticulatum, F. flocciferum, F. tricinctum, F. brachygibbosum, F. torulosum* and *F. proliferatum* were non- pathogenic. The result of aggressiveness test showed that *Fusarium culmorum* isolates differed in their aggressiveness on the susceptible durum wheat variety Kızıltan-91. The most aggressive *Fusarium culmorum* isolate Fc2 was used as inoculum to screen 165 spring wheat lines for their reaction. Thirteen percent of the lines tested showed promising and consistently resistant/moderately resistant reaction to *Fusarium culmorum*.

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Key Words: Crown rot, *Fusarium,* translation elongation factor, wheat, pathogenicity, aggressiveness, genotype, resistance

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LISTS OF SYMBOLS

g	Gram
µm	Micrometer
µl	Microliter
µg	Microgram
ng	Nanogram
mg	Milligram
ml	Milliliter
l	Liter
mm	Millimeter
cm	Centimeter
mM	Millimol
rpm	Revolution per minute
m/s	Minute per second
h	Hour
min	Minute
s	Second
^{o}C	Degree Celsius
^{o}	Degree
$MgCl_2$	Magnesium chloride
dNTPs	Deoxyribonucleotide triphosphates
NaOCl	Sodium hypolchlorite
bp	Base pair
kb	Kilo base
km	Kilometer

LISTS OF ABRREVIATIONS

CLA	Carnation Leaf Agar
DNA	Deoxyribonucleic acid
EPPO	European and Mediterranean Plant Protection Organization
FAO	Food and Agriculture Organization
GPS	Global Positioning System
HSD	Honestly Significant Difference
NCBI	National Center for Biotechnology Information
PCNB	Pentachloronitrobenzene
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
RCBD	Randomized Complete Block Design
SNA	Synthetic Nutrient-poor Agar
sp	Species (singular)
spp	Species (plural)
SPSS	Statistical Package for the Social Sciences
TBE	Tris Borate EDTA
UV	Ultraviolet
v/v	Volume per volume
w/v	Weight per volume
WA	Water agar

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1. INTRODUCTION

Wheat (*Triticum* spp.) plays a tremendous role in human nutrition. It serves as a staple food for 40% of the world's population (Bockus *et al.,* 2010). Its high yield and nutrition, ease of grain storage and processing it into different food forms made wheat the major diet component (Curtis, 2002; Shewry, 2009). The gluten protein in wheat causes the dough to rise and helps to produce light bread (Reitz, 1967; Hanson *et al.,* 1982). Wheat is consumed in different forms which includes; leavened breads and rolls, flat breads, porridge, biscuits, cakes, pasta and noodles (Hanson *et al.,* 1982; Shewry, 2009). It provides essential amino acids, minerals, vitamins and dietary fiber (Reitz, 1967; Shewry, 2009; Bockus *et al.,* 2010). Wheat serves as a source of more calories and protein to the world's diet than any other food crop (Hanson *et al.,* 1982). It provides about 55% of the carbohydrates (Breiman and Graur, 1995) and 20% of the food calories (Reitz, 1967; Bockus *et al.,* 2010) consumed globally. In Turkey, wheat contributes for more than half of the calories and protein in the diet (Hanson *et al.,* 1982).

Wheat is classified on the basis of species, commercial type, and growth habit. The genus *Triticum* comprises 16 recognized wheat species, among which *Triticum aestivum* L. and *Triticum durum* Desf. are cultivated on a large scale (Hanson *et al.,* 1982). The two main commercial types of wheat are bread wheat (*T. aestivum*) and durum wheat (*T. durum*). Based on the number of repeated genomes wheat is classified as diploid (AA, 2n=14), tetraploid (AA and BB, 2n=28) or hexaploid (AA, BB, and DD, 2n=42) (Shewry, 2009). Durum and bread wheat are tetraploid and hexaploid species, respectively. About 95% of the wheat grown worldwide is hexaploid bread wheat while most of the rest 5% being tetraploid durum wheat (Shewry, 2009). Based on growth habit wheat is classified as winter, spring and facultative type of wheat (Hanson *et al.,* 1982).

About 20% of the cultivated area of the world is planted with wheat (Bockus *et al.,* 2010). In spite of its big role in the world's diet, wheat production has fallen in recent years (Bockus *et al.,* 2010). In the world, over 500 million metric tons of wheat is

produced from a production area of about 200 million ha (Bockus *et al.,* 2010). The potential yield of wheat is limited by environmental factors including moisture, temperature, soil nutrient and pests (Hanson *et al.,* 1982). About 25 to 30% of the wheat crop is lost due to abiotic and biotic stresses, the latter due to diseases (Bockus *et al.,* 2010). The potential yield of wheat can exceed 10 tons' ha^{-1}; however, deficiencies in water and nutrients and the effects of pests and diseases reduce the global average yield to about 2.8 tons ha^{-}1 (Shewry, 2009).

Turkey is the tenth largest wheat producer in the world with annual production of around 22.1 million tons from a total wheat production area of 7.77 million ha in 2013 (Anonymous, 2014). The average yield of wheat in Turkey is 2.8 tons ha^{-1} (Anonymous, 2014), however, the yield varies from 1 ton ha^{-1} in the Eastern region to 3 tons ha^{-1} in the European part of Marmara region (Braun *et al*., 2001). In Turkey wheat accounts for 3.9% of total world wheat production, more than 32% of total cultivated land and 60% of cereal production (Geçit *et al.,* 2009). In Turkey three different wheat environments exist which produce the three wheat types; winter wheat, spring wheat and facultative wheat (Hanson *et al.,* 1982). Winter wheat is the widely grown wheat type in Turkey (Hanson *et al.,* 1982). About 90% of the wheat is grown under rain fed or semi-supplementary irrigation conditions (Braun *et al.,* 2001). The cropping system is mainly wheat-fallow rotation but grain legume rotation is practiced in some regions. Central Anatolian Plateau (CAP), Thrace region, and Southeast Anatolia (SEA) are the major wheat producing areas in Turkey, of which, CAP is the main winter wheat producing area. In the Thrace region winter wheat is produced under high rainfall conditions and intensive cropping systems in rotation with sunflower, while Southeast Anatolia is the primary area for spring wheat cultivation, although facultative wheat is also grown in the region.

The world food production is not in balance with the growing population. Among the factors contributing for this fact, plant diseases which reduce production and yield of crop plants are important ones. Cereal diseases exist wherever the crops are grown. In Turkey, bunt (*Tilletia foetida* and *T. caries*), loose smut (*Ustilago nuda*) and rusts

(*Puccinia striiformis*, *P. graminis* f. sp. *tritici* and *P. recondita* f. sp. *tritici*) are among the major diseases of wheat (Geçit *et al.,* 2009; Mamluk *et al*., 1997). Root and foot rots caused by *Fusarium* spp., *Drechslera sorokiniana, Pseudocercosporella herpotrichoides, Alternaria alternata, Sclerotium* spp., and *Rhizoctonia* spp. are common soilborne diseases of wheat in Turkey (Iren, 1981). Other diseases include; downy mildew, powdery mildew, septoria blotch, damping off and bacterial head blight (Mamluk *et al.,* 1997; Geçit *et al.,* 2009). Among the viral diseases, barley yellow dwarf (PAV and RMV serotypes) (Mamluk *et al.,* 1997; Geçit *et al.,* 2009), wheat streak mosaic virus (WSMV) and barley yellow striate mosaic virus (BYSMV) were reported from Central Anatolia Plateau at relatively lower frequencies (Mamluk *et al.,* 1997).

Soilborne diseases including crown rot are important diseases of cereals in the world, particularly in areas where cereal based rotations, marginal growing conditions and or cultural practices are common. Despite their economic importance, some soilborne diseases are given less attention because of the difficulty in working with them (Wallwork, 2000; Singleton, 2002). Soilborne pathogens of cereals invade crown and root tissues and interfere with nutrient and water uptake which lead to economic yield losses (Singleton, 2002). Yield loss caused by Fusarium crown rot disease is difficult to estimate as infection occurs at or near the soil surface (Strausbaugh *et al.,* 2004), and is not clearly visible until the formation of whiteheads shortly before harvest (Burgess *et al.,* 2001; Paulitz *et al.,* 2002).

Damage caused by *Fusarium* species on small grain cereals include, rotting seeds, seedlings, roots, crowns, basal stems or heads (Paulitz *et al.,* 2002). Fusarium crown rot also causes pre - and post emergence damping off, reduction in straw production, grain yield and grain quality (Smiley *et al.,* 2005b). Crown rot (CR) pathogens cause yield losses due to damaged seedlings, lodging and improper grain filling (Schilling *et al.,* 1996). Wet conditions shortly after seeding and dry conditions between anthesis and plant maturity are conditions that favor crown rot disease (Nelson *et al.* 1981; Paulitz *et al.,* 2002; Smiley *et al.,* 2005b). The disease is of economic importance in dryland wheat producing regions including Turkey, Australia, Europe,

North America, North and South Africa, West Asia, and South America (Smiley *et al.*, 2005b; Chakraborty *et al.,* 2006; Nicol *et al.*, 2007). Crown rot has been reported from different countries including Turkey (Aktaş *et al.,* 1999; Braun *et al.,* 2001; Tunali *et al.,* 2006), Australia (Wildermuth *et al.*, 1997; Burgess *et al.,* 2001; Akinsanmi *et al.,* 2004; Chakraborty *et al.,* 2006), USA (Smiley and Patterson, 1996; Gonzalez and Trevathan, 2000; Smiley *et al.,* 2005a; Moya-Elizondo *et al.*, 2011), Canada (Fernandez and Chen, 2005; Fernandez and Holzgang, 2009), Germany (Mishra, 1973), Italy (Rossi *et al.,* 1995), Croatia (Postic *et al.,* 2012), Norway (Kosiak *et al.,* 2003), Argentina (Carranza, 1961), Iran (Saremi *et al.,* 2007; Hajieghrari, 2009), New Zealand (Bentley *et al.,* 2006b), Poland (Weber *et al.,* 2001), South Africa (Klaasen *et al.,* 1991) and Tunisia (Gargouri-Kammoun *et al.,* 2009). World-wide losses exceeding 30% have been documented (Cook, 1968, 1992; Mishra, 1973; Klein *et al*., 1991; Burgess *et al*., 2001; Hekimham *et al.,* 2004). Smiley *et al*. (2005b) reported yield loss as high as 61% in the USA following artificial inoculation of wheat with a mixture of five *F. pseudograminearum* isolates. Yield losses ranging from 24% to 43% caused by crown rot diseases have been recorded on common bread wheat cultivars in Turkey (Nicol *et al.,* 2001; Hekimhan *et al.,* 2004).

Management of crown rot has relied on cultural practices that only provide partial control and are not reliable for limiting damage caused by the disease (Cook, 1981; Smiley and Patterson, 1996; Paulitz *et al*., 2002). Although there are no fully resistant wheat cultivars to crown rot disease (Pereyra *et al*., 2004; Wisniewska and Kowalczyk, 2005), use of genotypes that show some degree of resistance/tolerance is the most efficient and reliable approach to reduce yield losses due to Fusarium crown rot (Cook, 2001).

Turkey is characterized by diverse climatic conditions (Braun *et al.,* 2001). Güler *et al.,* (1990) classified Turkey into seven agro-ecological regions; Marmara, Aegean, Central Anatolia, Southeast Anatolia, Eastern Anatolia, Black Sea and Mediterranean regions. Particular pathogens associated with crown rot may dominate in different areas, and

different pathogens may be predominant during successive growing seasons in a particular region. Temperature, moisture and cropping practices are among the factors contributing for the difference in distribution or existence of a particular pathogen in a given region.

Although several surveys have been carried out to study crown rot of wheat in Turkey, most of them covered limited geographic areas and have been more than five years (Aktaş*et al.,* 1996; Mamluk *et al.,* 1997; Aktaş*et al.,* 1999; Aktaş*et al.,* 2000; Demirci and Dane, 2003; Bentley *et al.,* 2006a; Akgül and Erkılıç, 2007, Tunali*et al.,* 2008; Arıcı and Koç, 2010).

Objectives

General Objective:

The present research was carried out with a general objective of studying the diversity and pathogenicity of *Fusarium* species associated with crown rot of wheat in the different agro-ecological regions of Turkey and screening wheat lines for their resistance to the most aggressive *Fusarium culmorum* isolate identified.

Specific Objectives:

1. Identify *Fusarium* species associated with crown rot of wheat in different agro-ecological regions of Turkey using morphological and molecular methods.
2. Study morphological features of all *Fusarium* species identified in this research.
3. Study the pathogenicity of all identified *Fusarium* species on susceptible wheat cultivar.
4. Test the aggressiveness of *Fusarium culmorum* isolates on susceptible wheat cultivar.
5. Screen wheat lines for their reaction to the most aggressive isolate of *Fusarium culmorum.*

2. LITERATURE REVIEW

2.1. Crown Rot Caused by *Fusarium* spp.: Biology, Survival and Life Cycle, Host Range, Distribution and Management Options

Biology

Crown rot is a major problem facing dryland cereal production worldwide. Wet conditions favor initial infection of plants by crown rot pathogens, however, dry conditions near plant maturity which predisposes plants to water stress lead to severe damage (Wallwork, 2000). After infection, water stress in the affected plants can increase the degree of colonization, probably by disrupting host defense mechanisms (Burgess *et al.,* 2001).

The disease crown rot is also known by different common names including, Fusarium crown rot, dryland foot rot, dryland root rot, Fusarium root rot and common root rot (Paulitz *et al*., 2002). Crown rot is caused by a complex of fungal pathogens which include; *F. culmorum* (W. G. Smith.) Sacc., *F. pseudograminearum* (O'Donnell and Aoki) (= *F. graminearum* group 1, = *Gibberella coronicola*), *F. graminearum* (Sch.) (= *F. graminearum* group 2, = *Gibberella zea)*, *F. avenaceum* (Fr.) Sacc. (= *Gibberella avenacea*), *F. acuminatum* (Ell. and Ever.)*, F. crookwellense* (Burg. Nels. and Tous.)*, Microdochium nivale* (Fr.) Samuels and I. C. Hallett (= *Monographella nivalis; = F. nivale*) and *Bipolaris sorokiniana* (Sacc.) Shoemaker (= *Cochliobolus sativus*) (Cook, 1968; Smiley and Patterson, 1996; Aktaş *et al.,* 1999; Burgess *et al.,* 2001; Paulitz *et al.,* 2002; Fernandez and Chen 2005; Tunali *et al.,* 2006; Bockus *et al.,* 2010). These pathogens may occur singly, but they often exist together in the same fields and even within individual plants, and there may be difference in dominance of different pathogens at a specific location from year to year (Smiley and Patterson, 1996). In Turkey and other parts of the world *F. culmorum* and *F. pseudograminearum* are the two most commonly reported damaging *Fusarium* species causing the disease (Cook, 1992; Aktaş *et al*., 1999; Burgess *et al.,* 2001; Tunali *et al.,* 2006; Nicol *et al.,* 2007). In Turkey, *F. pseudograminearum* is relatively common in the Marmara region, where

spring wheat is grown in winter under mild temperatures and ripens under hot, dry conditions in spring, while *F. culmorum* is more prevalent in the Central Anatolian Plateau where winter wheat is grown through a cold winter (Burgess *et al*., 2010).

Scattered whiteheads with shrivelled white grains which are results of premature ripening of tillers are the first observable symptom of crown rot disease in a crop (Cook, 1968, 1980; Wallwork, 2000; Burgess *et al.* 2001; Singleton, 2002; Scherm *et al*., 2013). The whiteheads may contain few or no seeds (Cook, 1968; Wallwork, 2000; Burgess *et al.* 2001; Bockus *et al.,* 2010), pinched grain, or normal grain depending on the development of crown rot in relation to crop maturity (Burgess *et al.* 2010). Under the leaf sheaths of plants with whiteheads, a chocolate brown discoloration at the crown and base of plants that extends one to three internodes up the stem is observed (Cook, 1968; Cook, 1980; Singleton, 2002; Bockus *et al.,* 2010; Burgess *et al*., 2010; Scherm *et al*., 2013). Brown discoloration is also observed on subcrown internode (Scherm *et al*., 2013). As a result of the plant response to infection, symptom of basal browning may be observed prior to the presence of the fungus in these portions (Beccari *et al*., 2011). Formation of whitehead and chocolate-brown lesion in the lower stem of wheat are the key symptoms of the disease (Burgess *et al.,* 2001; Bockus *et al.,* 2010). However, compared with whiteheads, browning of the lower culm is considered a more reliable symptom of the disease (Dodman and Wildermuth, 1987; Klassen *et al*., 1992) but requires a labor-intensive assessment procedure. The pathogen may progress up the culm internally or externally, through leaf sheath. Cottony pink discoloration within the hollow of infected wheat culm or enclosing leaf sheaths is considered as a diagnostic sign of Fusarium crown rot (Cook, 1968, 1980; Bockus *et al.,* 2010). *Fusarium pseudograminearum* and *F. culmorum* typically produce a pinkish discoloration around or in the crown or under leaf sheath (Wallwork, 2000). Formation of pink coloration along with a mass of white fungal growth when infected plants are left in a damp plastic bag for few days is a clear indication of the disease (Wallwork, 2000). The pinkish coloration of diseased culms is as a result of an accumulation of Fusarium mycelium under leaf sheaths (Cook, 1968; Wallwork, 2000; Scherm *et al*., 2013). In seedlings, the first visual symptoms of the disease are a uniform browning of the stem bases (Burgess *et al*., 2001). Crown rot also causes brown discoloration on roots and coleoptiles of

infected seedlings (Scherm *et al*., 2013). However, Burgess *et al*. (2001) reported that infection of roots does not appear to be common. The formation of whitehead and stem browning symptoms depends on the level of host plant resistance and environmental conditions (Burgess *et al.,* 2010). In most climates, the development of whitehead symptoms increases with increasing drought stress (Singleton, 2002).

Survival and life cycle

Members of the genus *Fusarium* may produce three kinds of spores, ascospore (sexual spore), conidia (asexual spore) and chlamydospore. In *Fusarium* species sporulation (reproduction) may occur sexually by ascospores formed in perithecia or asexually by conidia (conidiospores) formed on sporodochia. In case of *Fusarium culmorum* sporulation occurs asexually by conidia (Cook, 1981). Under unfavorable conditions conidia change into chlamydospores, resistant spores which help the fungus to overwinter.

Fusarium crown rot pathogens survive unfavorable conditions on plant residues and organic matter as hyphae or as chlamydospore in soil (Burgess, 1981). Several species of *Fusarium* produce airborne conidia which colonize different plant parts including stems, leaves and flowers (Burgess, 1981). *Fusarium pseudograminearum* and *F. avenaceum* survive mostly as mycelium in plant residue (Cook, 1981; Paulitz *et al.,* 2002). *Fusarium culmorum* remain viable as mycelium in crop residues and survive as chlamydospore in soil for 2-4 years (Cook, 1980; Inglis and Cook, 1986; Bateman *et al*., 1998). *Fusarium graminearum* overwinters as perithecia on host tissue, especially in corn stalks (Bockus *et al.,* 2010). Alternate hosts and/or weeds serve as source of inoculum during the off season (Postic *et al.,* 2012). The result of a study conducted by Inch and Gilbert (2003) indicated that wild grasses harbour several species of *Fusarium*, causing Fusarium head blight in cereal crops in Manitoba, Canada.

On the onset of favorable environmental condition and presence of susceptible host, propagule of virulent pathogen starts infection of newly sown plants. Fusarium

crown rot pathogens enter stem bases directly, near the soil surface, approximately 2-3 cm below the soil surface through openings around emerging crown roots, or by infection of the newly emerging crown roots around 4-6 weeks after planting (Cook, 1968, 1980; Bockus *et al.,* 2010). Successful infection then leads to the colonization of crowns and subcrown internodes, which progress up the culm under conditions of water stress (Nelson *et al.,* 1981; Bockus *et al.,* 2010). Colonization of tissue takes place initially as intercellular apoplastic pathway between cells of the epidermis and cortex, and subsequently, complete colonization of tissue is achieved intracellularly in the symplast (Burgess *et al.,* 2001). Macroconidia is formed on infected host parts or crop residue located above ground where light is available (Cook, 1981). Water splashed conidia serve as sources of inoculum to produce crown rot and/or head blight in the next disease cycle, or macroconidia may enter the soil and serve as long term inoculum sources (Nelson *et al*., 1981). However, soilborne inoculum serves as a primary cause of infection under conditions of low humidity. Under conditions of high moisture and aggressive inoculum, infection prior to emergence can lead to seedling death and damping off (Bockus *et al.,* 2010).

Host range and distribution

Fusarium crown rot pathogens have a wide host range, most of which are cereals including wheat, barley, oats, rye, corn, sorghum and various grasses (Wallwork, 2000; Paulitz *et al.,* 2002; Scherm *et al*., 2013). *Fusarium graminearum, F. pseudograminearum, F. culmorum* and *F. avenaceum* have a wide range of host plants in addition to cereal grains (Cook, 1981). *Fusarium culmorum* was isolated from sugar beet, flax, carnation, bean, pea, asparagus, red clover, hop, leeks, Norway spruce, strawberry and potato tubers (Scherm *et al*., 2013). Bentley *et al.,* (2006a) isolated *F. culmorum, F. pseudograminearum, F. graminearum, F. semitectum* (synonym *F. incarnatum*) and *F. acuminatum* from grass stem bases in Northern Turkey.

Although crown rot pathogens may occur singly, they usually exist together in the same fields and even in the same plants. Temperature, moisture and cropping practices are among the factors contributing for the difference in the distribution or

occurrence of a particular crown rot pathogen in a particular area. Within a given region, *F. pseudograminearum* and *F. graminearum* are more common in areas with warmer temperature, whereas *F. culmorum* is prevalent in areas of intermediate temperature condition (Bockus *et al.,* 2010). Areas with temperatures favorable to *F. graminearum* are those suitable for production of rice or corn, while areas with temperatures suitable for *F. culmorum* are those favorable for production of wheat, barley and oats (Cook, 1981).

Management Options

Management of soilborne diseases is one of the biggest challenges in agriculture. Knowledge and understanding of ecology of the pathogen and predisposing factors help in management of soilborne diseases without reducing the potential yield of a crop (Cook, 1980). Integrated management practices which include, cultural, host plant resistance, chemical and other agronomic practices are used in control of most soilborne diseases (Singleton, 2002). Management practices used to reduce incidence of crown rot includes, control of grass weeds and host plants, crop rotation with at least a two year break from susceptible cereals, use of varieties having some degree of resistance, removal/ breakdown of infested plant residue, shallow seeding to avoid soilborne inoculum, use of pathogen free seeds, seed treatments, delayed planting and use of appropriate nitrogen application rates to avoid late season water stress (Wallwork, 2000; Paulitz *et al*., 2002; Burgess *et al.,* 2010; Bockus *et al*., 2010). However, delayed planting and optimized nitrogen application help to control the disease only partially (Cook, 1981; Smiley and Patterson, 1996; Paulitz *et al.*, 2002). Control of crown rot pathogens using chemicals is not an option (Paulitz *et al*., 2002; Burgess *et al*., 2010).

Wheat varieties vary in their reaction to crown rot, ranging from very susceptible to moderately resistant (Wallwork, 2000). There are no wheat varieties with full resistance to the disease (Pereyra *et al*., 2004; Wisniewska and Kowalczyk, 2005). Durum wheat varieties are more susceptible to crown rot than bread wheat (Wallwork, 2000; Fernandez and Jefferson, 2004; Fernandez *et al*., 2007; Bockus *et al.,* 2010; Burgess *et al*., 2010). Although wheat varieties do not have good resistance to root/crown rot, the

use of varieties/cultivars having some degree of resistance is the most effective, economic and reliable approach to reduce yield losses and carryover of inoculum to the subsequent years (Wallwork, 2000).

Adult and seedling resistance are the two types of host plant resistance. Seedling resistance can be identified using seedling bioassays during the first 45 days of the seedling growth. While, adult plant resistance involves evaluation of mature plants for symptoms of crown rot. Unlike evaluation of matured plant resistance in the field, seedling bioassay is time saving and avoids effects of other seasonal or environmental factors. Positive correlations between scores of crown rot in greenhouse tests and field trials have been documented (Klein *et al.*, 1985; Wildermuth and McNamara, 1994; Mitter *et al.*, 2006; Li *et al.*, 2008). Since seedling screening test speeds up selection of resistant progeny in breeding programs, it can be used to screen large quantities of germplasms in short periods of time and take only promising materials to field testing.

Breeding for crown rot resistance has been difficult, partly due to variability associated with phenotyping and also due to an incomplete understanding of resistance genes. Probably differences in genetic resistance exist for the various pathogens involved in the crown rot disease complex. Therefore, study for resistance initially should focus on only one species and expanded later to include other species (Paulitz *et al.*, 2002; Miedaner *et al.*, 2012). Miedaner (1997) reported a high correlation between resistance to *F. graminearum* and *F. culmorum* in wheat and rye.

2.2. The Genus *Fusarium*; Taxonomy, Host Range and Distribution and Identification

Taxonomy

Leslie and Summerell (2006) stated that the genus name *Fusarium* was erected by Link in 1809 for species with fusiform, non-septate spores borne on a stroma (sporodochium) and was based on *Fusarium roseum.* The genus *Fusarium* belongs to Kingdom Fungi;

Phylum Ascomycota; Subphylum Pezizomycotina; Class Sordariomycetes; Subclass Hypocreomycetidae; Order Hypocreales; Family Nectriaceae. Many of the most important *Fusarium* species form their sexual stage (teleomorph) in the genus *Gibberella*, and a number of other species have their teleomorph in the genus *Nectria*.

Fusarium taxonomy has been changing and many species in the genus remained not well defined. The factors contributing for the changing Fusarium taxonomy includes lack of clear morphological characters to separate species, morphological and physiological variation and mutation in culture (Geiser *et al*., 2004; Leslie and Summerell, 2006). Some of the variation in culture may be due to differences in media, temperature and light (Leslie and Summerell, 2006). Development of species specific oligonucleotide primers made species identification easy and well-defined unlike morphological studies. *Fusarium* is a genus with many species, strains, and metabolites which play important role in science or agriculture. There are more than 80 recognized species in the genus *Fusarium* (Leslie and Summerell, 2006).

Host range and Distribution

The genus *Fusarium* is one of the most important genera of fungi which include many economically important pathogens of plants (Booth, 1971; Nelson *et al.,* 1981; Leslie and Summerell, 2006). Many members of the genus are soil saprophytes and some are mycotoxigenic (Marasas *et al.,* 1984; Nelson *et al*., 1990; Wallwork, 2000). Some members of the genus cause infections in humans and other animals (Rebell, 1981). *Fusarium* species cause a wide range of diseases on many plants (Summerell *et al*., 2003). Many plants have at least one *Fusarium* associated disease (Leslie and Summerell, 2006). *Fusarium* species cause diseases including crown and root rots, stalk rots, head blights and vascular wilt (Nelson *et al.,* 1981). Some *Fusarium* species are more adapted to tropical, subtropical and temperate climates, while others are cosmopolitan (Windels, 1992; Summerell *et al.,* 2003).

Identification

The knowledge and ability to identify plant pathogenic organism (s) is the cornerstone to understand and control the disease. Fusarium identification has been carried out using morphological, biological and molecular tools (Leslie and Summerell, 2006). Gerlach and Nirenberg (1982) and Nelson *et al*. (1983) defined morphological species concepts during the 1980's. Morphological tools remain the only option and the most commonly used method for identifying *Fusarium* species for laboratories that do not have facilities and expertise to undertake molecular species identification (Leslie and Summerell, 2006). Shape of macroconidia is the most important morphological feature used for *Fusarium* species identification (Windels, 1992; Burgess *et al.,* 1994; Leslie and Summerell, 2006; Scherm *et al*., 2013). Usually the morphology of conidia alone is sufficient to identify a given culture to species (Leslie and Summerell, 2006). *Fusarium* species produce distinctly shaped macroconidia, usually with a foot shaped basal cell (Booth, 1984). The other morphological features used in *Fusarium* species identification include presence or absence of microconidia, shape or mode of formation of microconidia, the nature of the conidiogenous cells (phialides) on which microconidia are borne and presence or absence of chlamydospores (Leslie and Summerell, 2006). Presence or absence of sclerotia can also be used in species identification but it is not important taxonomic criteria (Windels, 1992). The aforementioned morphological characters used for identification of *Fusarium* species are observed on carnation leaf agar (CLA) or synthetic nutrient-poor agar (SNA), however; conidia formed on CLA are more suitable for species identification as they are stable in size and shape (Burgess *et al.,* 1994; Summerell *et al.,* 2003; Leslie and Summerell, 2006). Colony morphology (pigmentation on agar, color and abundance of aerial mycelium) and growth rate on potato dextrose agar (PDA) are important secondary characters used for *Fusarium* species identification (Burgess *et al.,* 1994; Summerell *et al.,* 2003; Leslie and Summerell, 2006).

With the development of species specific oligonucleotide primers, molecular techniques have become common and enabled well defined species identification. β-tubulin, Internal Transcribed Spacer (ITS), mitochondrial Small Subunit (mtSSU) and

translation elongation factor 1-alpha (TEF1-α) sequences have been widely used in the taxonomic studies of *Fusarium* species (O' Donnell *et al*., 1998; Leslie and Summerell, 2006). The TEF1-α gene sequence has been widely used as identification tool in *Fusarium* because it occurs consistently as single-copy in *Fusarium*, and shows a high level of sequence polymorphism among closely related *Fusarium* species (Geiser *et al.*, 2004). O'Donnell *et al*. (1998) developed primers ef1 and ef2 to study lineages within the *F. oxysporum* complex. Geiser *et al.* (2004) created the FUSARIUM- ID v.1.0, which is a publicly available database consisting sequences representing a phylogenetically diverse selection of TEF gene sequences from the genus and placed it on a local BLAST server, which can be accessed online at http://fusarium.cbio.psu.edu (Geiser *et al.*, 2004). FUSARIUM- ID v.1.0 contains methods for identification of *Fusarium* species by amplifying the TEF gene (~700bp) using primers ef1 (5'-ATGGGTAAGGARGACAAGAC-3') and ef2 (5'-GGARGTACCAGTSATCATG-3') (O' Donnell *et al.,* 1998) following a standard PCR procedure with an annealing temperature of 53°C.

2.3. History and review of surveys on crown rot of wheat

2.3.1. Review of surveys on crown rot of wheat in the world

Crown rot of wheat was first recorded on wheat in 1951 in Australia by McKnight (Burgess *et al.,* 2001). Since then the disease has been reported from different regions of the world including Turkey, North Africa, South Africa, Australia, USA and Canada (Klaasen *et al.,* 1991; Smiley and Patterson, 1996; Aktaş *et al*, 1999; Burgess *et al.,* 2001; Nicol *et al*., 2001; Fernandez and Chen, 2005; Saremi *et al.,* 2007). Some of the surveys on crown rot from the different countries are summarized below.

Cook (1968) conducted a detailed survey to study crown rot in winter wheat in the Pacific Northwest (PNW) of USA in 1964 for the first time. In this study, yield losses of up to 50% in individual fields of winter wheat were reported. More than 90% of the isolates from diseased plants were *F. roseum* f.sp. *cerealis* ' Culmorum'(=*F. culmorum*) and *F. reseum* f.sp. *cerealis* 'Graminearum'(= *F. graminearum*) with 'Culmorum' being

the common species. Although *F. roseum* f.sp. *cerealis* ‘Avenaceum’(=*F. avenaceum*) was isolated occasionally from crowns of plants from the region, it rarely killed infected plants.

In a study conducted by Burgess *et al*. (1975) in the eastern wheat belt of Australia, *Fusarium roseum* 'Graminearum' was found to be the predominant *Fusarium* associated with crown rot of wheat in the region. Although several *F. roseum* ‘Graminearum’ group 2 (=*F. graminearum*) isolates were obtained from wheat stem bases, most of the isolates were *F. roseum* ‘Graminearum’ group 1 (=*F. pseudograminearum*). The result of the study showed that the incidence and severity of crown rot caused by *F. roseum* 'Graminearum' was greater in areas where plants were affected by moisture stress.

Klein *et al*. (1990) conducted surveys during the periods of 1976 to 1981 to study the incidence of whiteheads in wheat in the northern areas of the wheat belt in New South Wales. Whiteheads which were associated with crown rot were common in wheat crops in the survey areas. However, the incidence of whiteheads was low (<5%) in most crops. The predominant crown rot pathogen was *Fusarium graminearum* Group 1(=*F. pseudograminearum*) which was isolated from 97% of stem bases collected from plants showing whitehead symptoms.

During the years 1987, 1988 and 1989, Parry (1990) investigated the incidence of pathogenic *Fusarium* and *Microdochium* species in stem bases of winter wheat in the Midlands, UK. The four *Fusarium* species isolated were *F. nivale* (=*Microdochium nivale*), *F. avenaceum*, *F. culmorum* and *F. graminearum* with *F. nivale* being the predominant species followed by *F. avenaceum* and *F. culmorum*.

Smiley and Patterson (1996) conducted surveys during the years 1993-1994 in 288 fields in Oregon and Washington of PNW of USA. Total of 831 *Fusarium* isolates representing 19 species and 487 *Fusarium* isolates representing similar 19 species were obtained from wheat crowns and subcrown internodes during 1993 and 1994,

respectively. *Fusarium pseudograminearum* was the most predominant pathogen in the region followed by *F. culmorum*. There was difference in prevalence among the crown rot pathogens during the two years in which *F. avenaceum* was the third most prevalent pathogen in 1993 (wet year). However, *Michrodochium nivale* was the third most prevalent species in 1994 (dry year). *Bipolaris sorokiniana* and *F. avenaceum* were the least prevalent pathogens during 1993 and 1994, respectively. Other *Fusarium* species isolated included *F. acuminatum, F. equiseti, F. oxysporum, F. proliferatum, F. reticulatum, F. solani* and *F. tricinctum*.

Backhouse *et al.* (2004) studied *Fusarium* species associated with crown rot of wheat and barley crops from the Eastern Australian Grain Belt between 1996 and 1999. *Fusarium pseudograminearum* was the most common species isolated from crops in Queensland and New South Wales, Victoria and South Australia. More than 70% of isolates obtained from the Victorian high-rainfall (>500 mm) region and the South-East region of South Australia were *F. culmorum*. *Fusarium culmorum* accounted for 18% of isolates from the Victorian medium-rainfall (350-500 mm) region, and 7% of isolates from each of the Victorian low-rainfall region and the Mid-North region of South Australia. Other less frequently isolated species were *F. avenaceum*, *F. crookwellense* and *F. graminearum*.

Akinsanmi *et al*. (2004) recovered a total of 415 isolates from wheat heads, crown and other plant parts collected from wheat fields in Queensland and northern New South Wales, Australia. The isolates were identified into 20 *Fusarium* species using morphological and molecular tools. They identified 332 isolates as, *F. pseudograminearum, F. graminearum, F. culmorum, F. avenaceum, F. acuminatum, F. oxysporum* and *F. poae* using molecular tools. Eighty - three isolates were identified as *F. equiseti, F. lateritium, F. nygamai, F. polyphialidicum, F. proliferatum, F. subglutinans, F. torulosum, F. tricinctum* and *F. verticillioides* using morphological and cultural characters. Their findings indicated that different *Fusarium* species dominated different plant parts, where *F. pseudograminearum* was the most dominant species (48%) and was more frequently isolated from crown, whereas *F. graminearum*

constituted 28% of all isolates, and was more frequently isolated from the head. *F. crookwellense* (8%), *F. avenaceum* (4%), and *F. poae* (2%) were among the less frequently isolated species. The remaining 16 species were isolated with frequencies less than 2%.

Strausbaugh *et al*. (2004) did extensive survey in 2001 and 2002 to identify soilborne pathogens from 81 wheat and 52 barley fields in 13 South-eastern Idaho counties. *Bipolaris sorokiniana* and *Fusarium culmorum* were the most frequently isolated and most virulent pathogens. *Fusarium* spp., *Rhizoctonia solani* and *Gaeumannomyces graminis* var. *tritici* were among the other pathogenic fungi obtained from root lesions.

Bentley *et al.* (2006b) carried out a survey to assess the frequency of isolation of *Fusarium* species associated with wheat stems in New Zealand. A total of 11 *Fusarium* species were isolated from wheat stem bases. *Fusarium oxysporum* was the most frequently isolated species followed by *F. culmorum*. Their finding showed the presence of a number of important pathogenic *Fusarium* species occurring on wheat in New Zealand. *F. culmorum* and *F. pseudograminearum* were isolated from 16% and 1.5% of the wheat stems, respectively.

Postic *et al*. (2012) reported the recovery of 300 isolates from grass weeds and plant debris in Croatia. The isolates were identified into 14 *Fusarium* species on the basis of morphological features and molecular tools (sequencing beta-tubulin and TEF1-α genes). *Fusarium graminearum* was the most frequently isolated species (20.3%), followed by *F. verticillioides* (18.4%), *F. oxysporum* (15.7%), *F. subglutinans* (12.7%), *F. proliferatum* (11%) and *F. avenaceum* (7.7%). *Fusarium acuminatum* (4%), *F. solani* (2.6%), *F. semitectum* (2.3%), *F. equiseti* (1.7%) and *F. crookwellense* (0.3%) were among the less commonly isolated *Fusarium* species.

2.3.2. Review of surveys on crown rot of wheat in Turkey

Although most were geographically limited, several surveys were carried out to study crown rot of wheat in Turkey. The surveys conducted so far are summarized as follows.

Muratçavuşoğlu and Hancıoğlu (1995) conducted a survey during May 1994 to determine *Fusarium* species causing root and crown rot of wheat in Ankara province. They collected diseased samples from 70 representative wheat fields. Isolation of fungi was carried out on PDA and 31 isolates of *Fusarium* species were obtained. Pathogenicity of the isolates was tested on wheat cultivar Gerek 79 using soil inoculation method. The findings of their study indicated that two isolates of *Fusarium culmorum,* 8 isolates of *F. acuminatum,* 4 isolates of *F. graminearum* and 1 isolate of *F. heterosporum* were pathogenic.

Aktaş *et al.* (1996) studied root and/or crown rot pathogens of wheat in Sakarya province of Turkey. Samples were collected from a total of 38 fields. Fungal pathogens were identified using morphological characters. *Rhizoctonia cerealis* (24.9%), *Alternaria alternata* (15.57%), *Fusarium graminearum* (10.9 %), *F. moniliforme* (10.9%), *F. equiseti* (9.72%) and *F. culmorum* (8.17%) were among the fungal pathogens obtained from wheat in the study area.

In 1992, 1993 and 1994, Mamluk *et al.* (1997) carried out extensive surveys to study wheat and barley diseases in the Central Anatolian Plateau. Samples were collected from a total of 299 and 79 wheat and barley fields, respectively. The most common disease of wheat in the study area was foot and root rot caused mainly by *Fusarium* species.

Aktaş *et al.* (1999) conducted a survey to study pathogens associated with root and crown rots of cereals in Konya province. Twenty-nine different fungi including *Fusarium culmorum* (23.88%), *Rhizoctonia cerealis* (12.95%), *Alternaria alternata*

(8.81%), *Drechslera sorokiniana* (7.32%), *F. moniliforme* (6.58%), *F. equiseti* (2.44%), *F. solani* (0.74%), *F. oxysporum* (0.74%) and *F. acuminatum* (0.63%) were identified in the study. *Fusarium culmorum* was the most dominant species comprising 23.88% of the isolates.

Aktaş *et al.* (2000) collected samples from 218 barley and wheat fields in Eskişehir province of Turkey to study root and crown rot diseases. Out of the 218 fields studied, 194 had disease incidences. Isolation from diseased samples yielded 24 species belonging to 8 genera including *Fusarium*, *Drechslera, Alternaria, Ophiobolus,* and *Phoma*. Fourteen species identified in their study were members of the genus *Fusarium*.

In a study conducted in the Erzurum province of Turkey by Demirci and Dane (2003), 468 isolates were obtained from crowns and subcrown internodes of winter wheat. *Fusarium acuminatum* (34.8%), *F. equiseti* (32.3%), *F. oxysporum* (16.9%), *Microdochium nivale* (15%), *F. tabacinum* (0.6%) and *F. solani* (0.4%) were fungi associated with foot rot of winter wheat in Erzurum province. In the pathogenicity tests conducted on wheat, the highest disease severity was caused by isolates of *M. nivale* while isolates of *F. acuminatum, F. equiseti, F. oxysporum* and *F. solani* were weakly to moderately pathogenic.

Uçkun *et al*. (2004) conducted a research to study root and crown rot pathogens of wheat in İzmir, Aydın and Denizli provinces of Turkey. *Fusarium* spp. (113 isolates) were the most predominant fungal pathogens identified followed by *Rhizoctonia cerealis* (16.6%) and *Alternaria alternata* (9.4%), respectively. The pathogenicity test showed that *Rhizoctonia cerealis* and *F. culmorum* were the most pathogenic fungi. *Fusarium culmorum* was the most common among the important pathogenic *Fusarium* species and comprised 11.5% of *Fusarium* isolates.

Bentley *et al*. (2006a) studied *Fusarium* species associated with wheat stem bases in Northern Turkey (the West coast of Marmara, the West Black Sea region, East Central

Anatolia regions). Fifteen *Fusarium* species including *F. oxysporum*, *F. equiseti*, *F. acuminatum*, *F. culmorum*, *F. torulosum*, *F. avenaceum*, *F. proliferatum*, *F. reticulatum*, *F. pseudograminearum* and *F. solani* were obtained from wheat stem bases. *Fusarium culmorum* was the most frequently isolated pathogenic species being isolated from 28% of the sites sampled whereas *F. pseudograminearum* was isolated from only 8% of the sites. *Fusarium culmorum* was the most commonly isolated species in the West coast of Marmara region.

In a study carried out in wheat growing areas of Adana, Mersin and Osmaniye provinces of Turkey by Akgül and Erkılıç (2007), crown rot disease was found in all the wheat fields surveyed with disease incidence and severity ranging from 8.0-100% and 2.0-33.4%, respectively. *Fusarium culmorum*, *F. equiseti*, *F. oxysporum*, *F. semitectum* (synonym *F. incarnatum*) and *F. moniliforme* were the *Fusarium* species isolated from diseased wheat plants.

Tunali *et al.* (2008) collected samples from 518 fields in the different cereal producing regions of Turkey during 2000 and 2001 to study the distribution frequency of fungi associated with root and crown rot of wheat. They reported more than 20 *Fusarium* species including *F. culmorum, F. pseudograminearum, F. acuminatum, F. avenaceum, F. equiseti, F. proliferatum, F. equiseti, F. semitectum*, *F. solani* and *F. tricinctum*. Among the commonly reported dryland root rot pathogens, *F. culmorum* was the most predominant species being isolated from 14% of the fields surveyed followed by *Bipolaris sorokiniana* (10%) and *F. pseudograminearum* (2%). *Fusarium culmorum* was the dominant pathogen in Mediterranean region while *F. pseudograminearum* was predominant in Marmara and Southeast Anatolia regions. Other less or non-pathogenic *Fusarium* species were also found in high frequencies, (*F. oxysporum*, *F. chlamydosporum,* 11%), (*F. sporotrichioides,* 10%) and (*F. avenaceum* and *F. solani,* 8%).

Araz *et al*. (2009) studied root and foot rot diseases of wheat in 4 districts of Sakarya province during 2007-2008 growing seasons. Forty-four diseased wheat root samples

were collected from research sites of Sakarya Agricultural Research Institute and Hanlı, Esence, Esenler and Kirazca villages of Sakarya province. *Fusarium graminearum*, *F. culmorum*, *F. subglutinans, F. crookwellense, F. oxysporum, F. moniliforme, F. solani, F. equiseti, F. acuminatum, F. sporotrichoides, Rhizoctonia* spp. and *Alternaria* spp. were identified using morphological characters. *Fusarium graminearum* and *F. culmorum* were obtained from 10 and 5 wheat cultivars, respectively. Both *F. graminearum* and *F. culmorum* were pathogenic on the cultivars they were isolated from.

Arıcı and Koç (2010) conducted a 2-year survey to study genetic diversity of *Fusarium graminearum* and *F. culmorum* isolated from wheat in Adana province of Turkey. A total of 32 *Fusarium* isolates were obtained from seeds and basal stem nodes of wheat showing disease symptoms. The isolates were identified as *F. culmorum*, *F. graminearum*, *F. avenecaum* and *F. croockwellense*. *Fusarium graminearum* was the predominant pathogen isolated, followed by *F. culmorum*, *F. avenecaum* and *F. croockwellense*, respectively. The result of RAPD-PCR analysis indicated *F. graminearum* and *F. culmorum* isolates obtained from Adana province of Turkey were genetically different.

3. MATERIALS and METHODS

3.1. Survey and Isolation

3.1.1. Survey

An extensive survey was conducted to collect samples from the main wheat growing regions in Turkey. Physiologically mature wheat samples were collected from Aegean, Black Sea, Central Anatolia, Southeast Anatolia, Eastern Anatolia and Mediterranean regions (Figure 3.1) during May, June and July 2013. Wheat samples were collected near plant maturity (growth stage 92 of the Zadoks scale) (Zadoks *et al.,* 1974). Sites were selected arbitrarily with a separation distance of 10-40 km. Samples were taken by pulling up about 100 tillers of wheat from 15-20 representative sites in the same field. Sampling was done in a zigzag pattern starting from some distance away from the edge of road. Plant samples were kept in paper bags labelled with relevant information and transported to the laboratory in Eskişehir (Transitional Zone Agricultural Research Institute). GPS was used to provide sites with coordinates of the location and elevation.

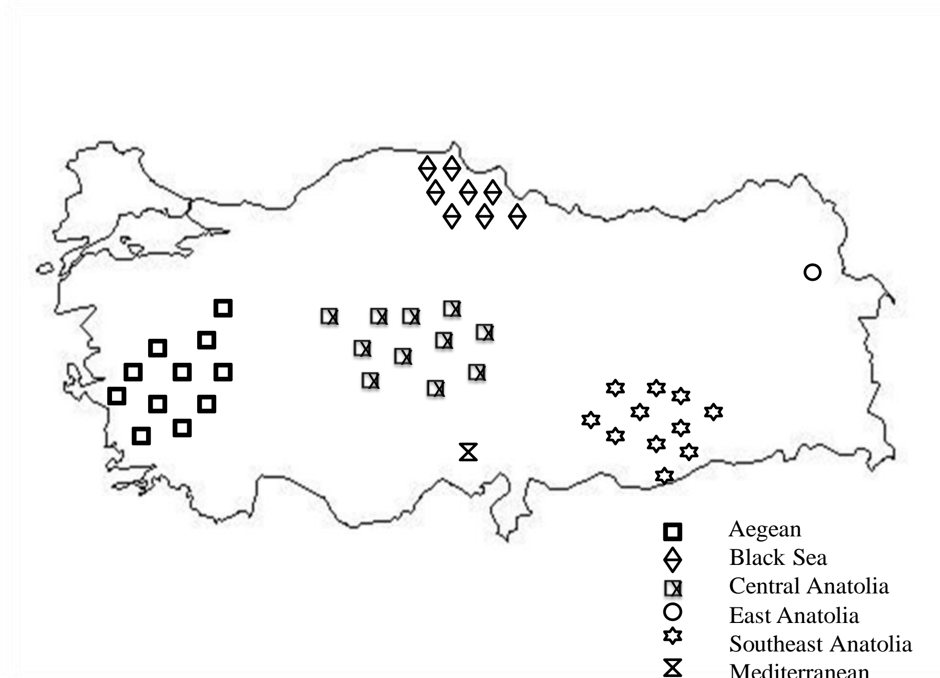

Figure 3.1. Map of Turkey showing agro-ecological regions where samples were collected

3.1.2. Fungal isolation

Soils and outer leaf sheaths were removed from stem bases and crowns. Stems and crowns were then washed thoroughly in running tap water and left on tissue paper for drying. Representative wheat plants showing crown rot symptoms were selected. Crown and stem (5-10 cm away from roots) tissues were sectioned into pieces approximately 1-2 cm in length. The diseased sections were surface sterilized using 1% NaOCl solution (v/v) for 3 min, rinsed three times in sterile distilled water and dried on sterile filter paper (Figure 3.2A).

Peptone PCNB agar (15 g peptone, 1 g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$, 0.75 g PCNB, and 20 g agar per 1 l distilled H_2O) (Nash and Synder, 1962; Booth, 1971; Burgess *et al.*, 1994; Leslie and Summerell, 2006) amended with antibiotics (100 mg/l each of streptomycin sulphate and ampicillin) was used for initial culturing of diseased samples. Isolation was carried out from 10 representative plants from each site. Sterilized individual stem or crown sections were transferred to sterile Petri dishes containing approximately 15-20 ml of Peptone PCNB agar amended with antibiotics (Figure 3.2B) and cultures were incubated for 5-7 days at 25°C day/20°C night temperatures under a 12 h photoperiod under cool white and black fluorescent light. Cultures obtained from peptone PCNB agar (Figure 3.2C) were transferred to SNA (1 g KH_2PO_4, 1 g KNO_3, 0.5 g $MgSO_4.7H_2O$, 0.5 g KCL, 0.2 g glucose, 0.2 g sucrose, 20 g agar per 1 l distilled H_2O) (Burgess *et al.*, 1994; Leslie and Summerell, 2006) (Figure 3.2D) to initiate spore formation and incubated for 7-10 days at the same incubation conditions mentioned above. Conidial suspension was made by putting a small scrap of macroconidia obtained from SNA in sterile distilled water. The spore suspension was poured onto 2% water agar (WA) (Burgess *et al.*, 1994; Leslie and Summerell, 2006) and the excess poured off immediately. The WA plates were incubated in an inclined position (30-40°) in the dark at 25°C for about 18 to 20 h (Burgess *et al.*, 1994). Single germinated conidium from the WA was carefully removed on a small square of agar using sterile flattened tip needle and transferred onto fresh SNA (Figure 3.3), kept for 7-10 days at light and temperature conditions mentioned above. Monosporic *Fusarium* cultures obtained were stored in glycerol (15%) in deep freezer at a temperature of (-80 ^{o}C) until

needed for further studies (morphologic and molecular identification, pathogenicity/aggressiveness and screening studies).

Figure 3.2. Isolation of fungal pathogens from crown/stem base sections; drying surface sterilized stem pieces in Petri dish containing sterile filter paper **(A)**, stem pieces plated on peptone PCNB agar **(B)**, cultures grown on peptone PCNB agar **(C)**, sub culturing cultures to SNA **(D)**

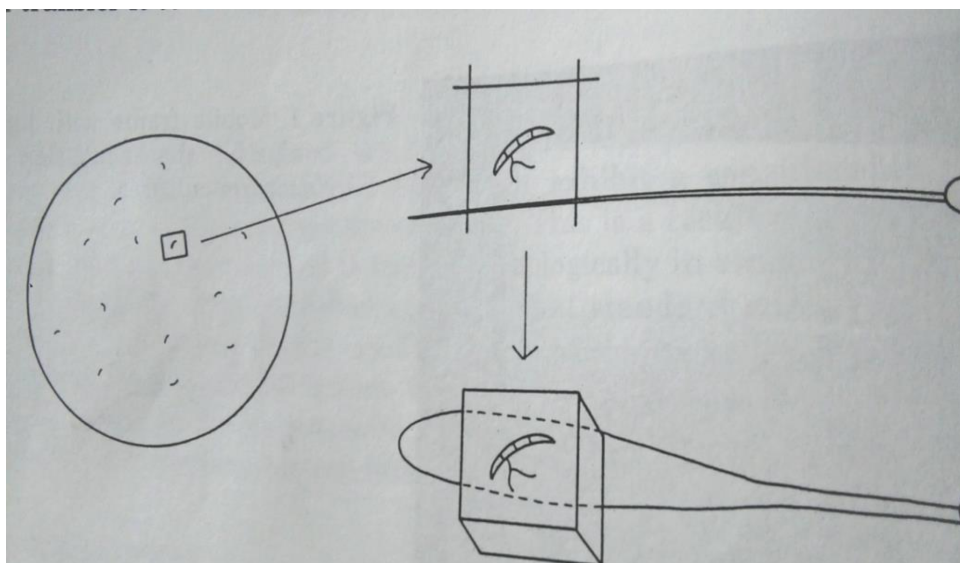

Figure 3.3. A diagram illustrating the procedure followed in single spore isolation
Source: Burgess *et al*. (1994)

3.2. *Fusarium* species identification

3.2.1. Morphological identification

Morphological identification of *Fusarium* species was based on macro- and microconidial morphology, phialide structure, mycelial characteristics, pigmentation on agar and growth rate using keys in Fusarium identification manuals (Booth, 1971, 1977; Burgess *et al*., 1994; Summerell *et al*., 2003; Leslie and Summerell, 2006). Color and abundance of aerial mycelium and pigmentation on agar were studied after incubation of monosporic *Fusarium* cultures on PDA (Merck) for 7 days at a temperature of 25°C day/20°C night, with 12 h photoperiod under cool white and black fluorescent light. Measurements for growth rate were taken after growing cultures on PDA for 72 h at a temperature of 25°C in complete darkness. Two measurements were taken at 90° angles (perpendicular) for each plate (Figure 3.4). Three plates per isolate were used to measure growth rate. To study macro- and microconidial morphology, phialide structure and presence or absence of chlamydospore, monosporic *Fusarium* isolates were plated on SNA and incubated for 7-10 days at a temperature of 25°C day/20°C night with 12 h photoperiod under cool white and black fluorescent light.

Figure 3.4. Measuring growth rate of *Fusarium* culture on PDA after 72 h in the dark at 25°C

3.2.2. Molecular identification

3.2.2.1. Mycelium collection

The method followed in mycelium collection is illustrated in Figure 3.5. Mycelia were harvested from 7-10 day-old *Fusarium* cultures in full strength Potato Dextrose Broth (PDB) medium (Difco). The mycelial mat was spooled out with sterile 1 ml pipette tips, and pressed against the tube to squeeze out excess medium. Remaining media was poured off and about 10 ml of sterile distilled water was added to wash the mycelial mat. Mycelial mat was pressed repeatedly against the plate to remove excess water and transferred to sterile filter paper to remove remaining water. After water was removed, the mycelial mat was transferred to a sterile 1.5 ml tube and stored at -20°C until DNA extraction.

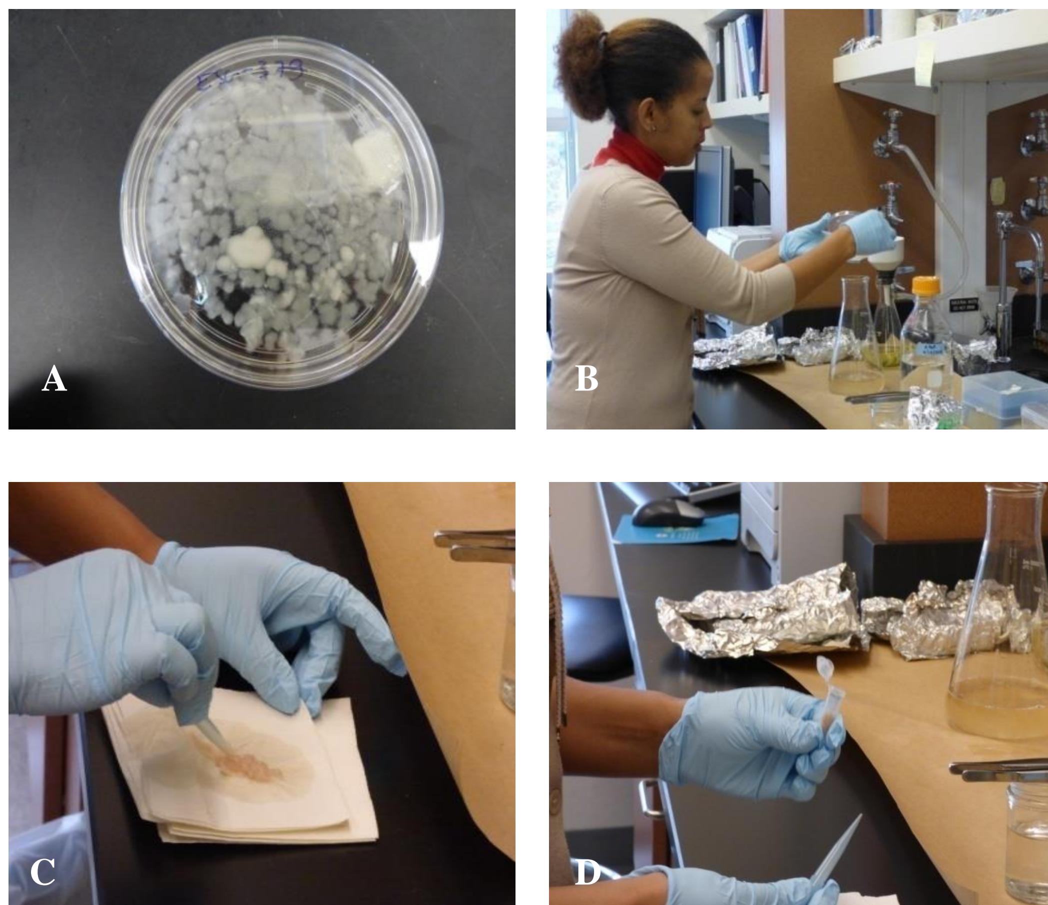

Figure 3.5. Methods followed for mycelium collection; *Fusarium* sp. culture on Potato Dextrose Broth **(A)**, pouring excess media and collecting mycelium **(B)**, removing excess water on sterile filter paper **(C)**, and putting mycelia in 1.5 ml tube for short term storage **(D)**

3.2.2.2. DNA extraction

Genomic DNA was extracted from each of *Fusarium* isolates using a FastDNA kit (MP Biomedicals, Santa Ana, CA, USA) following the manufacturer's instructions (Figure 3.6). Cell lysis solution (CLS-Y) from the kit was used as an extraction buffer. Mycelial tissue (~ 100 mg wet weight) was transferred to FastDNA tubes containing a ceramic bead (1.4 mm). One ml of CLS-Y was added to the tubes and tissue maceration was carried out using FastPrep-24 instrument (MP Biomedicals) (Figure 3.6C) at a speed of 4 m/s for 45 s. Samples were put on ice for 5 min and centrifuged at 14,000 g (12,300 rpm on Eppendorf 5415D centrifuge) (Figure 3.6D) for 5 minute. A volume of 600 µl of each of the supernatant was mixed with 600 µl binding matrix in a 1.5 ml centrifuge

tube. The content was mixed by inverting tubes and incubated at room temperature for 5 minute. Samples were centrifuged at 14,000 g (12,300 rpm) for 1 min to pellet the binding matrix. The supernatant was discarded. A volume of 500 µl salt/ethanol wash solution (SEWS-M) was added to the centrifuge tubes, to purify and release DNA bound in the silica membrane of the filter. Samples were centrifuged at 14,000 g for few seconds (9 to 10 s) and the supernatant was discarded. Samples were rinsed again with SEWS-M. After rinsing samples were centrifuged again for 1 min and the supernatant was discarded. DNA was eluted by gently re-suspending the binding matrix in 100 µl of DNA elution solution (DES) and incubated for 2 to 3 min at room temperature. Samples were centrifuged at 14,000 g for 1 minute. The supernatant containing eluted DNA was transferred to a clean 0.6 ml microcentrifuge tube (Axygen) (Genesee Scientific, San Diego, CA, USA) and stored at 4°C for further use.

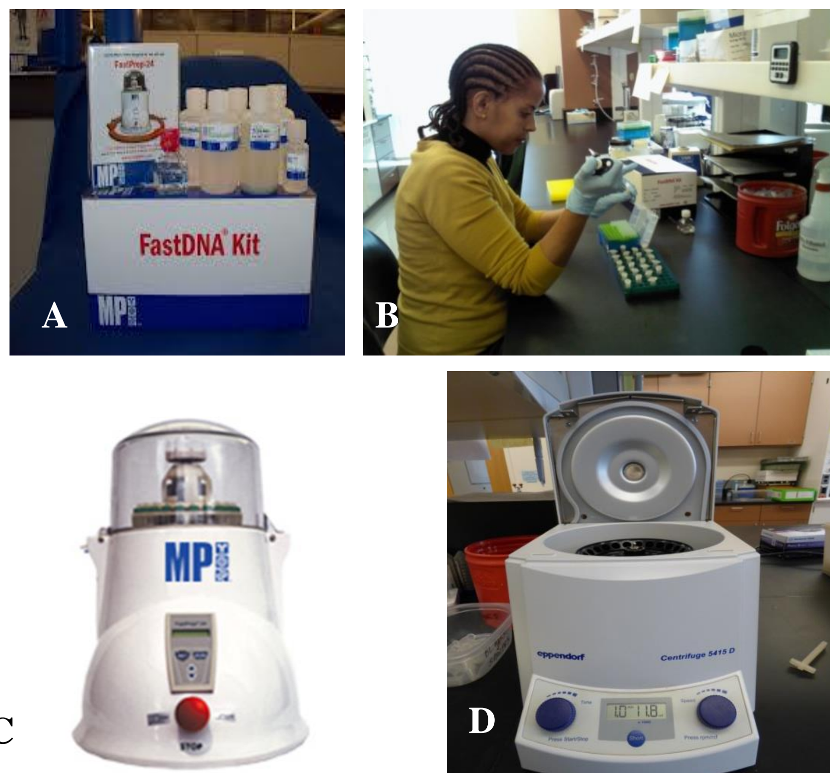

Figure 3.6. DNA isolation using FastDNA Kit (**A** and **B**), FastPrep-24 used for tissue maceration **(C),** Eppendorf 5415D centrifuge used for pelleting samples **(D)**

3.2.2.3. PCR amplification

The translation elongation factor-1 alpha (TEF-1α) gene region of *Fusarium* isolates was amplified using primers using ef1 (5'-ATGGGTAAGGARGACAAGAC-3') and ef2 (5'-GGARGTACCAGTSATCATG-3') (O'Donnell *et al*., 1998). For each 30 µl PCR reaction, a mixture was made containing 25-50 ng fungal DNA, 5X buffer (Promega), 1.5 mM $MgCl_2$, 0.13 mM dNTPs, 0.4 µM ef1 (forward primer), 0.4 µM ef2 (reverse primer), 1.5 unit Taq polymerase (Go TaqFlexi DNA polymerase, Promega) and PCR water. PCR amplification was carried out using thermal cycler (BioRad, T100Thermal Cycler, BIO-RAD, Hercules, CA, USA) with an initial denaturing temperature of 94°C for 3 min. A total of 35 cycles were performed with temperature profile in each cycle consist of 92°C for 45 s, an annealing temperature of 53°C for 45 s (Geiser *et al.,* 2004), an extension temperature of 72°C for 1 min and one final extension temperature of 72 °C for 5 min (Figure 3.7).

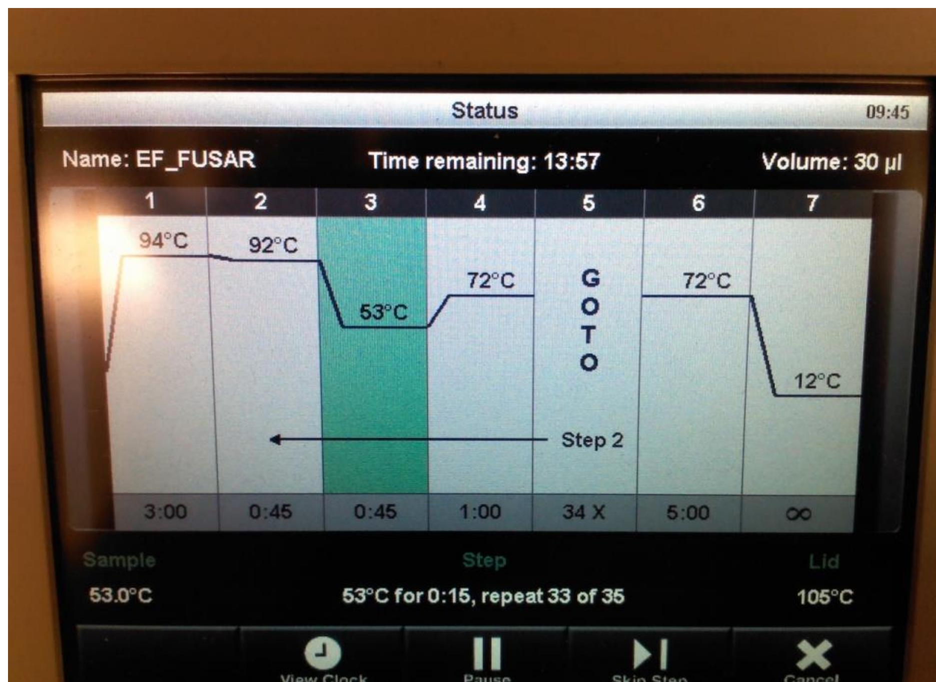

Figure 3.7. Snapshot of BioRad, T100Thermal Cycler for PCR amplification of TEF region of *Fusarium* species

3.2.2.4. Gel electrophoresis

The PCR products were separated by electrophoresis on 1% agarose gels in 0.5X TBE buffer. To prepare 1% agarose gel, 1.6 g of agarose was added into 160 ml of 0.5X TBE buffer. The mixture was melted in an oven for about 2 min until it formed a clear solution and was cooled for few minutes. Once it was cooled, 1 drop of ethidium bromide (10 mg/µl) was added into the solution. The solution was then well mixed and poured into gel tray (24.5 cm wide by 10 cm long). The gel was kept in the gel box, and floated in 0.5X TBE buffer solution connected with electric source (Figure 3.8). Ten µl of 1 kb DNA ladder (0.1µg/µl) (Invitrogen 1kb Plus DNA Ladder) was added into the first and last wells in the gel. 1.5 µl of 10X loading buffer was mixed with 10 µl of PCR product and loaded into wells starting from the second well. The gel box was covered and connected to an electric power (about 100 volts) to provide electric current which allows the negatively charged DNA to move towards the positively charged cathode. After 45 min, the gel was carefully removed and put onto the UV box to take pictures to see the amplified DNA bands in the gel.

Figure 3.8. Running gel to confirm PCR amplification for *Fusarium* isolates

3.2.2.5. Measuring PCR product concentration

Once the amplification was confirmed positive, PCR product concentration was measured in Tecan A 5082 micro plate reader (Tecan Australia Pty Ltd., Melbourne, Australia) using Hoechst 3385 fluorescent DNA quantification kit (BIO-RAD, Hercules, CA, USA) following the manufacturer's procedure (Figure 3.9, 3.10).

Fluorescence cuvette used with Hoechst 3385 fluorometer kit which contains 96 well is shown in Figure 3.10. One mg/ml Hoechst 33258 stock solution was prepared by diluting 1 ml of 10 mg/ml Hoechst 33258 solution with 9 ml of sterile distilled water. A solution containing 22.5 ml sterile distilled water, 2.5 ml of 10X TNE buffer and 50 µl of 1mg/ml Hoechst 33258 dye was prepared in sterile 50 ml tube and 200 µl of the solution was added into each of the 96 well in fluorescence cuvette. 500, 100, 50, 20, 0 ng/ml of calf thymus DNA standard was added into each of the first three wells of A, B, C, D, E, respectively, where as 5 µl of the PCR product was added in to wells A5 to H12. By measuring the fluorescence (absorbance) of each of the standard DNA concentration using fluorometer (TECAN, SAFIRE) (Figure 3.9B), a simple linear equation was developed to predict the PCR concentration. The simple linear equation was used to calculate the concentration of the each of the PCR products from their respective absorbance.

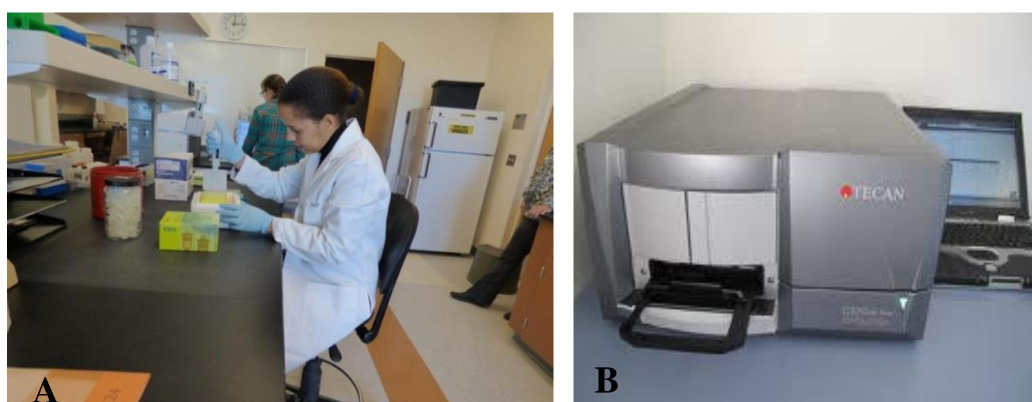

Figure 3.9. Measuring concentration of PCR product using Hoechst 3385 fluorometer kit; preparing PCR products for concentration measurement **(A)**, fluorometer (TECAN) used to measure PCR product concentration **(B)**

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Figure 3.10. The 96 well fluorescence cuvette of Hoechst 3385 fluorometer kit

3.2.2.6. Sequencing and sequence analysis

After calculating the concentration of PCR product for each sample (ng/µl), PCR product (µl), PCR water and forward primer (ef1) volume (µl) were calculated to make a final volume of 15 µl for sequencing following protocol of Elim Biopharmaceuticals, Inc. (Hayward, CA, USA). Thus prepared PCR premix was sent to Elim Biopharmaceuticals, Inc. for forward sequencing. The TEF1–α gene sequences were manually edited with ChromasLite software V.2.1 (Technelysium Pty Ltd, South Brisbane, Australia). The edited sequences (~ 650 bp) were then blasted in NCBI BLAST (http://blast.ncbi.nlm.nih.gov) for similar reference sequences to identify the isolates to corresponding *Fusarium* species.

3.3. Pathogenicity and aggressiveness tests

3.3.1. Pathogenicity test

Monosporic *Fusarium* isolates were grown on half strength PDA to prepare PDA plugs for inoculum in pathogenicity test. Pathogenicity experiments were conducted on a susceptible durum wheat cultivar Kızıltan-91.

A pre trial was carried out to check the time (1, 2, and 3 min) for surface disinfestation of wheat seeds with 1 % NaOCl solution (v/v). In all the three treatments, wheat seeds were able to germinate. Thus 1 % NaOCl solution for 3 min was used for surface disinfestation of wheat seeds. Surface disinfested seeds were rinsed twice in sterile distilled water and dried on sterile filter paper. Seeds were then placed in Petri dishes with a stack of filter paper saturated with sterile distilled water and kept in an incubator at a temperature of 25°C for 3-4 days for germination.

Sterile potting mixture of 50:40:10 sand, soil and organic matter (v/v/v) were used for growing wheat seedlings for pathogenicity test. Plastic tubes (2.5 cm x 16 cm) were filled with soil mixture up to 5 cm bellow the top of the tube. A one centimeter diameter half strength PDA agar disc prepared from the periphery of about 7 days old cultures were placed into the tubes containing potting mixture. A single pregerminated seed was placed on the agar plug and covered with a thin layer of steril potting mixture (Figure 3.11). Agar plug with no fungus was used for control treatments. Each treatment (each fungal isolate) was replicated 3 times (each pot represents one replicate) and treatments were arranged in Randomized Complete Block Design (RCBD). Plants were kept in a growth chamber at a condition of 16 h of artificial light and temperatures of 25/15 ($\pm$5) ^{o}C day/night and relative humidity of 60/80 ($\pm$10) % (Mitter *et al*., 2006). The plants were watered whenever necessary. The experiment was repeated to confirm the results.

Figure 3.11. Agar disc inoculation method used for pathogenicity test; planting pre-germinated wheat seeds on agar disc (plug) with fungal mycelium (left) and covering them with a light layer of soil mixture (right)

Nine weeks after inoculation plants were washed off soils and leaf sheaths were removed. Scoring for the typical symptoms of browning on the crown and the main stem base was carried out using a 1-5 scale (Figure 3.12) (1: 1-9 %, 2: 10-29 %, 3: 30-69 %,4:70-89 %,5: 90-99 %) modified from Wildermuth and McNamara (1994) according to Nicol *et al*. (2001).

Figure 3.12. 1 to 5 scale used for scoring crown rot disease severity caused by *Fusarium* species

The pathogens were re-isolated from crowns of inoculated plants and control plants to fulfill the requirement for Koch's postulates, for representative isolates of each pathogenic species. The re-isolated cultures were confirmed as the corresponding *Fusarium* species by comparing their morphology with known cultures of the species and no culture growth was observed from control plants.

3.3.2. Aggressiveness test

Fusarium culmorum isolates were tested for their aggressiveness to choose the most aggressive one for screening test. The procedure followed in this experiment is illustrated in Figure 3.13. Monosporic cultures of *F. culmorum* isolates were grown on SNA for 10-14 days at a temperature of 25ºC day/20ºC night, with 12 h photoperiod under cool white and black fluorescent light to initiate spore formation. Wheat bran was kept in autoclavable plastic bags and moistened with distilled water. It was then sterilized at a temperature of 121ºC for 15 min. The sterilization was repeated two times at an interval of 24 h. Little amount of sterile distilled water was poured into Petri dishes containing *Fusarium culmorum* cultures with spores, obtained from SNA. The cultures were cut into pieces and put into plastic bags containing sterile wheat bran and incubated for 10-14 days under the same incubation conditions mentioned above. The wheat bran colonized by spores of *Fusarium culmorum* isolates was air dried under aseptic conditions before use. Spore suspension was made by putting some amount of wheat bran colonized by spores of the isolates in sterile distilled water, mixed well to let the spores suspense in water, filtered using several layers of cheesecloth and the concentration adjusted to $1x10^6$ spore/ml after counting spore number using a haemocytometer.

Seeds of durum wheat cultivar Kızıltan-91 were surface disinfested and pregerminated following the same procedure mentioned under section 3.1.5.1. Pregerminated seeds were then placed on stacks of plastic sheet and moist filter paper. Spore suspension of 500 µl (with a concentration of $1x10^6$ spore/ml) amended with 0.1% v/v Tween 20 was applied to each seedling using an aseptic pipet, rolled and tied with rubber band and

kept in tubes containing some amount of water to provide the seedlings with moisture. Same amount of sterile distilled water was applied for control treatments. Each treatment (isolate and control) was replicated 9 times. The treatments were arranged in RCBD and kept in a growth chamber to provide them with optimum conditions of humidity, temperature and light for growth. After covering inoculated seedlings with plastic sheet for 24 h to provide humidity and darkness required for fungal incubation, seedlings were kept in growth room at a condition of of 16 h photoperiod under artificial light, a temperature of 25/15 ($\pm$5)^{o}C day and night temperature and relative humidity of 60/80 ($\pm$10) % (Mitter *et al*., 2006). Plants were provided with appropriate amount of water every day for the duration of the experiment. The experiment was repeated to confirm the results.

Scoring for disease severity was carried out 21 days after inoculation using 1-5 scale (Figure 3.14) (1: 1-9%, 2: 10-29%, 3: 30-69%, 4:70-89%, 5: 90-99%) modified from Wildermuth and McNamara (1994) according to Nicol *et al*., (2001).

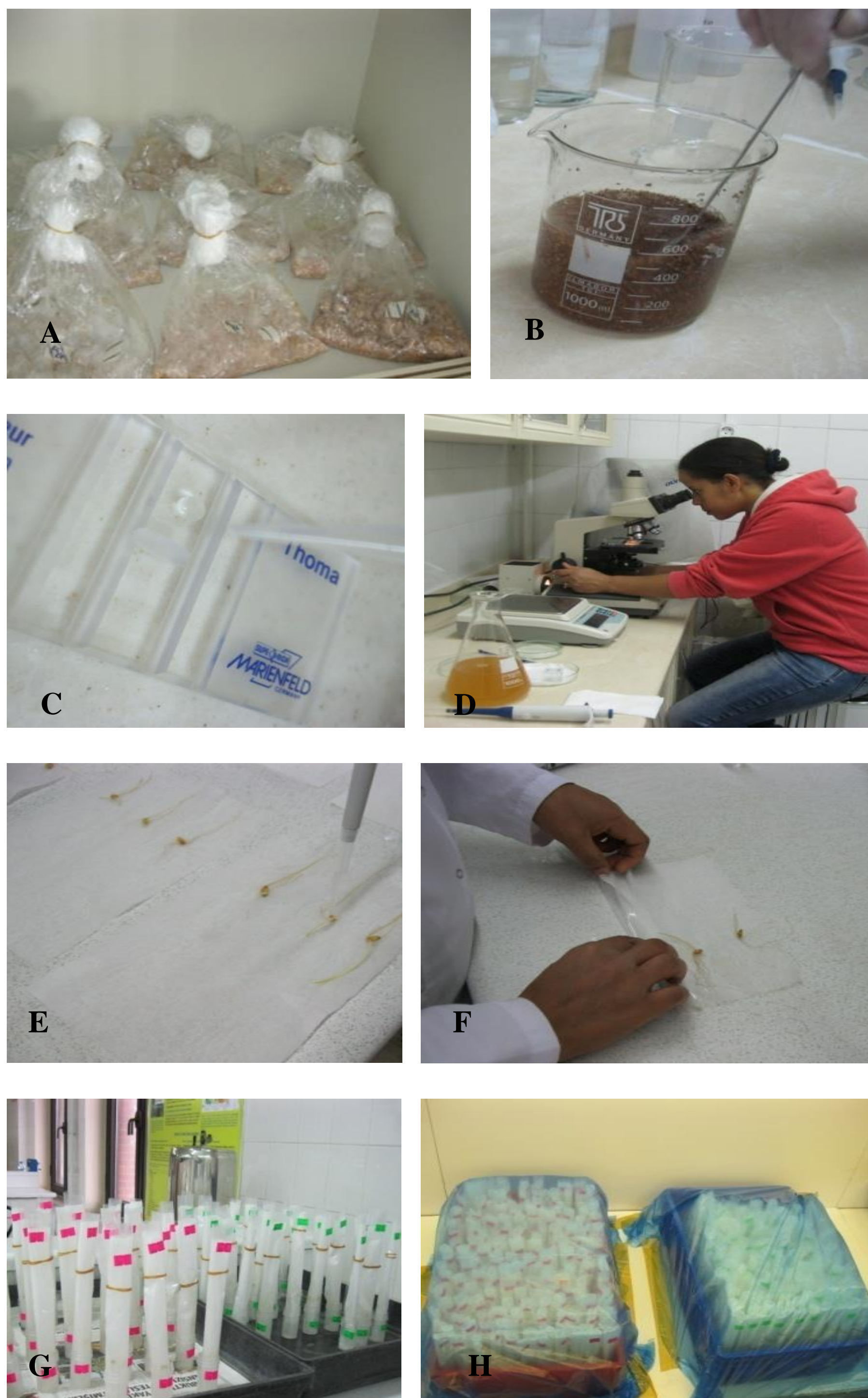

Figure 3.13. The procedure followed in aggressiveness test; growing Fusarium inoculum on wheat bran **(A)**, preparing inoculum from colonized wheat bran **(B)**, preparing fungal suspension for counting using haemocytometer **(C)**, counting spore number under microscope **(D)**, inoculation of pregerminated wheat seeds with fungalsuspension **(E)**, rolling inoculated seedlings (pregerminated seeds) after inoculation **(F)**, inoculated seedlings placed in small tubes containing water **(G)**, inoculated seedlings covered with polyethylene sheet for 24 h to provide moisture **(H)**

Figure 3.14. 1 to 5 scale used for scoring aggressiveness of *Fusarium culmorum* isolates on durum wheat cultivar Kızıltan-91

3.4. Screening wheat germplasms for their reaction to *Fusarium culmorum*

The result of aggressiveness test revealed that *Fusarium culmorum* isolate number two (Fc2) was the most aggressive *Fusarium culmorum* isolate. Therefore, Fc2 was used as inoculum for screening experiment. The screening experiment was conducted to assess 165 lines of spring wheat (Appendix 2) for their reactions to the most aggressive *F. culmorum* isolate, Fc2. The procedure followed in the screening experiment is illustrated in Figure 3.15. Inoculum was prepared following the same procedure mentioned under section 3.1.5.2. Surface sterilization and pre-germination of seeds were carried out following the same procedure mentioned under section 3.1.5.1. Single pre-germinated seed was placed in each tube containing sterile potting mixture of sand: soil: organic matter (50:40:10 v/v/v), covered with thin layers of same soil mixture and moistened. Plants were then kept in a growth chamber at a condition of 16 h photoperiod under artificial light, 25/15($\pm$5) ºC day and night temperatures and relative humidity of 60/80 ($\pm$10) % (Mitter *et al*., 2006). Plants were supplied with water whenever necessary. One week after planting (10 to 11 days after sowing), plants were inoculated with 1 ml of spore suspension (1x10^6 spore/ml) amended with 0.1% v/v Tween 20 on stem bases (~ 0.5 cm above the soil) (Mitter *et al*., 2006) using aseptic

pipette. Nine wheat cultivars (Table 3.1) were used for control. The control cultivars were inoculated with the same amount and concentration of spore suspension. Each treatment (each wheat germplasm) was replicated 5 times. Treatments were arranged in RCBD and plants were covered with a plastic sheet for 48 h to maintain high humidity and darkness required for fungal incubation (Mitter *et al.,* 2006). Plants were then placed at the same light, temperature and humidity conditions mentioned above. Plants were provided with appropriate amount of water every day for the duration of the experiment. The experiment was repeated to confirm the results.

Nine weeks after inoculation plants were washed off soils and leaf sheaths were removed. Scoring for the typical symptoms of browning on the crown and the main stem base was carried out using a 1-5 scale (Figure 3.12) (1: 1-9%, 2: 10-29%, 3: 30-69%, 4:70-89%, 5: 90-99%) modified from Wildermuth and McNamara (1994) according to Nicol *et al*., (2001).

Table 3.1. Wheat genotypes used as control in the screening experiment

Wheat genotype	Accession No.	CID [1]	Reaction [2]	Wheat type [3]	Sources [4]
Adana-99			MS	SW	TK
Altay-2000	010627		MR/MS	WW	TK
Carisma			MR	WW	IT
Suntop		200000963	MR	SW	AUS
Emu Rock		200000805	MS	SW	AUS
Janz	960370	4982215	MS	WW	AUS
Seri-82	951027		S	SW	MX
Kutluk-94	950660		S	WW	TK
Süzen-97	950283		S	WW	TK

[1]CID= Cross Identification
[2]MS=moderately susceptible, MR=moderately resistant, MS=moderately susceptible, S=susceptible
[3]SW=spring wheat, WW=winter wheat
[4] TK=Turkey, IT=Italy, AUS=Australia, MX=Mexico

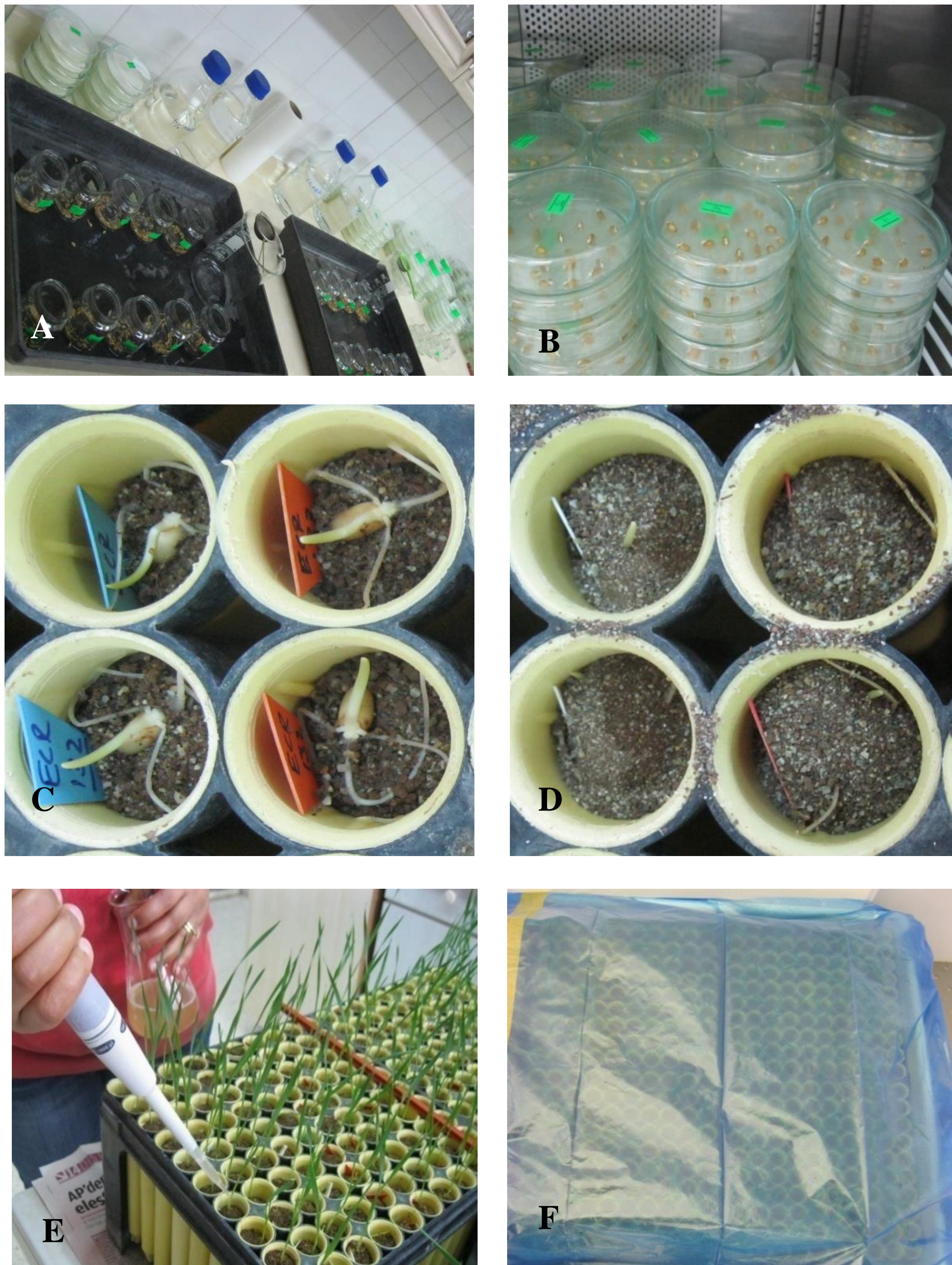

Figure 3.15. Illustration of methods followed in the screening experiment; surface disinfestation of wheat seeds (**A**), pregermination of seeds (**B**), planting pregerminated seeds in tubes containing sterile soil mixture (**C**), covering seeds with thin layer of sterile soil mixture (**D**), inoculation of one week old plants with 1 ml of spore suspension (1x10^6 spore/ml) (**E**), inoculated plants covered with polyethylene sheet for 48 h to provide suitable conditions required for fungus incubation (**F**)

3.5. Data analysis

Data were subjected to analysis of variance (ANOVA) using general linear models (GLM) procedure of SPSS (IBM SPSS Statistics 21) and means were compared using Tukey's HSD test at $P = 0.05$ level of significance.

4. RESULTS

4.1. Survey

In the present study a total of 342 *Fusarium* isolates were obtained from samples collected from 200 wheat fields in the different wheat growing regions of Turkey. The highest number of *Fusarium* isolates (113) was obtained from Southeast Anatolia region from which 54 fields surved followed by Aegean region (95 isolates from 42 fields). Total of 73 isolates were obtained from 41 fields surveyed in the Black Sea region. In the Central Anatolia region, samples were collected from 61 wheat fields which yielded 58 *Fusarium* isolates. Only few isolates were obtained from Mediterranean (2) and Eastern Anatolia (1) regions.

4.2. *Fusarium* species identification

4.2.1. Morphological identification

***Fusarium culmorum* (W.G. Smith) Saccardo**

Almost all *F. culmorum* isolates showed similar colony characteristics on PDA. They were fast growing, with growth rates ranging from 41 to 57 mm (Table 4.1). *Fusarium culmorum* formed abundant white mycelia which completely covered the Petri dish in one week (Figure 4.1A), and produced carmine red pigment on PDA (Figure 4.1B). The species produced macroconidia which were very uniform in shape (Figure 4.1E). The macroconidia were borne on monophialides (Figure 4.1C). The macroconidia produced were wider, with width of 6-8 µm and relatively short, with length of 35-39 µm (Table 4.2). *Fusarium culmorum* produced thick walled macroconidia which were 3 to 4 septate, with most of them being 4 septate (Figure 4.1E). The macroconidia produced lacked a distinctive foot-shaped basal cell, but had notched basal cells and rounded and blunt apical cells. *Fusarium culmorum* formed chained chlamydospores in hyphae (Figure 4.1D) and macroconidia, two weeks after incubation on SNA. It did not form microconidia on SNA.

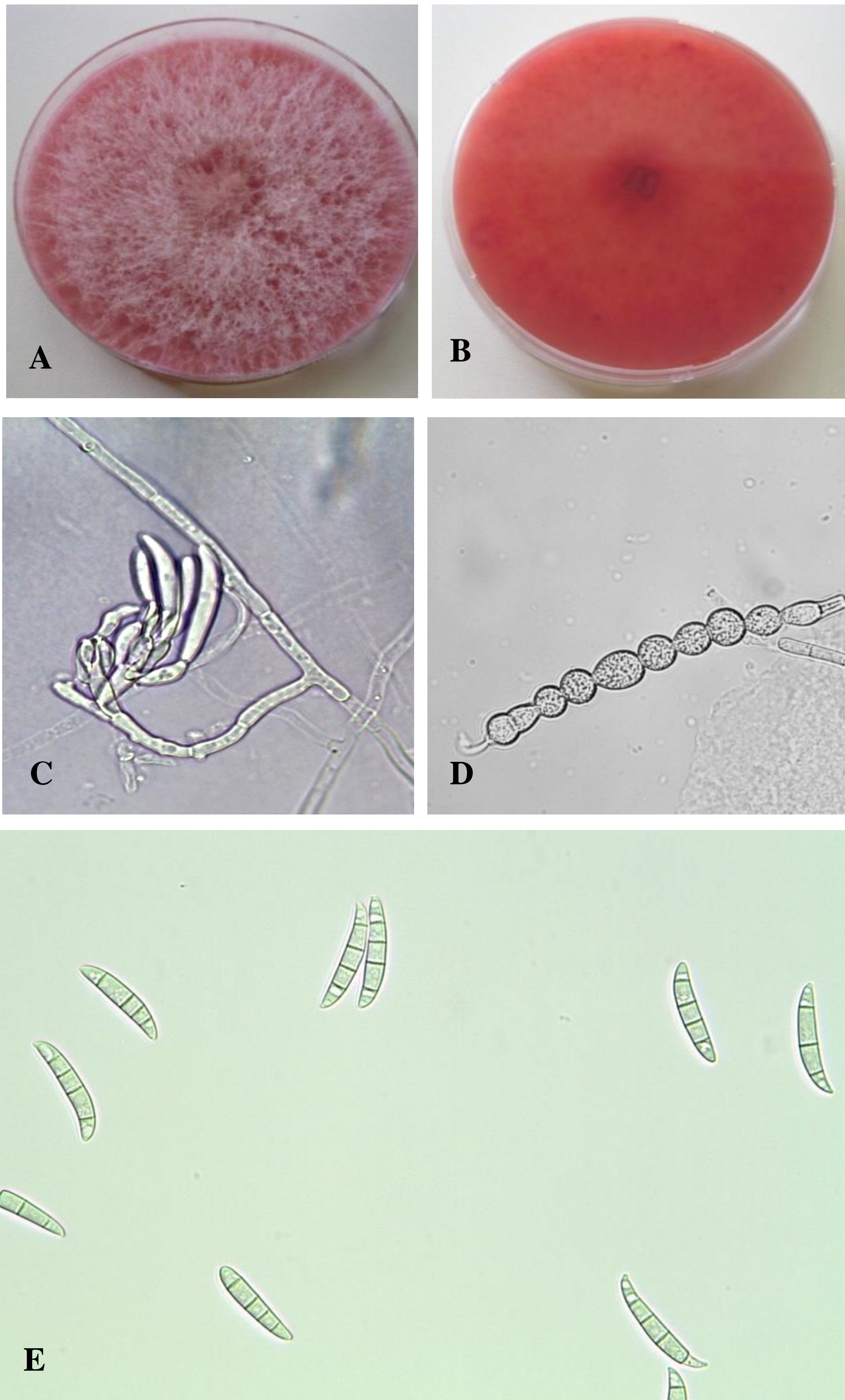

Figure 4.1. *Fusarium culmorum*; surface **(A)** and reverse **(B)** of culture on PDA; phialide **(C)**, chlamydospores **(D)** and macroconidia **(E)**

***Fusarium graminearum* Schwabe**

Fusarium graminearum isolate grew rapidly on PDA and had growth rate of 44-47 mm (Table 4.1). It produced dense mycelia which were pale orange in color (Figure 4.2A) and formed pale orange pigment on PDA (Figure 4.2B). *Fusarium graminearum* formed relatively slender (4-7 µm), thick walled macroconidia which had length ranging from 48to 63 µm (Table 4.2). The macroconidia were moderately curved to straight, with well developed foot shaped basal cell and tapered apical cells (Figure 4.2C). Although the macroconidia produced were 5 to 6 septate, most of them were 5 septate (Figure 4.2C). Macroconidia were borne on monophialides (Figure 4.2D).

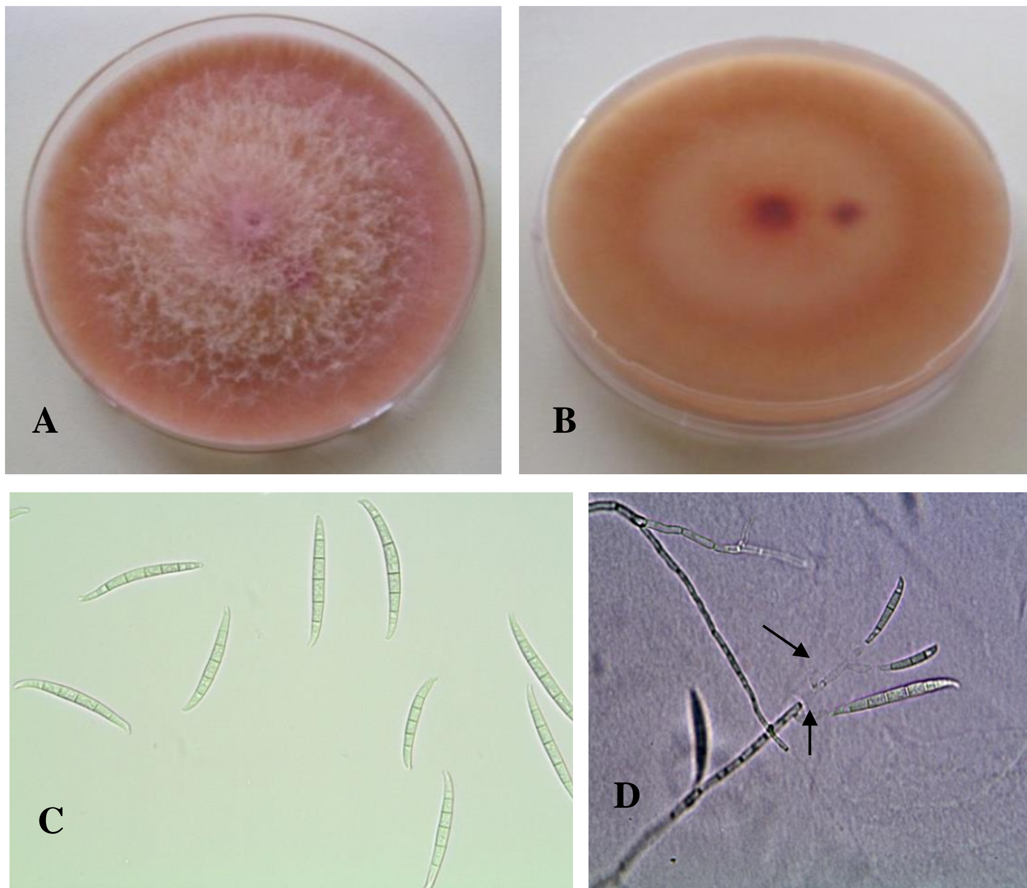

Figure 4.2. *Fusarium graminearum*; surface **(A)** reverse **(B)** of culture on PDA; macroconidia **(C)** and phialide structure **(D)**

***Fusarium pseudograminearum* Aoki & O'Donnell**

The three *F. pseudograminearum* isolates showed similar colony characteristics on PDA. The isolates were fast growing, with growth rates ranging from 35 to 41 mm (Table 4.1). They formed abundant mycelia which were white in the periphery and yellowish at the centre. Isolates of *F. pseudograminearum* produced pink pigment on PDA (Figure 4.3B). *Fusarium pseudograminearum* produced medium to long (50-61 µm), relatively slender (5-7 µm) macroconidia (Table 4.2), which were almost straight to moderately curved, with foot shaped basal cells and curved apical cells (Figure 4.3C). Macroconidia were 5 to 6 septate, with most of them being 5 septate (Figure 4.3C). Macroconidia were borne on phialides (Figure 4.3D).

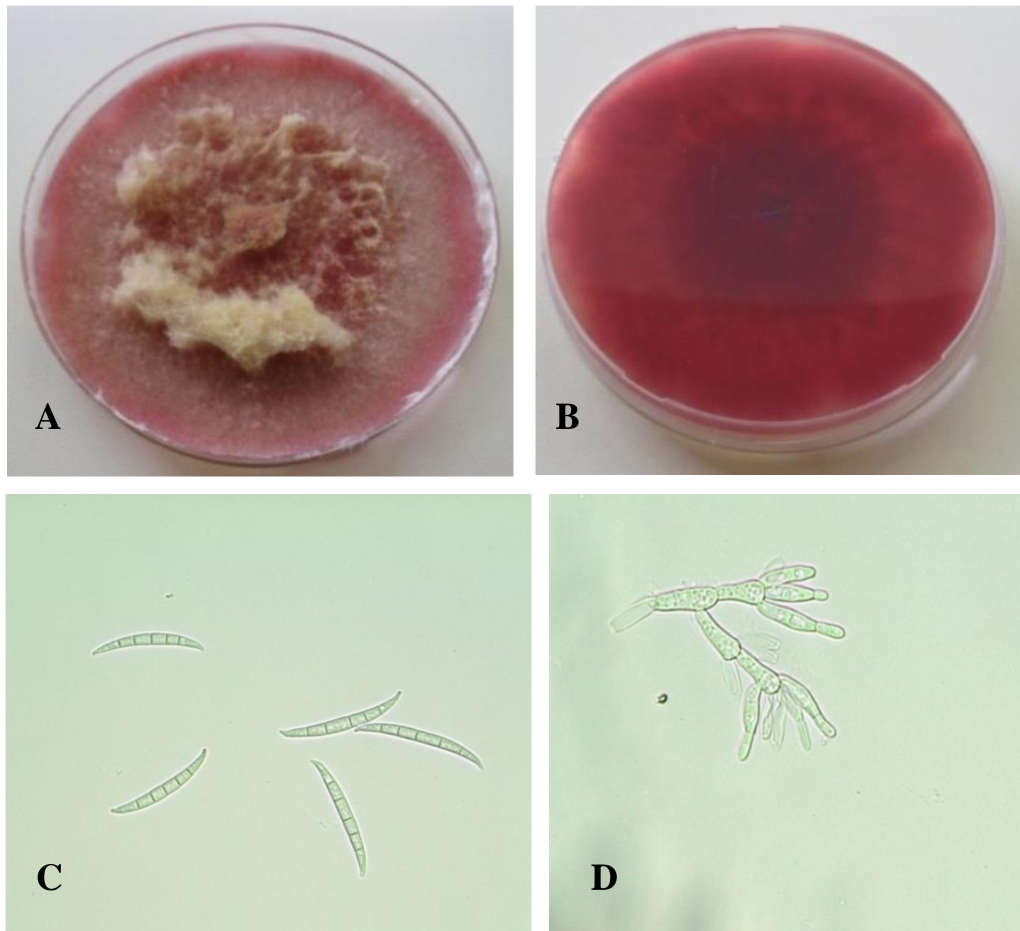

Figure 4.3. *Fusarium pseudograminearum*; surface **(A)** and reverse **(B)** of culture on PDA; macroconidia **(C)** and phialide structure **(D)**

***Fusarium hostae* Geiser & Juba**

Fusarium hostae isolates had intermediate growth rates, ranging from 20 to 36 mm (Table 4.1). The isolates produced limited aerial mycelium with light purple, violet and yellow color. Most of them did not produce pigment on PDA although few formed violet pigment which was similar in color with the one formed on mycelium (Figure 4.4A and 4.4B). *Fusarium hostae* produced abundant microconidia on SNA (Figure 4.4C). Although most of the microconidia were 0 septate, few were 1 septate. Macroconidia with curved or hooked apical cells and foot shaped basal cells were produced (Figure 4.4C). Macroconidia had size of 20-40 µm and 3-4.75 µm length and width, respectively (Table 4.2). Most of the macroconidia were 3 septate although few were 4 septate (Figure 4.4C).

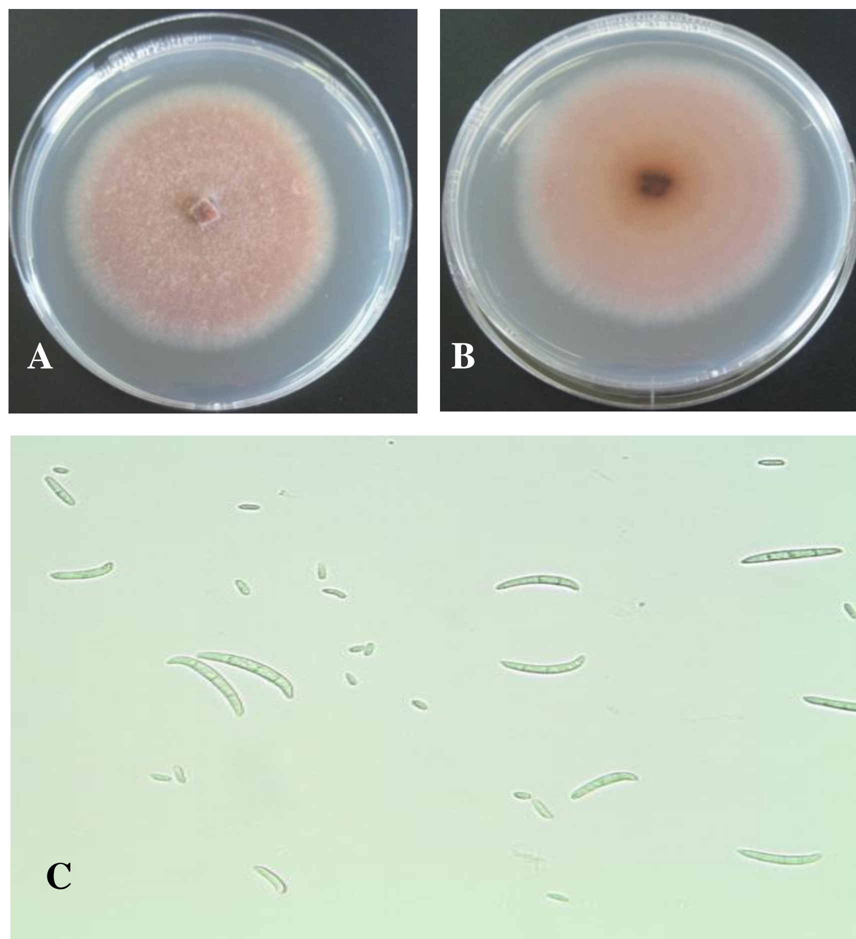

Figure 4.4. *Fusarium hostae*; surface **(A)** and reverse **(B)** of culture on PDA; macro- and microconidia **(C)**

***Fusarium redolens* Wollenweber**

Isolates of *F. redolens* were slow to moderately growing (20-32 mm) (Table 4.1). They produced sparse white mycelium (Figure 4.5A). Although most of them did not form pigment on PDA, few produced very light brown pigment (Figure 4.5B). Macroconidia produced by *F. redolens* are shown in Figure 4.5C. The species produced robust and thick walled macroconidia with the upper third of the conidia being the widest. The macroconidia produced had hooked apical cells and foot shaped basal cells and were 3 to 5 septate, with most of them being 5 sepate. They had size of 30-55 µm and 3-5 µm length and width, respectively (Table 4.2).

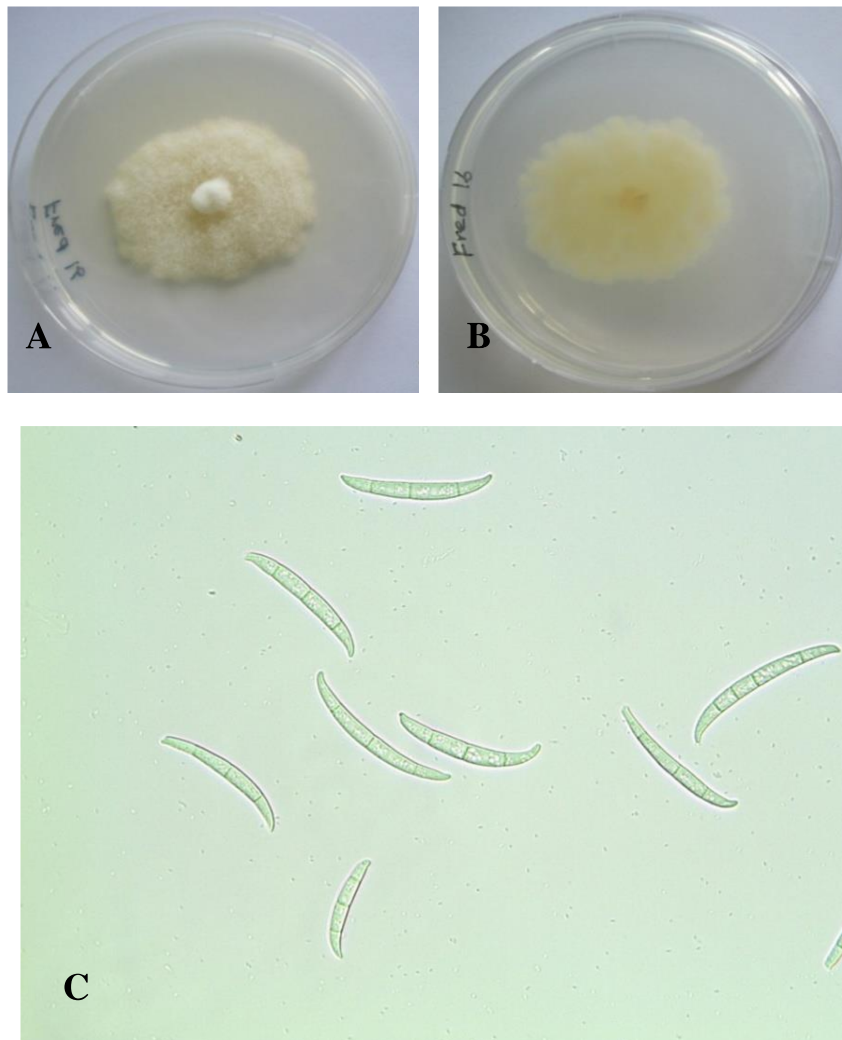

Figure 4.5. *Fusarium redolens*; surface **(A)** and reverse **(B)** of culture on PDA; macroconidia **(C)**

***Fusarium avenaceum* (Fries) Saccardo**

Fusarium avenaceum isolates showed slow to moderate growth (15-30 mm) (Table 4.1). They produced relatively abundant floccose mycelium which ranged from white to light yellow in color (Figure 4.6A) and formed yellowish brown pigment on PDA (Figure 4.6B). *Fusarium avenaceum* produced thin walled, long (with length of 42.5-72.5 µm) and slender (with width of 3-4.5 µm) macroconidia (Table 4.2). Macroconidia were straight to slightly curved with long and tapering apical cells and foot-shaped basal cells and 5 septate (Figure 4.6C).

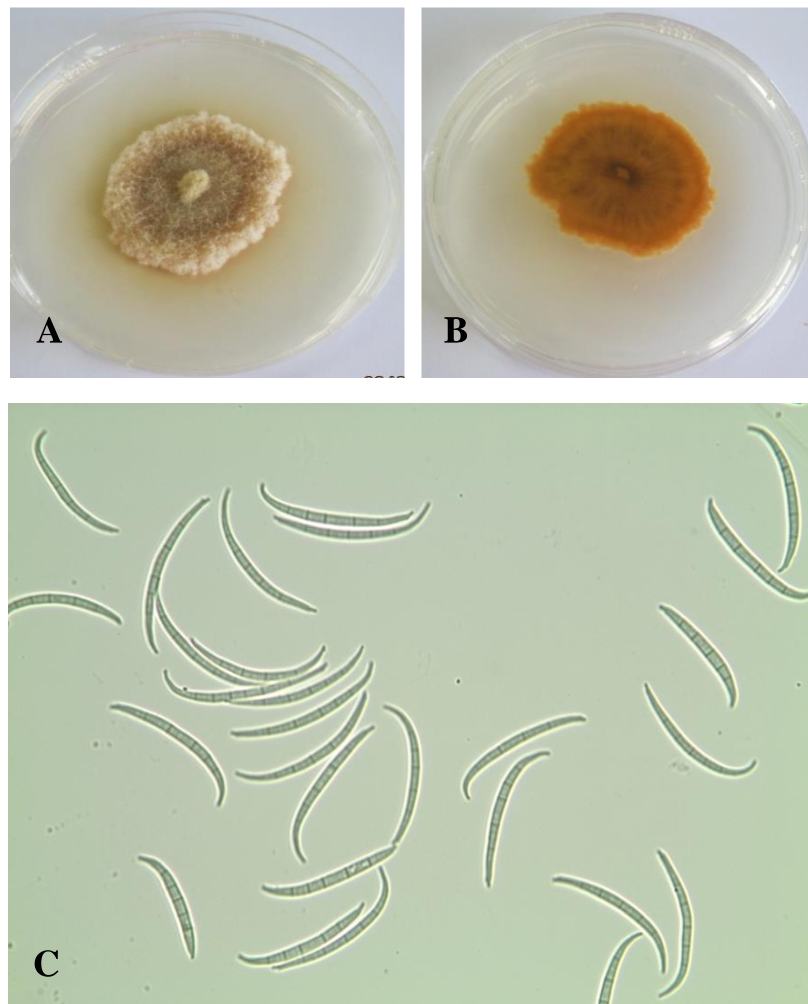

Figure 4.6. *Fusarium avenaceum*; surface **(A)** and reverse **(B)** of culture on PDA;   macroconidia **(C)**

Fusarium acuminatum **Ellis & Everhart**

Fusarium acuminatum isolates were relatively slow to moderately growing with growth rates of 17-36 mm (Table 4.1). Most of the isolates produced rose to burgundy floccose mycelium which was abundant in some isolates, while others produced rose to burgendy floccose mycelium which was grayish at the periphery (Figure 4.7A). They produced honey brown pigment on PDA (Figure 4.7B). *Fusarium acuminatum* produced thick walled macroconidia which had sizes of 24-58 µm and 4-5.5 µm length and width, respectively (Table 4.2). Macroconidia produced were moderately curved, with distinct foot shaped basal cell and long tepering apical cell and were 3 to 5 septate (Figure 4.7C). Microconidia were 0 to 1 sepate (Figure 4.7C).

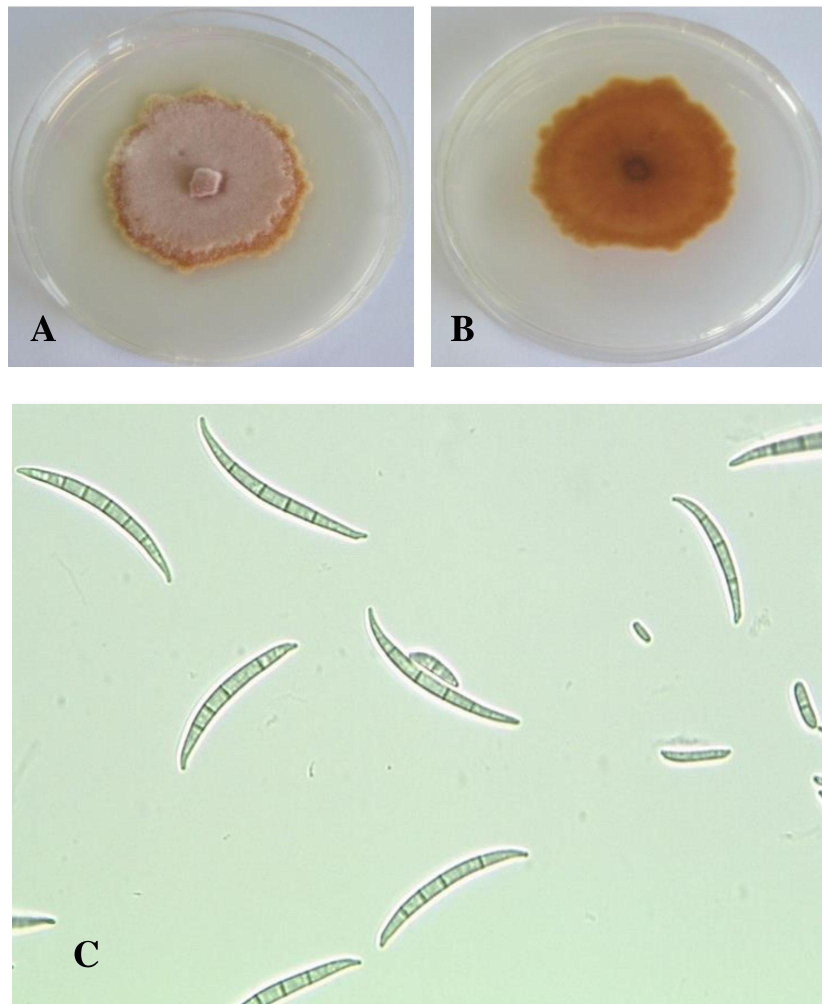

Figure 4.7. *Fusarium acuminatum*; surface **(A)** and reverse **(B)** of culture on PDA; macro- and microconidia of **(C)**

***Fusarium equiseti* (Corda) Saccardo**

Isolates of *Fusarium equiseti* were slow to moderately growing with growth rates ranging from 24 to 47 mm (Table 4.1). They formed uniform floccose mycelia which were initially white and changed to light greyish color with age (Figure 4.8A). Most of the isolates did not form pigment on PDA, although few produced pale brown pigment (Figure 4.8B). *Fusarium equiseti* produced thick walled 5 to 7 septate macroconidia which have strong dorsiventral curvature with a distinctly foot-shaped basal cell and tapering elongated apical cell (Figure 4.8C). The macroconidia produced were slender (with width of 3.5-6 µm), medium to long (with length of 35-60 µm) (Table 4.2).

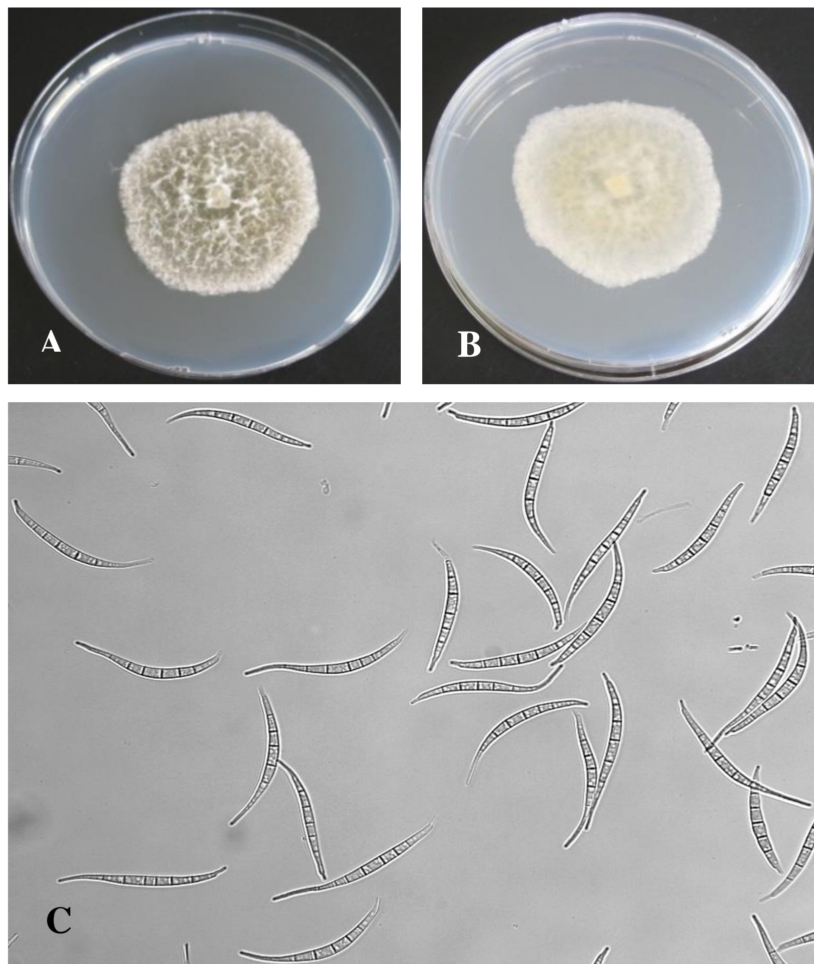

Figure 4.8. *Fusarium equiseti*; surface **(A)** and reverse **(B)** of culture on PDA; macroconidia **(C)**

***Fusarium brachygibbosum* Padwick**

Fusarium brachygibbosum isolates were moderately to fast growing (39-51 mm) (Table 4.1). They produced sparse to abundant aerial mycelium ranging in color from white, pink to light pink (Figure 4.9A). Some isolates produced light pink (Figure 4.9B) and pinkish brown pigment while others did not form pigment on PDA. Macroconidia produced by *F. brachygibbosum* were wider in the middle and had distinct foot shaped basal cells (Figure 4.9C). They were 3 to 5 septate and had sizes of 25-42.5 µm and 3-5 µm length and width, respectively (Table 4.2).

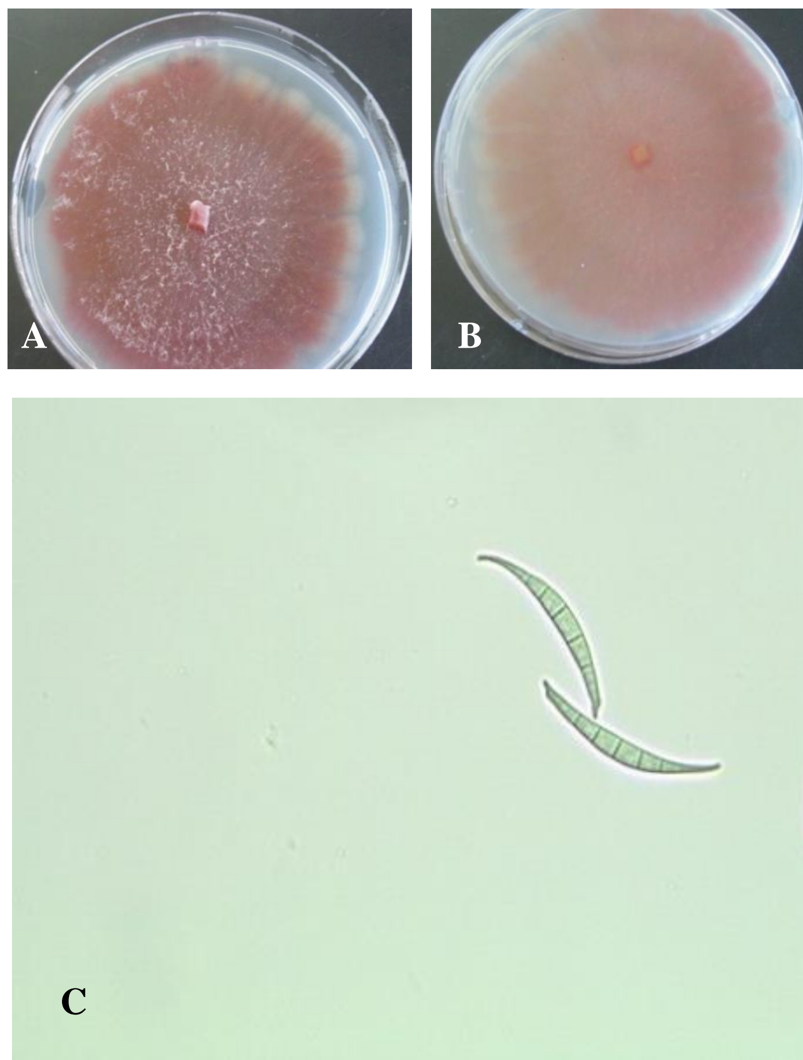

Figure 4.9. *Fusarium brachygibbosum*; surface **(A)** and reverse **(B)** of culture on PDA; macroconidia **(C)**

Fusarium torulosum **(Berkeley & Curtis) Nirenberg**

Isolates of *Fusarium torulosum* were slow growing with growth rate of 14-21 mm (Table 4.1). They produced sparse yellow aerial mycelium (Figure 4.10A), red, yellow and brown pigment on PDA (Figure 4.10B). *Fusarium torulosum* produced thick walled macroconidia with length of 40-48 µm and width of 3.75-4.75 µm (Table 4.2). The macroconidia had foot shaped basal cells and pointed apical cells and were 3 to 5 septate with most of them being 5 septate (Figure 4.10C). Microconidia were 1 septate (Figure 4.10C).

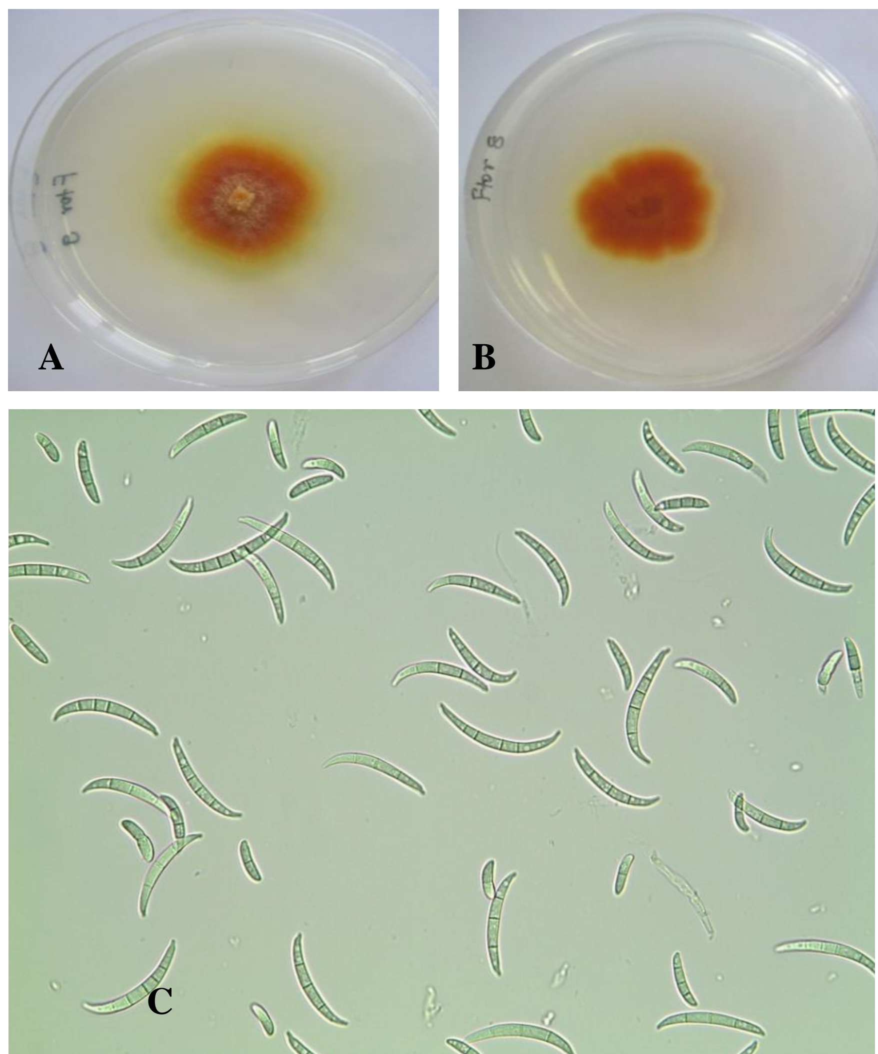

Figure 4.10. *Fusarium torulosum*; surface **(A)**, reverse **(B)** of culture on PDA; macro- and microconidia **(C)**

***Fusarium oxysporum* Schlechtendahl emend. Snyder & Hansen**

Fusarium oxysporum isolates showed widely varying colony characterstics on PDA. They were slow to moderately growing with growth rates ranging from 26 to 33 mm (Table 4.1). The isolates produced floccose, sparse white aerial mycelium (Figure 4.11A), which became purple-violet with age. Some isolates produced dark magenta pigment on PDA while others produced no pigment (Figure 4.11B). *Fusarium oxysporum* produced abundant microconidia which were 0 to 1 septate on SNA (Figure 4.11C, 4.11D). Short to medium sized macroconidia with length and width of 22.5-37.5 µm and 2.5-4.5 µm, respectively were produced (Table 4.2). The macroconidia produced were thin walled and 2 to 3 septate, but most of them were 3 septate (Figure 4.11D). The isolates formed chlamydospores in two weeks on SNA.

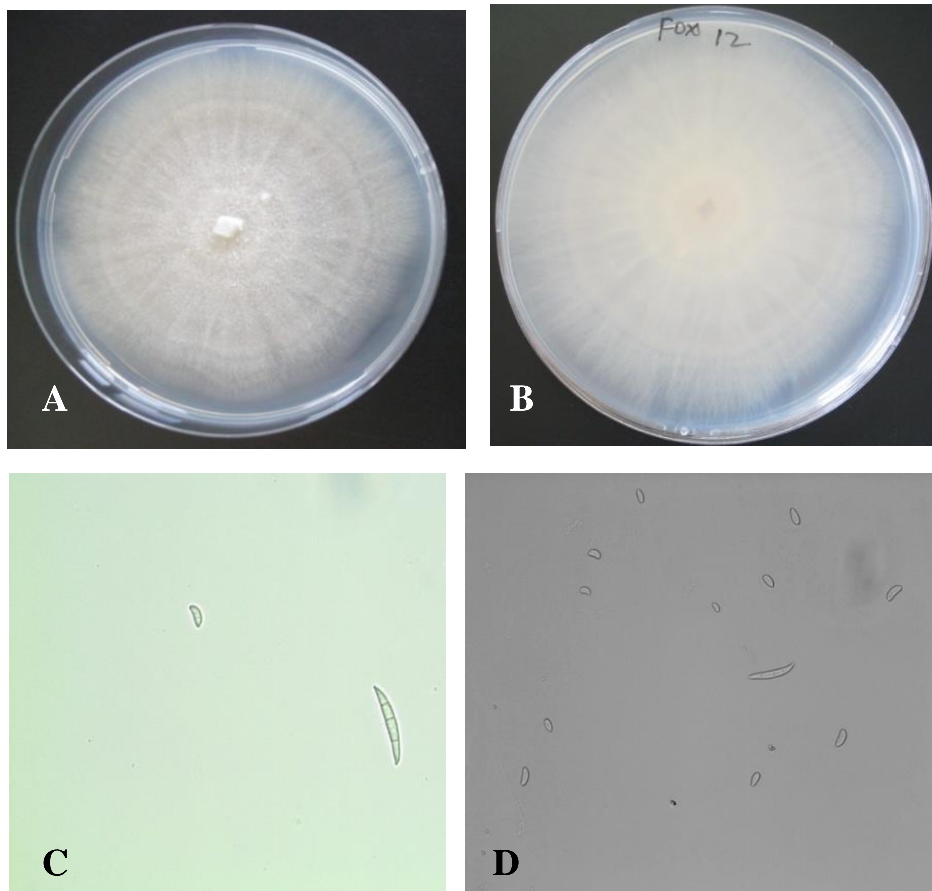

Figure 4.11. *Fusarium oxysporum*; surface **(A)** and reverse **(B)** of culture on PDA*;* micro- and macroconidia (**C** - **D**)

Fusarium solani (Martius) Appel & Wollenweber emend. Snyder & Hansen

Fusarium solani isolates were slow to moderately growing with growth rates ranging from 22 to 26 mm (Table 4.1). They produced sparse white to cream aerial mycelium (Figure 4.12A). The isolates did not form pigment on PDA (Figure 4.12B). *Fusarium solani* produced 3 to 5 septate macroconidia. The macroconidia produced were relatively wider with width and length ranging from 3.75-5 µm and 15-27.5 µm, respectively (Table 4.2). Macroconidia had poorly developed basal cells and blunt apical cell. The species also formed microconidia on SNA (Figure 4.12D).

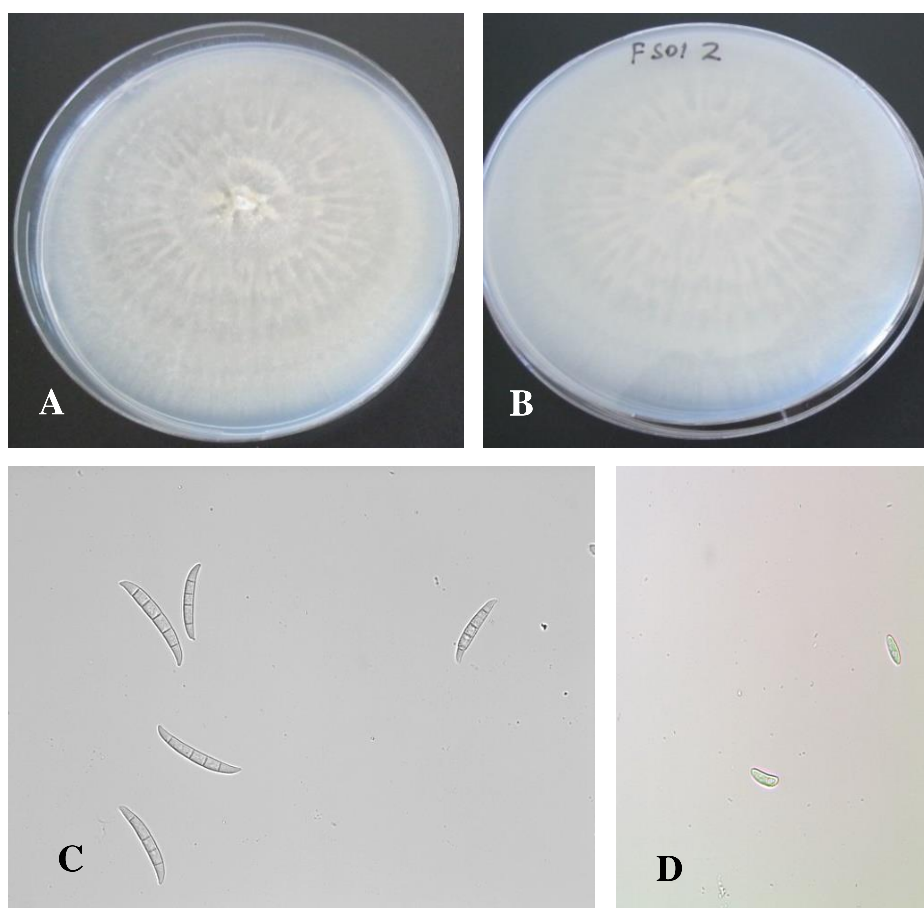

Figure 4.12. *Fusarium solani*; surface **(A)** and reverse **(B)** of culture on PDA; macroconidia **(C)** and microconidia **(D)**

Fusarium proliferatum **(Matsushima) Nirenberg**

Isolates of *Fusarium proliferatum* were slow to moderately growing (22-30 mm) (Table 4.1). They produced abundant floccose mycelium which was initially white and turned purple violet with age (Figure 4.13A). They formed violet and light violet pigment on PDA (Figure 4.13B). Thin walled slender macroconidia with size of 23.75-35 µm and 2.5-3 µm length and width, respectively were produced (Table 4.2). The macroconidia produced were relatively straight with curved apical cell and 3 to 5 septate (Figure 4.13C). *Fusarium proliferatum* produced abundant 0 to 1 septate microconidia on SNA one week after incubation.

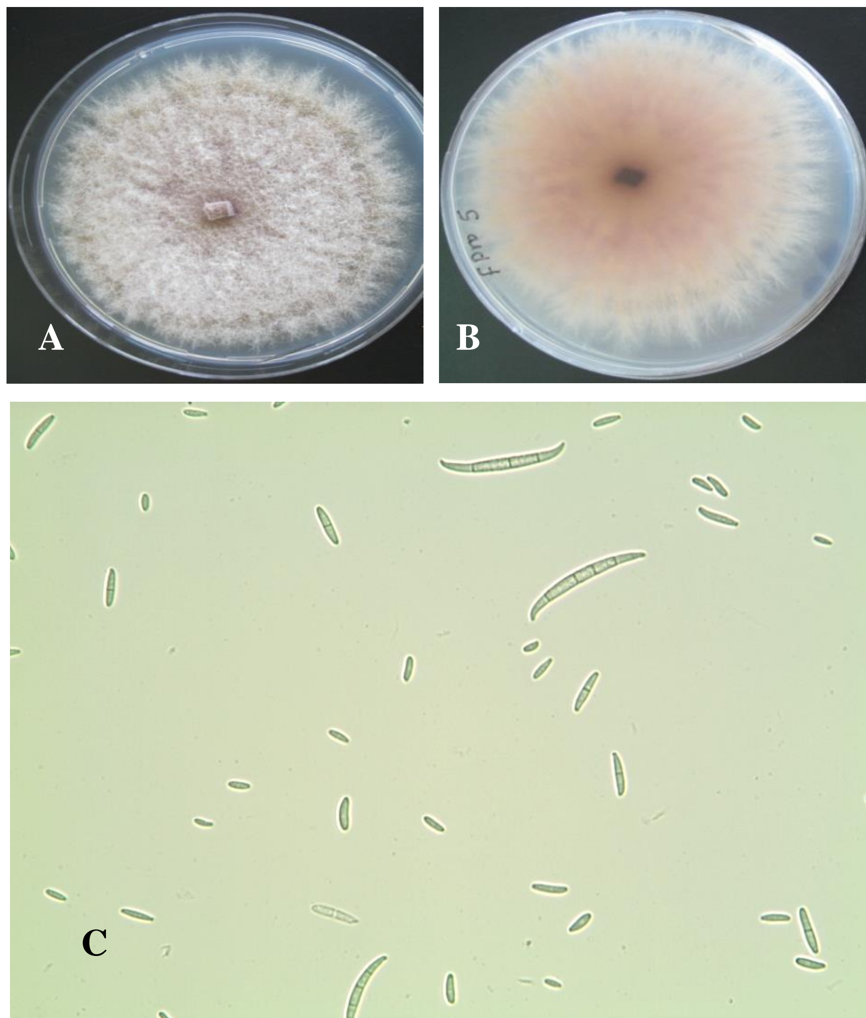

Figure 4.13. *Fusarium proliferatum*; surface **(A)** and reverse **(B)** of culture on PDA; macro- and microconidia **(C)**

Fusarium flocciferum **Corda**

The six *F. flocciferum* isolates showed almost similar colony charateristics on PDA. The isolates were slow growing with growth rate ranges of 14-17 mm (Table 4.1). They formed sparse mycelium which was yellow in the center and white in the periphery (Figure 4.14A) and produced reddish brown pigment on PDA (Figure 4.14B). The isolates also produced yellow flourescent pigment on agar other than where culture growth was observed (Figure 4.14A and 4.14B). *Fusarium flocciferum* produced relatively curved 3 to 5 septate macroconidia with foot shaped basal cell (Figure 4.14C) and with length and width of 22.5-37.7 μm and 3.25-3.8 μm, respectively (Table 4.2).

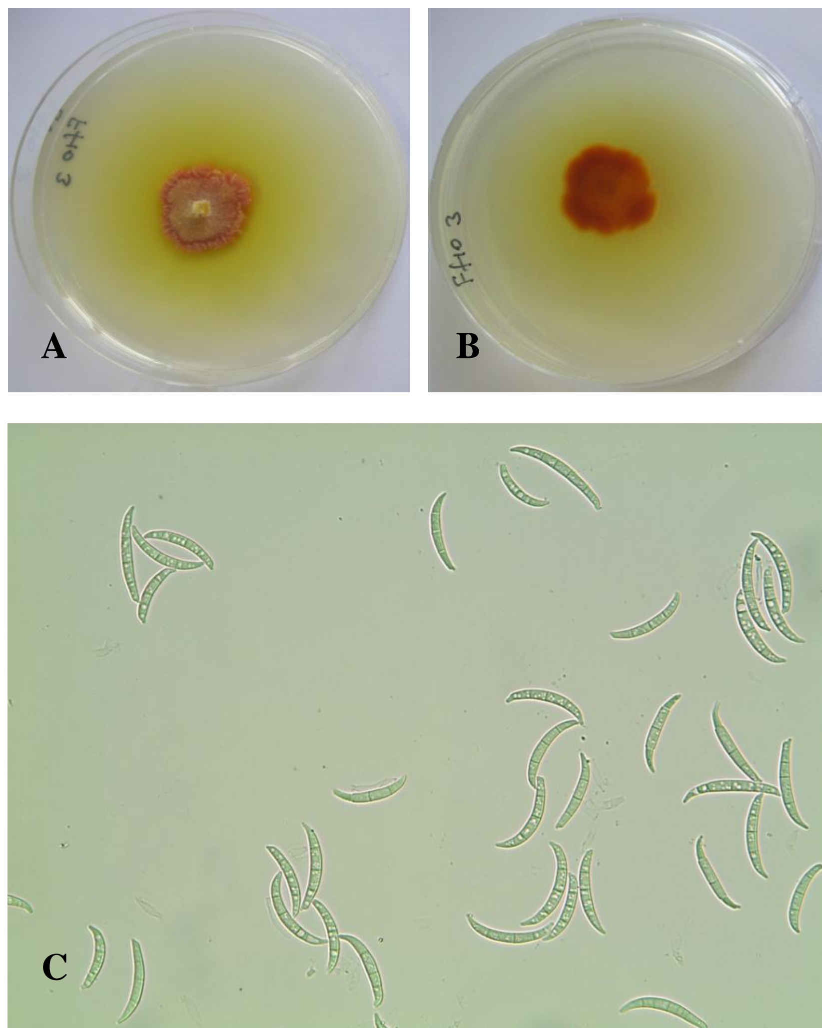

Figure 4.14. *Fusarium flocciferum*; surface **(A)** and reverse **(B)** of culture on PDA; macroconidia **(C)**

Fusarium incarnatum **Berkeley & Ravenel**

The three *F. incarnatum* isolates showed differences in their colony characteristics on PDA. The isolates were slow to moderatley growing with growth rates ranging from 21 to 39 mm (Table 4.1). Two of the isolates produced abundant light orange aerial mycelium and produced light orange pigment on PDA (Figure 4.15B). One of the isolates formed mycelium which was yellow at the center and cream at the periphery. Thick walled macroconidia with length and width of 23.75-37.5 μm and 2.75-4.5 μm, respectively were observed (Table 4.2). Macroconidia produced were 3 to 5 septate (Figure 4.15C).

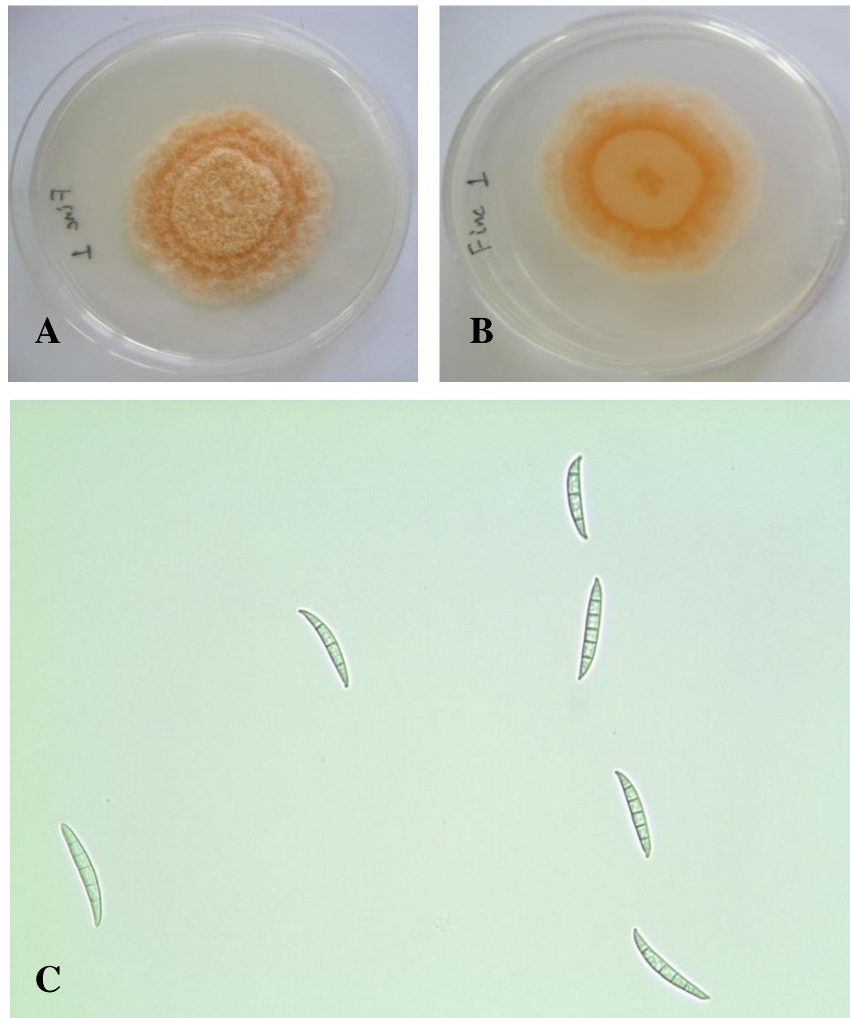

Figure 4.15. *Fusarium incarnatum*; surface **(A)** and reverse **(B)** of culture on PDA; macroconidia **(C)**

***Fusarium tricinctum* (Corda) Saccardo**

Fusarium tricinctum was slow growing and had growth rate of 20-21 mm (Table 4.1). It produced floccose mycelium which was yellowish at the center and white at the periphery (Figure 4.16A) and formed olive brown pigment on PDA (Figure 4.16B). *Fusarium tricinctum* produced 3 to 5 septate macroconidia with curved to tapering apical cell and well marked foot cells (Figure 4.16C). Macroconidia had size of 22.5-42.5 μm and 2.5-4.8 μm length and width, respectively (Table 4.2). The species also produced 0 to 1 septate microconidia (Figure 4.16D).

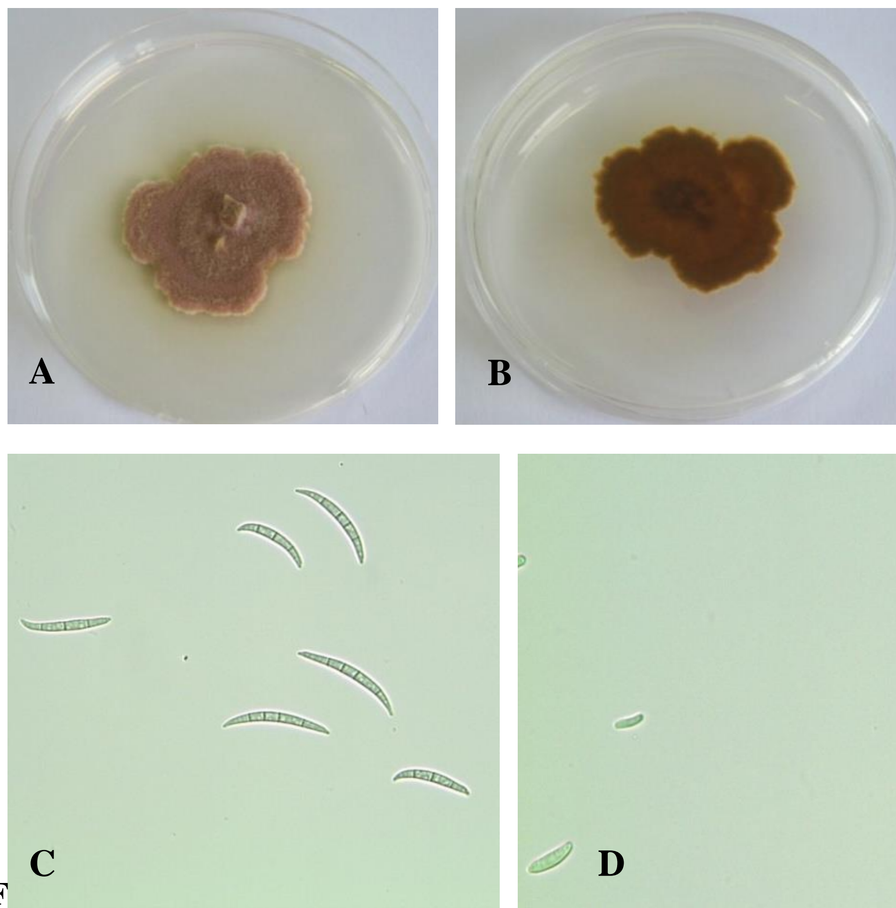

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Figure 4.16. *Fusarium tricinctum*; surface **(A)** and reverse **(B)** of culture on PDA; macroconidia **(C)** and microconidia **(D)**

Fusarium reticulatum Montagne

Fusarium reticulatum was a slow growing species with growth rates ranging from 14 to 17 mm (Table 4.1). It produced sparse yellowish mycelium which was white at the periphery (Figure 4.17A). The isolate produced light brown pigment on PDA (Figure 4.17B). *Fusarium reticulatum* produced relatively curved macroconidia which were 2-3 septate (Figure 4.17C). The macroconidia produced had length and width in the range of 23.75-37.5 µm and 3-5 µm, respectively (Table 4.2).

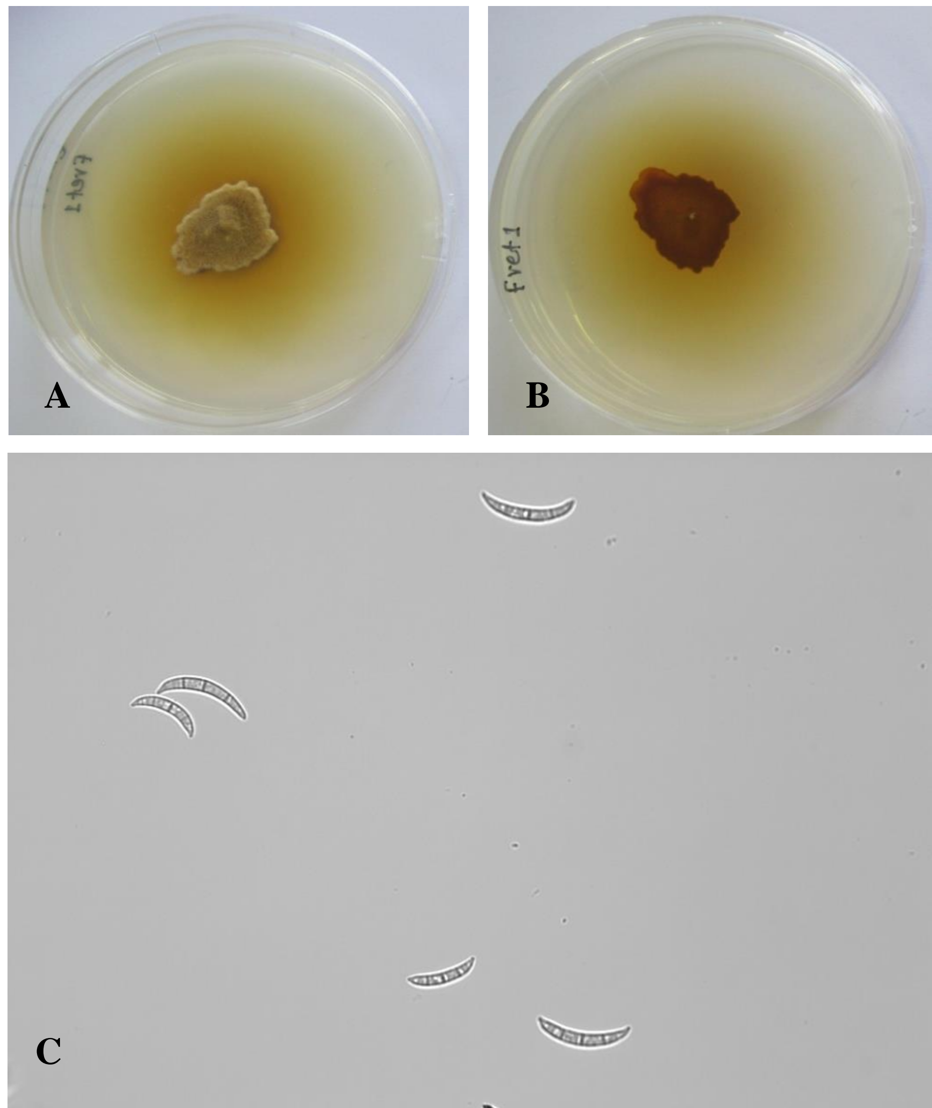

Figure 4.17. *Fusarium reticulatum;* surface **(A)** and reverse **(B)** of culture on PDA; macroconidia **(C)**

Table 4.1. Growth rate of *Fusarium* species isolated from crown/stem bases of wheat

Species	Growth rate (mm)[1]
F. culmorum	41-57
F. graminearum	44-47
F. brachygibbosum	39-51
F. pseudograminearum	35-41
F. equiseti	24-47
F. incarnatum	21-39
F. hostae	20-36
F. acuminatum	17-36
F. oxysporum	26-33
F. redolens	20-32
F. proliferatum	22-30
F. avenaceum	15-30
F. solani	22-26
F. tricinctum	20-21
F. torulosum	14-21
F. flocciferum	14-17
F. reticulatum	14-17

[1]Growth rate after 72 h on PDA at 25°C in complete darkness expressed as the average diameter of colony in mm

Table 4.2. Size of macroconidia of *Fusarium* species isolated from crown/stem bases of wheat

Species	Length (µm)*	Width (µm)*
F. avenaceum	42.5-72.5	3-4.5
F. graminearum	48-63	4-7
F. pseudograminearum	50-61	5-7
F. equiseti	35-60	3.5-6
F. acuminatum	24-58	4-5.5
F. redolens	30-55	3-5
F. torulosum	40-48	3.75-4.75
F. brachygibbosum	25-42.5	3-5
F. tricinctum	22.5-42.5	2.5-4.8
F. culmorum	35-39	6-8
F. hostae	20-40	3-4.75
F. reticulatum	23.75-37.5	3-5
F. incarnatum	23.75-37.5	2.75-4.5
F. oxysporum	22.5-37.5	2.5-4.5
F. flocciferum	22.5-37.5	3.25-3.8
F. proliferatum	23.75-35	2.5-3
F. solani	15-27.5	3.75-5

*Length and width of macroconidia measured at 40x magnification under light microscope after 7-10 days of incubation on SNA at a temperature of 25ºC day/20ºC night, with 12 h photoperiod under cool white and black fluorescent light

4.2.2. Molecular identification

4.2.2.1. Gel electrophoresis

The result of gel electrophoresis for PCR products of *Fusarium* isolates confirmed that DNA extraction and PCR amplification were successful. The bands produced by amplifying the TEF gene region of the first 20 *Fusarium* isolates had almost similar sizes ~700 bp (Figure 4.18).

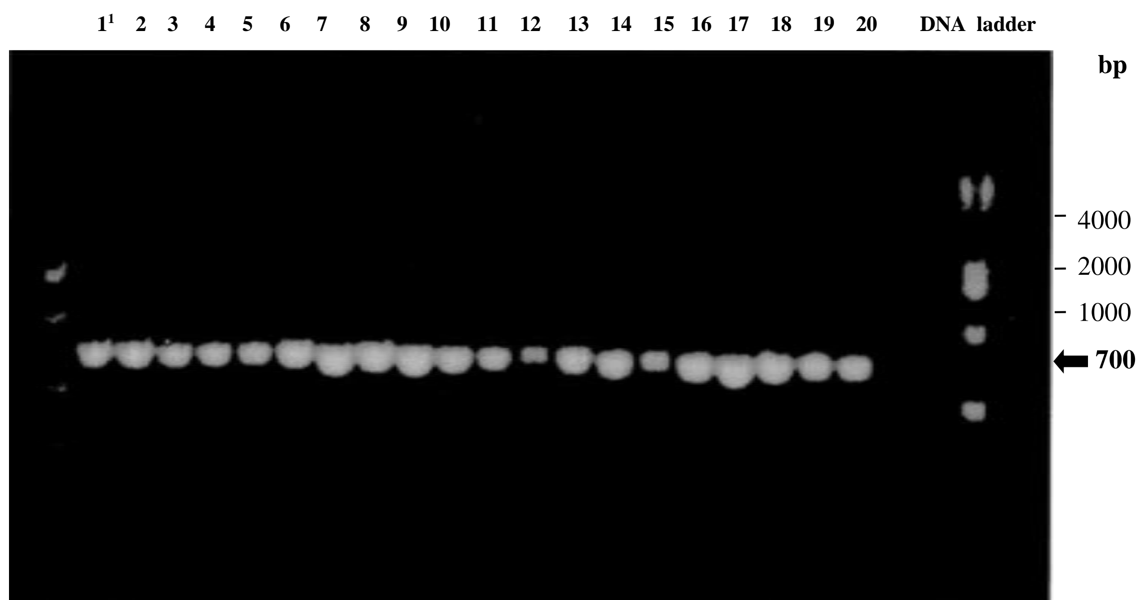

Figure 4.18. Result of gel electrophoresis for PCR products of the first 20 *Fusarium* isolates using 1kb DNA Ladder

[1]1= Fred1, 2= Fred2, 3= Fac1, 4= Fac1, 5= Fb1, 6= Fred3, 7= Fox1, 8= Fred4, 9= Fox2, 10= Fox3, 11= Fb2, 12= Fac2, 13= Fred5, 14= Feq1, 15= Feq2, 16= Feq3, 17= Fox4, 18= Fh1, 19= Fh2, and 20= Feq4

4.2.2.2. Sequencing and sequence analysis

For the 342 *Fusarium* isolates obtained from wheat crowns/stem bases, TEF gene fragments were amplified using primers ef1 and ef2 (O'Donnell *et al*., 1998). Summary of results of sequencing and BLAST analysis for the 17 *Fusarium* species identified is presented in Table 4.3.

Although TEF sequences ranging from 314 to 574 bp were amplified for *Fusarium culmorum* isolates, most of them yielded sequences of ~500 bp, while only two isolates produced sequences of 314 bp long. The isolates had matches ranging from 98 to 100% with GenBank accession JF740860, however; most had matches of 100% with the same accession. The three *F. pseudograminearum* isolates resulted TEF gene sequences of 500, 555 and 501 bp with the first two matches of 99% and the third 100% with accession of *F. pseudograminearum* JN862233.

Ninety-three percent of *F. equiseti* isolates produced sequences ranging from 400 to 655 bp. Seventy percent of the isolates had matches of 99% with accessions of *F. equiseti* DQ854855. TEF sequences ranging from 243 to 648 bp were obtained for *F. acuminatum* isolates, however most of the isolates produced ~540 bp with matches of 99 and 100% to the *F. acuminatum* accessions KC999530 and JX397863, respectively. *Fusarium avenaceum* isolates yielded TEF sequences ranging from 340 to 648 bp, with matches ranging from 97% to 100% with accession JX397827.

Except an isolate of *F. redolens* which yielded TEF sequence of 212 bp, the remaining 18 isolates had sequences ranging from 461 to 667 bp. Most of the isolates had matches of 99%, although some had 100% matches in identity with accession HQ731063. The twenty *F. hostae* isolates yielded TEF sequences ranging from 475 to 655 bp, in which sequence lengths > 600 bp were produced for 70% of them. Seventeen out of the twenty isolates had a 99% match with the accession of *F. hostae* DQ854862. All the 12 *F. oxysporum* isolates produced sequences ranging from 634 to 653 bp and eleven out of the twelve had matches of 99% with GeneBank accession GU170550 and one isolate had 100% match in identity with accession DQ435353. For the three *F. solani* isolates sequences of 599, 640 and 668 bp with matches of 99% with accessions HQ731056, JF7408666 and HQ731053, respectively were produced. Isolate of *F. solani* produced the largest TEF fragment which was 668 bp. Although sequences ranging from 594 to 654 bp were produced by *F. torulosum* isolates, eight out of nine isolates yielded sequences 609 to 654 bp long, with identity matches of 98 to 99% with accession KC999494.

Table 4.3. Summary of sequencing and BLAST analysis of TEF gene region for the 17 *Fusarium* species isolated from wheat in Turkey

Species	Number of isolates sequenced	Amplicon[1] size (bp)	Identity (%)	Closest matching accession
F. culmorum	46	314-574	98-100	JF740860
F. pseudograminearum	3	500-555	99-100	JN862233
F. graminearum	1	503	100	JF278573
F. hostae	20	475-655	96-100	DQ854862
F. redolens	19	212--667	99-100	GU250584
F. avenaceum	12	340-648	97-100	JX397827
F. acuminatum	50	243-648	98-100	KC999530
F. equiseti	123	243-655	97-100	DQ854855
F. brachygibbosum	25	363-651	94-99	GQ505418
F. oxysporum	12	634-653	99-100	GU170550
F. solani	3	599-668	99	HQ731056
F. torulosum	9	594-654	97-99	KC999494
F. flocciferum	6	514-645	99-100	KC999486
F. incarnatum	3	468-641	99-100	JN092338
F. proliferatum	8	400-650	99-100	AF291058
F. tricinctum	1	617	99	JX397845
F. reticulatum	1	621	100	DQ854864

[1] Only 5% of the isolates had amplicons less than 400 bp

4.3. Identity of *Fusarium* species

A total of 342 isolates were obtained from samples collected from the different wheat growing regions of Turkey during May, June and July 2013. The isolates were then identified into 17 *Fusarium* species by examining morphological characters and sequencing TEF1-α gene region. The *Fusarium* species identified were; *F. culmorum, F. pseudograminearum, F. graminearum, F. equiseti, F. acuminatum, F. brachygibbosum, F. hostae, F. redolens, F. avenaceum, F. oxysporum, F. torulosum, F. proliferatum, F. flocciferum, F. solani, F. incarnatum, F. tricinctum* and *F. reticulatum.*

The number of isolates and isolation frequency of each *Fusarium* species obtained from the different agro-ecological regions is shown in Figure 4.19 and Table 4.4,

respectively. *F. equiseti* was the most prevalent *Fusarium* species with isolation frequency of 35.96% (123 of 342) followed by *F. acuminatum* and *F. culmorum* with frequencies of 14.61% (50 of 342) and 13.45% (46 of 342), respectively. *Fusarium equiseti* was the most frequently isolated species from Southeast Anatolia, Aegean and Central Anatolia regions (Figure 4.20). *Fusarium acuminatum* was the second most frequently isolated species and was isolated from Aegean, Southeast Anatolia, Black Sea, Central Anatolia and Mediterranean regions (Table 4.5).

Fusarium culmorum (46 of 342) was the most predominant among the damaging *Fusarium* species and was isolated from Central Anatolia, Black Sea, Aegean, and Southeast Anatolia regions (Table 4.5). The least predominant pathogenic species were *F. pseudograminearum* (3 of 342) and *F. graminearum* (1 of 342) which were isolated only from Central Anatolia and Black Sea regions, respectively (Table 4.5).

Fusarium hostae (20) and *F. redolens* (19) were isolated from Aegean, Southeast Anatolia, Black Sea and Central Anatolia regions (Table 4.5), with isolation frequencies of 5.84% and 5.55%, respectively (Table 4.4). The least frequently isolated species were *F. graminearum* (1), *F. tricinctum* (1) and *F. reticulatum* (1), which were isolated from Black Sea, Black Sea and Aegean regions, respectively (Table 4.5). Other fungi that were isolated from crowns of wheat but not quantified and further studied included *Alternaria* species, *Bipolaris sorokiniana* and *Rhizoctonia* species.

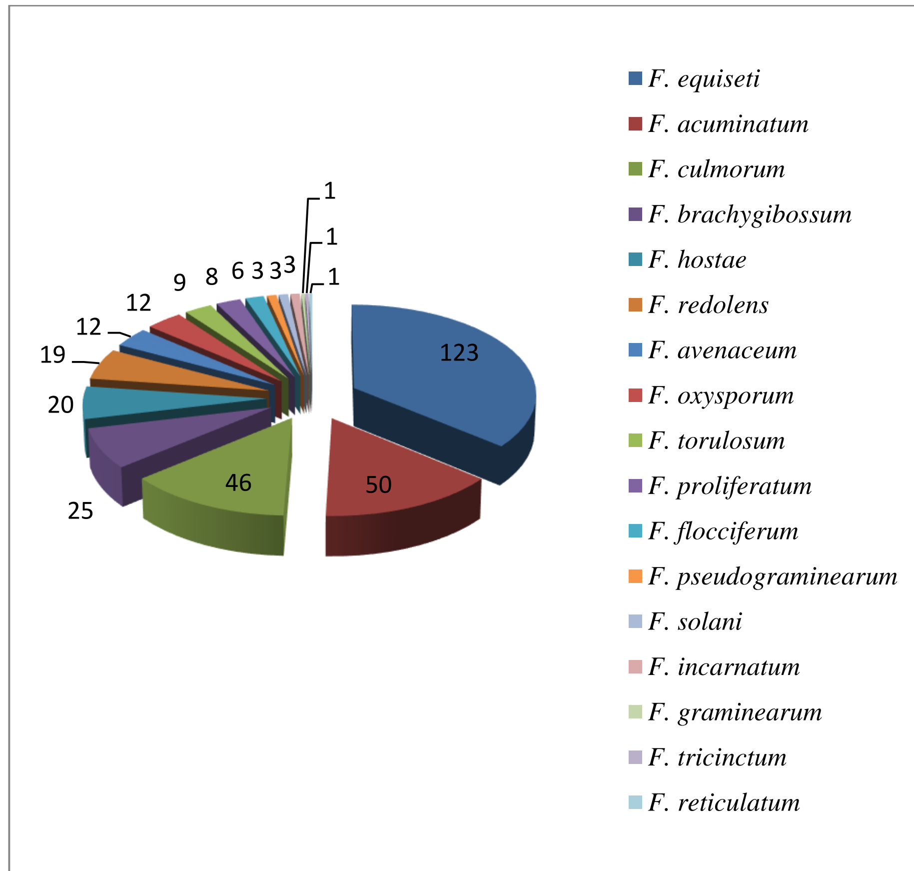

Figure 4.19. Isolation frequency of *Fusarium* species obtained from wheat crown/stem bases collected from Turkey during summer 2013

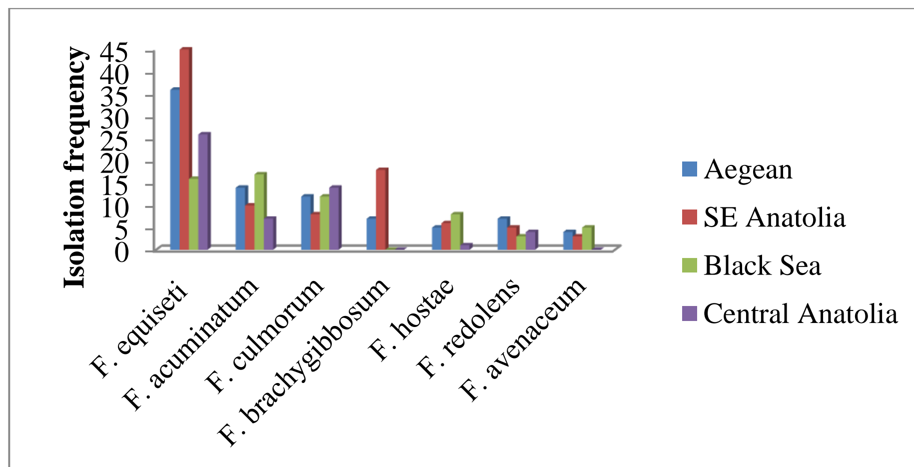

Figure 4.20. Isolation frequency of some of the predominant *Fusarium* species isolated from crown/stem bases of wheat from the different agro-ecological regions of Turkey

Table 4.4. Isolation frequency of *Fusarium* species isolated from wheat crown/stem bases collected from Turkey during summer 2013

Species	Isolation frequency (%)
F. equiseti	35.96
F. acuminatum	14.61
F. culmorum	13.45
F. brachygibossum	7.30
F. hostae	5.84
F. redolens	5.55
F. avenaceum	3.50
F. oxysporum	3.50
F. torulosum	2.63
F. proliferatum	2.33
F. flocciferum	1.75
F. pseudograminearum	0.87
F. solani	0.87
F. incarnatum	0.87
F. graminearum	0.29
F. tricinctum	0.29
F. reticulatum	0.29

Table 4.5. Number of *Fusarium* species isolated from wheat crown/stem bases collected from the different agro-ecological regions in Turkey during summer 2013

Species	**Agro-ecological region**					
	Aegean	**SE Anatolia**	**Black Sea**	**Central Anatolia**	**Eastern Anatolia**	**Mediterranean**
F. equiseti	36	45	16	26	-	-
F. acuminatum	14	10	17	7	-	2
F. culmorum	12	8	12	14	-	-
F. brachygibbosum	7	18	-	-	-	-
F. hostae	5	6	8	1	-	-
F. redolens	7	5	3	4	-	-
F. avenaceum	4	3	5	-	-	-
F. oxysporum	5	-	5	2	-	-
F. torulosum	-	6	1	1	1	-
F. proliferatum	1	7	-	-	-	-
F. flocciferum	2	2	2	-	-	-
F. pseudograminearum	-	-	-	3	-	-
F. solani	1	1	1	-	-	-
F. incarnatum	-	2	1	-	-	-
F. tricinctum	-	-	1	-	-	-
F. reticulatum	1	-	-	-	-	-
F. graminearum			1			
Total	**95**	**113**	**73**	**58**	**1**	**2**

4. 4. Pathogenicity and aggressiveness of *Fusarium* species on wheat

4.4.1. Pathogenicity of *Fusarium* species on wheat

Seven out of the 17 *Fusarium* species tested for their pathogenicity caused crown rot in different levels of severity. *Fusarium culmorum*, *F. pseudograminearum* and *F. graminearum* caused severe crown rot disease on susceptible durum wheat Kızıltan - 91. *Fusarium avenaceum* and *F. hostae* were moderately pathogenic. *Fusarium redolens* was weakly pathogenic. *Fusarium acuminatum* included isolates capable of causing necrosis on crown/stem bases of wheat. On the other hand *F. oxysporum*, *F. equiseti*, *F. solani*, *F. incarnatum*, *F. reticulatum, F. flocciferum, F. tricinctum, F. brachygibbosum, F. torulosum* and *F. proliferatum* were non- pathogenic.

The mean scores of disease severity for the 46 *F. culmorum* isolates and control treatment is shown in Table 4.6. Disease severity scores for *F. culmorum* isolates ranged from 2 to 4.3 with an average of 3.3. Twenty nine *F. culmorum* isolates had disease severity scores significantly greater (P<0.001) (Appendix 3) than non-inoculated treatments (Table 4.6). Among the 46 *Fusarium culmorum* isolates tested, Fc2 and Fc7 were the two most pathogenic ones with disease severity scores of 4.3. The isolates Fc2 and Fc7 were obtained from İzmir and Saruhanlı regions of Turkey, respectively (Appendix 1). Highly pathogenic *F. culmorum* isolates caused complete death of plants (Figure 4.21A) while no symptoms of necrosis were observed on crowns of non-inoculated control treatments (Figure 4.21B). The least pathogenic isolates were Fc45, Fc18 and Fc12 which had scores of 2. Fc45, Fc18, Fc12 were isolated from samples collected from Yozgat, Adıyaman and Pamukyazı regions, respectively (Appendix 1).

Table 4.6. Mean scores of disease severity caused by *Fusarium culmorum* isolates on durum wheat cultivar Kızıltan-91 in seedling test

Isolate [1]	Score [2]
Fc2	4.3 [a *]
Fc7	4.3 [a]
Fc38	4.0 [ab]
Fc30	4.0 [ab]
Fc28	4.0 [ab]
Fc27	4.0 [ab]
Fc5	4.0 [ab]
Fc1	4.0 [ab]
Fc48	3.7 [ab]
Fc44	3.7 [ab]
Fc41	3.7 [ab]
Fc31	3.7 [ab]
Fc25	3.7 [ab]
Fc23	3.7 [ab]
Fc17	3. 7 [ab]
Fc16	3. 7 [ab]

Table 4.6. Continued.

Fc14	3. 7 [ab]
Fc10	3. 7 [ab]
Fc9	3.7 [ab]
Fc4	3.5 [ab]
Fc47	3.3 [ab]
Fc39	3.3[ab]
Fc35	3.3 [ab]
Fc32	3.3 [ab]
Fc29	3.3 [ab]
Fc26	3.3 [ab]
Fc15	3.3 [ab]
Fc13	3.3 [ab]
Fc6	3.3 [ab]
Fc22	3.0 [abc]
Fc21	3.0 [abc]
Fc8	3.0 [abc]
Fc46	2.7 [ab]
Fc42	2.7 [ab]
Fc37	2.7 [ab]
Fc34	2.7 [ab]
Fc24	2.7 [ab]
Fc20	2.7 [ab]
Fc19	2.7 [ab]
Fc43	2.3 [ab]
Fc40	2.3 [ab]
Fc36	2.3 [ab]
Fc3	2.3 [ab]
Fc45	2.0 [bc]
Fc12	2.0 [bc]
Mean	**3.3**
Control	**1.0 [c]**

1Fc= *Fusarium culmorum*

[2] Score of each isolate is a mean of three replicates.

*Values that share a letter are not
significantly different at 0.05 level, according to Tukey HSD test

Figure 4.21. Wheat plant inoculated with *Fusarium culmorum* **(A)** and non-inoculated control treatment **(B)**

Fusarium pseudograminearum isolates were highly pathogenic and had mean disease severity scores ranging from 3.3 to 3.7 (with an average of 3.6) (Table 4.7). Disease severity scores of the three *F. pseudograminearum* isolates were significantly greater (P<0.001) (Appendix 4) than the score of the control treatment (Table 4.7). *Fusarium graminearum* isolate also caused severe disease and had mean score of 3.7.

Table 4.7. Mean scores of disease severity caused by *Fusarium pseudograminearum* isolates

Isolate [1]	**Score** [2]
Fpg2	3.7 [a*]
Fpg3	3.7 [a]
Fpg1	3.3 [a]
Mean	**3.6**
Control	**1.0** [**b**]

[1] Fpg = *Fusarium pseudograminearum*
[2] Score of each isolate is the mean of three replicates
*Values that share a letter are not significantly different at 0.05 level, according to Tukey HSD test

Disease severity scores of *F. hostae* isolates are presented in Table 4.8. The score for disease severity ranged from 1.0 to 3.0 with a mean of 2.2. The scores of seven isolates were significantly (P<0.001) (Appendix 5) greater than the non-inoculated control treatment, and six isolates had scores of 3.0 (Table 4.8). The moderately pathogenic isolates of *F. hostae* resulted brown discoloration on crown of inoculated plants (Figure 4.22A) while no disease symptom was observed on crowns of non-inoculated control treatments (Figure 4.22B). Isolates Fh20, Fh19, Fh17, Fh11, Fh10, Fh1 and Fh14 were obtained from Mesudiye-Konya, Samsun, Samsun, Adıyaman, Adıyaman, Mersinli and Yakakent regions, respectively (Appendix 1).

Table 4.8. Mean scores of disease severity caused by *Fusarium hostae* isolates

Isolate [1]	**Score [2]**
Fh20	3.0 [a *]
Fh19	3.0 [a]
Fh17	3.0 [a]
Fh11	3.0 [a]
Fh10	3.0 [a]
Fh1	3.0 [a]
Fh14	2.7 [ab]
Fh16	2.3 [abc]
Fh9	2.3 [abc]
Fh8	2.3 [abc]
Fh7	2.3 [abc]
Fh13	2.0 [abc]
Fh4	2.0 [abc]
Fh3	2.0 [abc]
Fh2	1.7 [abc]
Fh18	1.3 [bc]
Fh15	1.3 [bc]
Fh6	1.3 [bc]
Fh5	1.3 [bc]
Fh12	1.0 [c]
Mean	**2.2**
Control	**1.0 [c]**

[1] Fh = *Fusarium hostae,*
[2] Score of each isolate is the mean of three replicate
*Values that share a letter are not significantly different at 0.05 level, according to Tukey HSD test

Figure 4.22. Non-inoculated control treatment **(A)** and wheat plant inoculated with *Fusarium hostae* **(B)**

Brown discoloration was observed on the crowns of wheat plants inoculated with pathogenic *F. redolens* isolates (Figure 4.23A), while control treatments showed no disease symptoms (Figure 4.23B). Disease severity scores ranged from 1.0 to 3.0 with an average of 1.7. Only three out of the 19 *F. redolens* isolates had disease severity scores significantly greater (P<0.001) (Appendix 6) than the non inoculated control treatments (Table 4.9). Fred15 and Fred2 isolates which had the highest score of disease severity (3.0) were obtained from İceri çumra and Uşak provinces, respectively. On the other hand Fred18, Fred13, Fred7, Fred6 and Fred5 isolates had disease severity scores of 1.0, thus they were considered as non pathogenic.

Table 4.9. Mean scores of disease severity caused by *Fusarium redolens* isolates

Isolate [1]	Score [2]
Fred 15	3.0 [a*]
Fred 2	3.0 [a]
Fred 16	2.7 [ab]
Fred 19	2.3 [abc]
Fred 17	2.3 [abc]
Fred 14	2.3 [abc]
Fred 9	2.0 [abc]
Fred 12	1.7 [abc]
Fred 10	1.7 [abc]
Fred 11	1.3 [bc]
Fred 8	1.3 [bc]
Fred 4	1.3 [bc]
Fred 3	1.3 [bc]
Fred 1	1.3 [bc]
Fred 18	1.0 [c]
Fred 13	1.0 [c]
Fred 7	1.0 [c]
Fred 6	1.0 [c]
Fred 5	1.0 [c]
Mean	**1.7**
Control	**1.0 [c]**

[1] Fred = *Fusarium redolens,*
[2] Score of each isolate is the mean of three replicates
*Values that share a letter are not significantly different at 0.05 level, according to Tukey HSD test

Figure 4.23. Wheat plants inoculated with *Fusarium redolens* **(A)** and non-inoculated control treatment **(B)**

The results of pathogenicity test for isolates of *F. avenaceum* revealed that the isolates were moderately pathogenic. Brown discoloration in the crown and lower stem of wheat plant inoculated with moderately pathogenic *F. avenaceum* isolate and non inoculated control treatment is shown in Figure 4.24. There was no significant difference in disease severity caused by *F. avenaceum* isolates. Disease severity scores ranged from 3.0 to 2.0 (Table 4.10), with an average of 2.6. Four out of the twelve *F. avenaceum* isolates had scores of 3.0. Isolates Fav4, Fav7, Fav9 and Fav12 were obtained from Kızılcapınar-Aydın, Urfa, Durağan and Yakakent regions, respectively (Appendix 1).

Table 4.10. Mean scores of disease severity caused by *Fusarium avenaceum* isolates

Isolate [1]	Score [2]
Fav4	3.0
Fav7	3.0
Fav9	3.0
Fav12	3.0
Fav1	2.7
Fav2	2.7
Fav8	2.7
Fav10	2.7
Fav3	2.3
Fav5	2.3
Fav6	2.3
Fav11	2.0
Mean	**2.6**
Control	**1.0**

[1] Fav = *Fusarium avenaceum*
[2] Score of each isolate is the mean of three replicates

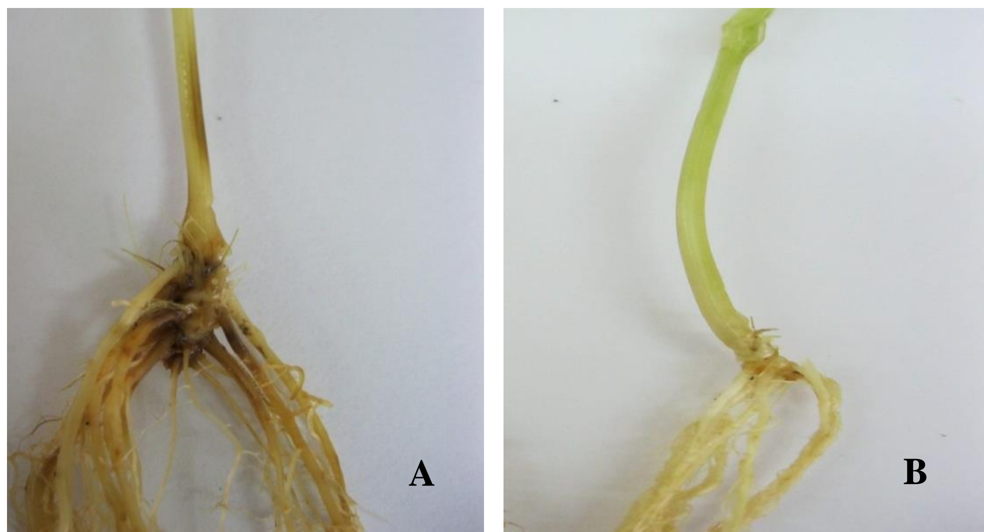

Figure 4.24. Wheat plant inoculated with *Fusarium avenaceum* **(A)** and non-inoculated control treatment **(B)**

Disease severity scores for *Fusarium acuminatum* ranged from 1.0 to 2.0, with an average of 1.4 (Table 4.11). Although most isolates did not cause necrosis on susceptible durum wheat cultivar Kızıltan-91, four out of the 50 *Fusarium acuminatum* isolates were weakly pathogenic which had scores of 2.0. No disease symptoms were observed on wheat plants inoculated with *F. oxysporum*, *F. equiseti*, *F. solani*, *F. incarnatum*, *F. reticulatum, F. flocciferum, F. tricinctum, F. brachygibbosum, F. torulosum* and *F. proliferatum.* Thus, these species were non pathogenic.

Table 4.11. Summary of results of seedling pathogenicity test for the 17 *Fusarium* species obtained from crown/stem bases of wheat

Species	**No. of isolates tested**	**No. of isolates significantly different from control**	**Mean scores of all isolates**	**Pathogenicity**
F. graminearum	1	1	3.7	Highly pathogenic
F. pseudograminearum	3	3	3.6	Highly pathogenic
F. culmorum	46	29	3.3	Highly pathogenic
F. avenaceum	12	0	2.6	Moderately pathogenic
F. hostae	20	7	2.2	Moderately pathogenic
F. redolens	19	3	1.7	Weakly pathogenic
F. acuminatum	50	0	1.4	Mostly non pathogenic few weak pathogens
F. brachygibossum	25	0	1.3	Non pathogenic
F. proliferatum	8	0	1.3	Non pathogenic
F. oxysporum	12	0	1.2	Non pathogenic
F. solani	3	0	1.2	Non pathogenic
F. torulosum	9	0	1.2	Non pathogenic
F. flocciferum	6	0	1.2	Non pathogenic
F. incarnatum	3	0	1.0	Non pathogenic
F. equiseti	123	0	1.0	Non pathogenic
F. tricinctum	1	0	1.0	Non pathogenic
F. reticulatum	1	0	1.0	Non pathogenic

4.4.2. Aggressiveness of *Fusarium culmorum* isolates on wheat

There was significant (P<0.001) difference in aggressiveness among *F. culmorum* isolates (Appendix 8). The mean score for aggressiveness of the 46 *F. culmorum* isolates is presented in Table 4.12. The mean disease severity scores ranged from 1.2 to 4.4, with an average of 3.0. *Fusarium culmorum* isolate number 2 (Fc2) which was collected from İzmir was the most aggressive isolate with a mean score of 4.4. It caused severe crown rot on susceptible durum wheat cultivar Kızıltan-91, and formed pink mycelium in the crown area of inoculated seedlings (Figure 4.25A). The least aggressive isolate was Fc45 which had mean score of 1.2. The control treatment had a mean score of 1.0 which means no disease symptom (Figure 4.25B).

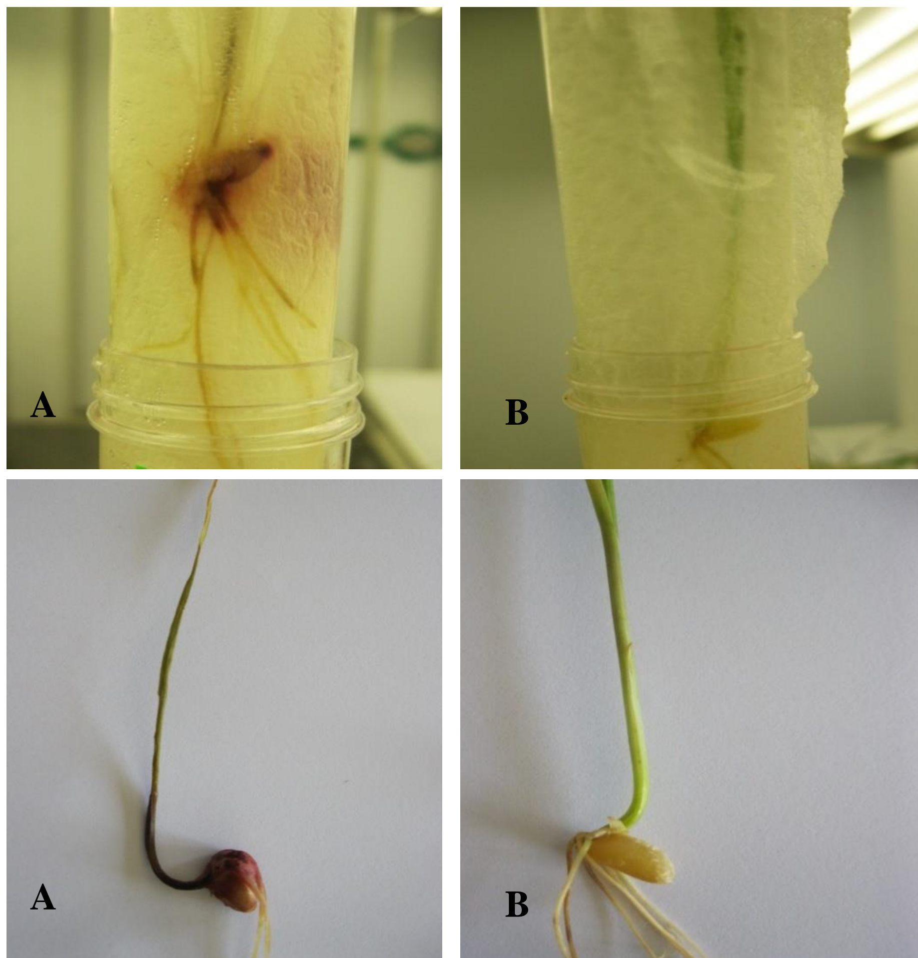

Figure 4.25. Wheat seedling inoculated with *Fusarium culmorum* (Fc2) (**A**, upper and lower) and non- inoculated control treatment (**B,** upper and lower)

Table 4.12. Mean scores for aggressiveness of *Fusarium culmorum* isolates on durum wheat cultivar Kızıltan-91

Isolate [1]	**Score** [2]
Fc2	4.4 [a*]
Fc9	4.3 [a]
Fc3	4.3 [a]
Fc6	4.3 [ab]
Fc14	4.2 [ab]
Fc7	4.0 [abc]
Fc15	4.0 [abc]
Fc13	3.9 [abcd]
Fc42	3.8 [abcde]
Fc34	3.6 [abcdef]
Fc29	3.6 [abcdef]
Fc25	3.6 [abcdef]
Fc24	3.6 [abcdef]
Fc10	3.4 [abcdefg]
Fc4	3.4 [abcdefg]
Fc35	3.3 [abcdefgh]
Fc5	3.3 [abcdefgh]
Fc17	3.2 [abcdefgh]
Fc36	3.1 [abcdefgh]
Fc27	3.1 [abcdefgh]
Fc40	2.9 [bcdefgh]
Fc48	2.8 [cdefgh]
Fc41	2.8 [cdefgh]
Fc20	2.8 [cdefgh]
Fc16	2.8 [cdefgh]
Fc8	2.8 [cdefgh]
Fc43	2.8 [cdefgh]
Fc44	2.7 [cdefgh]
Fc38	2.7 [cdefgh]
Fc30	2.7 [cdefgh]
Fc26	2.7 [cdefgh]
Fc23	2.7 [cdefgh]

Table 4.12. Continued

Fc19	2.7 cdefgh
Fc47	2.6 defghi
Fc31	2.6 defghi
Fc28	2.6 defghi
Fc1	2.6 defghi
Fc46	2.4 efghi
Fc39	2.4 efghi
Fc37	2.4 efghi
Fc21	2.4 efghi
Fc32	2.2 fghij
Fc22	2.2 fghij
Fc12	2.1 ghij
Fc18	2.0 hij
Fc45	1.2 ij
Mean	**3.0**
Control	**1.0 j**

[1]Fc= *Fusarium culmorum*
[2] Score of each isolate is the mean of nine replicates
*Values that share a letter are not significantly different at 0.05 level, according to Tukey HSD test

4.5. Screening wheat germplasms for their reaction to *Fusarium culmorum*

Based on the result of aggressiveness test, the most aggressive *Fusarium culmorum* isolate, Fc2 was used to screen wheat germplasms for their reaction. Mean scores of wheat lines and control cultivars and their reaction are presented in Table 4.13. Lists of lines tested and related information are presented in Appendix 2. ANOVA table for screening experiment is presented in Appendix 9.

The mean disease severity scores for the lines tested ranged from 1.4 to 4.4 with an average of 3.1. Two (147, 158) out of the 165 lines tested, were resistant (R) in their reaction and had scores of 1.4. Twenty lines (5, 100, 143, 163, 32, 138, 86, 89, 104,

123, 153, 161, 8, 34, 142, 9, 15, 47, 116, 146) showed moderately resistant (MR) reaction and had scores ranging from 1.6 to 2.4. The scores of both the resistant and moderately resistant lines were not significantly different from scores of moderately resistant control cultivars Suntop (1.6), Carisma (1.8) and Altay-2000 (2.4). Sixty-three percent of the lines were moderately susceptible (MS). The scores of moderately susceptible lines ranged from 2.6 to 3.4 which were not significantly different from the moderately susceptible control cultivars Adana-99 (2.6), Janz (2.6) and Emu Rock (2.6). Out of the 165 lines tested, 39 were susceptible (S) in their reaction. These susceptible lines had scores ranging from 3.6 to 4.4 which were not significantly different from the score of the susceptible control cultivars Süzen-97 (3.6) and Kutluk-94 (4.0).

Symptoms of crown rot (necrosis and/or brown discoloration) on resistant, moderately resistant, moderately susceptible and susceptible wheat lines are shown in Figure 4.26.

Table 4.13. Mean disease severity scores and reactions of wheat lines and control cultivars tested against *Fusarium culmorum* isolate Fc2

Germplasm [a]	Score [1]	Reaction [2] [3]
158	1.4 [a]	R
147	1.4 [a]	R
5	1.6 [ab]	MR
100	1.6 [ab]	MR
143	1.6 [ab]	MR
Suntop	1.6 [ab]	MR
163	1.8 [abc]	MR
32	1.8[abc]	MR
138	1.8 [abc]	MR
Carisma	1.8 [abc]	MR
86	2.0 [abcd]	MR
89	2.0 [abcd]	MR
104	2.0 [abcd]	MR
123	2.0 [abcd]	MR
153	2.0 [abcd]	MR

Table 4.13. Continued.

161	2.0^{abcd}	MR
8	2.2^{abcde}	MR
34	2.2^{abcde}	MR
142	2.2^{abcde}	MR
9	2.4^{abcdef}	MR
15	2.4^{abcdef}	MR
47	2.4^{abcdef}	MR
116	2.4^{abcdef}	MR
146	2.4^{abcdef}	MR
Altay-2000	2.4^{abcdef}	MR
33	$2.6^{abcdefg}$	MS
54	$2.6^{abcdefg}$	MS
57	$2.6^{abcdefg}$	MS
76	$2.6^{abcdefg}$	MS
78	$2.6^{abcdefg}$	MS
87	$2.6^{abcdefg}$	MS
115	$2.6^{abcdefg}$	MS
120	$2.6^{abcdefg}$	MS
135	$2.6^{abcdefg}$	MS
137	$2.6^{abcdefg}$	MS
156	$2.6^{abcdefg}$	MS
157	2.6^{abcdfg}	MS
169	$2.6^{abcdefg}$	MS
Adana-99	$2.6^{abcdefg}$	MS
Janz	$2.6^{abcdefg}$	MS
Emu Rock	$2.6^{abcdefg}$	MS
67	$2.8^{bcdefgh}$	MS
22	$3.0^{cdefghi}$	MS
28	$3.0^{cdefghi}$	MS
30	$3.0^{cdefghi}$	MS
43	$3.0^{cdefghi}$	MS
75	$3.0^{cdefghi}$	MS
82	$3.0^{cdefghi}$	MS
85	$3.0^{cdefghi}$	MS

Table 4.13. Continued.

93	3.0 cdefghi	MS
95	3.0 cdefghi	MS
102	3.0 cdefghi	MS
103	3.0 cdefghi	MS
109	3.0 cdefghi	MS
114	3.0 cdefghi	MS
117	3.0 cdefghi	MS
118	3.0 cdefghi	MS
124	3.0 cdefghi	MS
127	3.0 cdefghi	MS
130	3.0 cdefghi	MS
148	3.0 cdefghi	MS
152	3.0 cdefghi	MS
155	3.0 cdefghi	MS
159	3.0 cdefghi	MS
160	3.0 cdefghi	MS
165	3.0 cdefghi	MS
168	3.0 cdefghi	MS
Seri-82	3.0 cdefghi	MS
12	3.2 defghij	MS
13	3.2 defghij	MS
16	3.2 defghij	MS
21	3.2 defghij	MS
40	3.2 defghij	MS
41	3.2 defghij	MS
44	3.2 defghij	MS
61	3.2 defghij	MS
65	3.2 defghij	MS
66	3.2 defghij	MS
74	3.2 defghij	MS
79	3.2 defghij	MS
80	3.2 defghij	MS
88	3.2 defghij	MS
96	3.2 defghij	MS

Table 4.13. Continued.

105	$3.2^{defghij}$	MS
111	$3.2^{defghij}$	MS
112	$3.2^{defghij}$	MS
119	$3.2^{defghij}$	MS
121	$3.2^{defghij}$	MS
122	$3.2^{defghij}$	MS
129	$3.2^{defghij}$	MS
132	$3.2^{defghij}$	MS
134	$3.2^{defghij}$	MS
139	$3.2^{defghij}$	MS
145	$3.2^{defghij}$	MS
154	$3.2^{defghij}$	MS
2	3.4^{efghij}	MS
4	3.4^{efghij}	MS
10	3.4^{efghij}	MS
14	3.4^{efghij}	MS
17	3.4^{efghij}	MS
18	3.4^{efghij}	MS
23	3.4^{efghij}	MS
24	3.4^{efghij}	MS
26	3.4^{efghij}	MS
27	3.4^{efghij}	MS
29	3.4^{efghij}	MS
31	3.4^{efghij}	MS
35	3.4^{efghij}	MS
45	3.4^{efghij}	MS
46	3.4^{efghij}	MS
48	3.4^{efghij}	MS
55	3.4^{efghij}	MS
56	3.4^{efghij}	MS
63	3.4^{efghij}	MS
64	3.4^{efghij}	MS
77	3.4^{efghij}	MS
91	3.4^{efghij}	MS

Table 4.13. Continued.

94	3.4^{efghij}	MS
97	3.4^{efghij}	MS
98	3.4^{efghij}	MS
101	3.4^{efghij}	MS
107	3.4^{efghij}	MS
108	3.4^{efghij}	MS
113	3.4^{efghij}	MS
125	3.4^{efghij}	MS
126	3.4^{efghij}	MS
133	3.4^{efghij}	MS
141	3.4^{efghij}	MS
144	3.4^{efghij}	MS
149	3.4^{efghij}	MS
151	3.4^{efghij}	MS
162	3.4^{efghij}	MS
166	3.4^{efghij}	MS
3	3.6^{fghij}	S
68	3.6^{fghij}	S
71	3.6^{fghij}	S
73	3.6^{fghij}	S
128	3.6^{fghij}	S
136	3.6^{fghij}	S
Süzen-97	3.6^{fghij}	S
19	3.6^{fghij}	S
25	3.6^{fghij}	S
36	3.6^{fghij}	S
42	3.6^{fghij}	S
51	3.6^{fghij}	S
52	3.6^{fghij}	S
58	3.6^{fghij}	S
62	3.6^{fghij}	S
84	3.6^{fghij}	S
92	3.6^{fghij}	S
99	3.6^{fghij}	S

Table 4.13. Continued.

106	3.6^{fghij}	S
110	3.6^{fghij}	S
164	3.6^{fghij}	S
37	3.8^{ghij}	S
39	3.8^{ghij}	S
49	3.8^{ghij}	S
60	3.8^{ghij}	S
70	3.8^{ghij}	S
72	3.8^{ghij}	S
81	3.8^{ghij}	S
131	3.8^{ghij}	S
6	4.0^{hij}	S
20	4.0^{hij}	S
38	4.0^{hij}	S
69	4.0^{hij}	S
83	4.0^{hij}	S
150	4.0^{hij}	S
167	4.0^{hij}	S
Kutluk-94	4.0^{hij}	S
7	4.2^{ij}	S
11	4.2^{ij}	S
53	4.2^{ij}	S
59	4.4^{j}	S

* = Values that share a letter are not significantly different at 0.05 level, according to Tukey HSD test
[1] = Score of each germplasm is the mean of five replicates
[2] = R=Resistant, MR=Moderately Resistant, MS=Moderately Susceptible, S= Susceptible,
[3] Score ranges for corresponding reaction, R=1-1.4, MR=1.5-2.4, MS=2.5-3.4, S=3.5-4.4 and HS=4.5-5
[a] = Adana-99. Altay-2000, Seri-82, Kutluk-94, Süzen 97, Carisma, Janz, Emu Rock and Suntop are control cultivars

Figure 4.26. Reaction of wheat genotypes to *Fusarium culmorum* (Fc2); resistant **(A)**, moderately resistant **(B)**, moderately susceptible **(C)** and susceptible **(D)** wheat genotypes

5. DISCUSSION

Species identification is the important step in understanding and controlling plant diseases. Various morphological, analytical and molecular methods are used in species identification. However, morphological method is the only option for laboratories that that do not have facilities and expertise to carry out molecular species identification. Shape of macroconidia is the most important morphological feature used for Fusarium identification (Windels, 1992; Burgess *et al.,* 1994; Leslie and Summerell, 2006; Scherm *et al*., 2013), which in many cases alone is sufficient for identification (Leslie and Summerell, 2006). The *Fusarium* species identified in the present research produced distinctly shaped macroconidia on SNA after 7 to 10 days of incubation. As expected the different *Fusarium* species showed differences in morphological and cultural characteristics on PDA. There were also differences in cultural and morphological features within isolates of same species in some cases. However isolates within some species including *Fusarium culmorum* showed almost similar morphological and cultural characteristics. Almost all isolates of *Fusarium culmorum* were fast growing, produced abundant mycelium which completely covered the Petri dish in one week, formed carmine red pigment on PDA and produced very uniform thick walled macroconidia on SNA. The longest (72.5 µm) and shortest (15 µm) macroconidia were produced by isolates of *F. avenaceum* and *F. solani,* respectively. Isolates of *F. oxysporum*, *F. proliferatum* and *F. tricinctum* produced the thinnest macroconidia (with width of 2.5 µm) whereas the thickest macroconidia which had width of 10 µm were produced by *F. culmorum*.

Growth rate on PDA is a useful secondary character used in *Fusarium* species identification (Burgess *et al.,* 1994; Summerell *et al.,* 2003; Leslie and Summerell, 2006). Isolates of *F. culmorum, F. graminearum* and *F. pseudograminearum* were relatively fast growing and produced abundant mycelium which completely covered the Petri dish in seven days. Most of the isolates of these three species had similar growth rates. Slow growing *Fusarium* species included *F. flocciferum*, *F. reticulatum* and *F. torulosum. Fusarium equiseti* isoates varied widely in their growth rate. *Fusarium avenaceum* and *F. acuminatum* isolates showed almost similar growth patterns

exhibiting slow to moderate growth. Isolates of *F. hostae* and *F. redolens* had almost similar growth rate ranges of 20-36 mm and 20-32 mm, respectively. The growth rates of most of the *Fusarium* species studied in this experiment lied within the range of values of growth rate of the species reported in Leslie and Summerell (2006).

Although use of morphological features is the widely used method for species identification, it is time consuming and difficult in some *Fusarium* species. For these reasons molecular identification might be required to accurately identify species types within short period of time. In this study, 95% of the isolates produced TEF sequences ranging from 400 to 668 bp. However, the remaining 5% of the isolates produced sequences < 400 bp. The longest amplicon (668 bp) was produced by *F. solani* isolate. The findings of the present research is congruent with the reports of Geiser *et al.* (2004) in FUSARIUM-ID which states the possibility of amplification of ~700 bp TEF gene region using primers ef1 and ef2. Similarly, Wulff *et al*. (2010) used the genetic variability in the TEF gene (~700 bp) to identify *Gibberella fujikuroi* species complex associated with rice Bakanae disease. Rahjoo *et al*. (2008) amplified the TEF gene region of *Fusarium* isolates obtained from maize ears in Iran using primers ef1 and ef2 in which the sequence analysis confirmed the identity of *Fusarium* species which were morphologically similar. In a study carried out in Argentina, sequence analysis from amplification of the TEF1-α gene region using primers ef1 and ef2 enabled the researchers to detect the main toxigenic *Fusarium* species associated with cereal grains (Sampietro *et al*., 2010).

Our study which identified 17 *Fusarium* species from crowns/stem bases of wheat is the second nationwide survey after Tunali *et al*. (2008), which reported more than 20 *Fusarium* species from roots and crowns of wheat in Turkey. Bentley *et al.* (2006a) also reported 16 *Fusarium* species from wheat in Northern Turkey. In our research, the highest number of *Fusarium* isolates (113) was obtained from Southeast Anatolia region while only few isolates were obtained from Mediterranean and Eastern Anatolia regions. This is attributed to the limited number of samples collected from Mediterranean and Eastern Anatolia regions.

The finding of our study which indicated *F. equiseti* as the most predominant *Fusarium* species associated with crown rot of wheat agrees with previous reports from Turkey (Bentley *et al.,* 2006a) and Western Canada (Fernandez *et al.,* 2014). *Fusarium equiseti* has also been reported among the most dominant *Fusarium* species associated with root and crown rot of wheat in North West Italy (Rossi *et al.,* 1995), and Mississippi, USA (Gonzalez and Trevathan, 2000). In a study carried out in Syria *F. equiseti* was reported to be among the most frequently isolated *Fusarium* spp. from wheat kernels (Alkadri *et al*., 2013). *Fusarium equiseti* has been reported as wide spread and non pathogenic *Fusarium* species (Summerell *et al.,* 2003; Leslie and Summerell, 2006). In the present research *F. equiseti* was found to be non pathogenic on susceptible durum wheat seedlings. In contrast to the present finding, Gonzalez and Trevathan (2000), Demirci and Dane (2003) and Fernandez and Chen (2005) reported *F. equiseti* as a pathogen on wheat.

In this study*, F. culmorum* was the most predominant among the damaging *Fusarium* species. However *F. pseudograminearum* and *F. graminearum* were isolated only at very low frequencies. Our finding is congruent with the study of Tunali *et al.* (2008) which reported *F. culmorum* and *F. pseudograminearum* as the most and less predominant pathogenic species on wheat in Turkey, respectively. *Fusarium culmorum* has also been reported as the most frequently isolated crown rot pathogen in Central Anatolia Plateau and it comprised 23.88% of the isolates (Aktaş *et al.,* 1999). Uçkun *et al*. (2004) also reported *Fusarium culmorum* as the most important crown rot pathogen in İzmir, Denizli and Aydın Province of Turkey. Similar reports have been documented from North West Italy (Rossi *et al.,* 1995), Norway (Kosiak *et al*., 2003), New Zealand (Bentley *et al.,* 2006b). Alkadri *et al*. (2013) reported *F. culmorum* among the most frequently isolated *Fusarium* species from wheat kernels in Syria. However, *F. pseudograminearum* was the most predominant Fusarium crown rot pathogen in the northern areas of the wheat belt in New South Wales of Australia (Klein *et al*., 1990) and Oregon and Washington of North West Pacific of USA (Smiley and Patterson, 1996). In a study conducted in eastern Australian grain belt, *F. pseudograminearum* and *F. culmorum* were among the most common *Fusarium* species isolated from wheat or barley while *F. graminearum* was among the less frequently isolated species

(Backhouse *et al.,* 2004). In our study, the crown rot pathogens *F. pseudograminearum* and *F. graminearum* were detected at very low levels and at present, they are unlikely to limit cereal production in the surveyed areas of Turkey. The greater pathogenicity of *F. culmorum, F. pseudograminearum* and *F. graminearum* on wheat seedlings than that of the other *Fusarium* species tested in this study agrees with previous reports from Turkey (Demirci and Dane, 2003; Tunali *et al.,* 2006) and USA (Smiley and Patterson, 1996). Similarly Fernandez and Chen (2005) reported *F. culmorum* and *F. graminearum* as more pathogenic species on wheat than other *Fusarium* species tested.

Fusarium hostae is closely related to *F. redolens* (Geiser *et al*., 2001). *Fusarium hostae* was first isolated from *Hosta* sp. in the USA (Geiser *et al*., 2001). It has been reported on *Hyacinthus* sp. in the Netherlands (Baayen *et al*., 2001). Our study is the first report of *F. redolens* and *F. hostae* causing crown rot on wheat in Turkey (Gebremariam *et al*., 2015a, 2015b). These two species were isolated from the four (Aegean, Southeast Anatolia, Black Sea, Central Anatolia) regions surveyed. This suggests the two species look widely spread in the major wheat growing areas of Turkey. *Fusarium redolens* has been reported to cause crown rot on durum wheat in Saskatchewan, Canada (Taheri *et al*., 2011), Fusarium yellows on chickpea (*Cicer arietinum*) in Spain (Jimenez-Fernandez *et al*., 2011) and rot of onions (*Allium cepa*) in Turkey (Bayraktar and Dolar, 2011). In our study, *F. hostae* was more pathogenic (with mean score of 2.2) on durum wheat compared to *F. redolens* (with mean score of 1.7). Similarly Geiser *et al*. (2001) reported the greater pathogenicity of *F. hostae* on hostas plants than *F. redolens.*

The result of pathogenicity test revealed that isolates of *F. avenaceum* were moderately pathogenic on durum wheat seedlings. Similar with the present finding, intermediate pathogenic capability of *F. avenaceum* on wheat has been documented (Fernandez and Chen, 2005). Smiley and Patterson (1996) also reported *F. avenaceum* isolates capable of killing wheat seedlings in the greenhouse. However, in contrast to our results, Arseniuk *et al*. (1993) reported *F. avenaceum* as, or more, pathogenic to winter wheat seedlings than *F. culmorum* and *F. graminearum.*

In our study, *F. acuminatum* was the second most predominant *Fusarium* species next to *F. equiset*. Similarly *F. acuminatum* has been reported to be among the most dominant *Fusarium* species associated with root and crown rot of wheat in Mississippi, USA (Gonzalez and Trevathan, 2000) and in Western Canada (Fernandez *et al.,* 2014). In our study, most of the isolates of *F. acuminatum* did not cause crown rot, although the species included some isolates capable of causing brown discoloration in the crown/stem bases of wheat. *Fusarium acuminatum* was thus considered non pathogenic in our finding. *Fusarium acuminatum* isolates capable of killing wheat seedlings in the greenhouse have been documented (Smiley and Patterson, 1996).

In this study, *F. oxysporum* and *F. solani* were non pathogenic. However, Demirci and Dane (2003) reported *F. oxysporum* and *F. solani* as weakly pathogenic species on winter wheat. Gonzalez and Trevathan (2000) also reported isolates of *F. solani* capable of causing slight to moderate discoloration of crown and seminal roots in test tube experiments. *Fusarium oxysporum* isolates capable of killing wheat seedlings in the greenhouse have also been documented (Smiley and Patterson, 1996).

Out of the 17 *Fusarium* species tested for pathogenicity, three were weak to moderately pathogenic and 11 were non pathogenic. The present finding supports the results of earlier studies which reported relatively high levels of weak, secondary pathogens, or non pathogenic species of *Fusarium* on wheat in Turkey (Demirci and Dane, 2003; Bentley *et al.,* 2006a; Akgül and Erkılıç, 2007; Tunali *et al.,* 2008). Similar situation have been reported in wheat producing areas in the Pacific Northwest of the USA (Smiley and Patterson, 1996). It can be seen from the findings of our study that the aetiology of crown rot pathogens in the surveyed areas of Turkey is complex. Differences in presence and prevalence of *Fusarium* spp. in the different agro-ecological regions surveyed might be due to differences in environmental conditions, agronomic practices, genetic resistance in wheat cultivars to the different *Fusarium* species and/or limited isolation of *Fusarium* isolates.

Screening for resistance to Fusarium crown rot of wheat was started in Australia in 1960s (McKnight and Hart, 1966; Purss, 1966). Although most varieties are susceptible, partial resistance to crown rot occurs in wheat. Screening for resistance and susceptibility can be carried out using seedling and adult plant tests and positive correlation between crown rot ratings in greenhouse and field trials have been documented (Klein *et al.*, 1985; Wildermuth and McNamara, 1994; Mitter *et al.*, 2006; Li *et al.*, 2008). Therefore, seedling bioassay which is time saving and avoids effects of other seasonal or environmental factors can be used to screen large quantities of germplasms rapidly and promising materials can be taken to field testing. Wheat varieties vary in their reaction to crown rot, ranging from very susceptible to moderately resistant (Wallwork, 2000). However, there are no fully resistant wheat cultivars to this disease (Pereyra *et al.*, 2004; Wisniewska and Kowalczyk, 2005). The genotypes tested in our study showed differences in reaction ranging from resistant (R) to susceptible (S) to *Fusarium culmorum* (Fc2). Two lines (147, 158) were resistant in their reaction and had scores of 1.4. Twenty lines (5, 100, 143, 163, 32, 138, 86, 89, 104, 123, 153, 161, 8, 34, 142, 9, 15, 47, 116, 146) were moderately resistant and had scores ranging from 1.6 to 2.4. These 22 lines had scores ranging from 1.4 to 2.4 which were not significantly different from the scores of moderately resistant control cultivar Suntop (1.6), Carisma (1.8) and Altay-2000 (2.4). Thirteen percent of the lines tested showed consistently resistant/ moderately resistant (R/MR) reaction to *Fusarium culmorum* isolate Fc2. Differences in reaction ranging from moderately resistant (MR) to susceptible (S) in wheat genotypes against *Fusarium culmorum* have also been reported from Turkey (Demirci, 2003). The search for resistance initially should focus upon only one species and expand later to include other species (Paulitz *et al.*, 2002; Miedaner *et al.*, 2012). For crown rot a high correlation between the resistance to *F. graminearum* and *F. culmorum* in wheat and rye has been documented (Miedaner, 1997). Therefore the wheat lines that showed some degree of resistance to *Fusarium culmorum* in our research can serve as useful sources of genetic resistance in breeding for *Fusarium culmorum* in particular or can be expanded and used for search for resistance to other *Fusarium* species. These lines can also be used to reduce yield losses due to Fusarium crown rot and carryover of inoculum to the subsequent years.

Conclusions

A wide range of *Fusarium* species associated with wheat crown rot exist in major wheat growing regions of Turkey and the spread of the species is variable among these regions. *Fusarium equiseti* is the most ubiquitous species of *Fusarium* in wheat growing regions of Turkey. Among damaging *Fusarium* species, *F. culmorum* is widely spread and is relatively detectable in higher frequencies in most wheat growing regions of Turkey.

It is clearly seen that *Fusarium culmorum*, *F. graminearum* and *F. pseudograminearum* are the three most important pathogenic species of *Fusarium* in seedling test with durum wheat cultivar Kızıltan-91. However, *F. graminearum* and *F. pseudograminearum* were isolated in very low frequencies, thus they are unlikely to limit wheat production in Turkey. Although many other *Fusarium* species have been isolated in this research, their importance as crown rot pathogen seem to be limited.

Fusarium culmorum isolates differed in their aggressiveness on the susceptible durum wheat cultivar Kızıltan-91.

Thirteen percent of the lines tested showed promising and consistently resistant/moderately resistant reaction to *Fusarium culmorum*.

Recommendations

The lines that showed consistentely resistant/moderatly resistant (R/MR) reactions can serve as useful sources of genetic resistance in breeding for Fusarium crown rot. Plant breeders attempting to incorporate resistance to crown rot into cereal crops in Turkey should focus on screening with *Fusarium culmorum* isolates.

In areas where the damaging *Fusarium culmorum* is prevalent, integrated management options should include crop rotation with at least 2 years break from wheat, use of varieties showing some degree of resistance to the disease, selecting proper nitrogen fertilization rates and irrigation management to maintain continuous moisture throughout the growing season.

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APPENDIX

Appendix 1. Summary of survey information for the 342 Fusarium species isolates collected from wheat in Turkey during summer 2013

Species	Isolate Code	Location	Elevation ft	Address	Region
F. culmorum	**Fc1**	38º33'826"N, 27º2'701"E	38	İzmir (Ege TAE)	Aegean
	Fc2	38º33'826"N, 27º2'701"E	38	İzmir (Ege TAE)	Aegean
	Fc3	38º33'826"N, 27º2'701"E	38	İzmir (Ege TAE)	Aegean
	Fc4	38º33'826"N, 27º2'701"E	38	İzmir (Ege TAE)	Aegean
	Fc5	39º7'70"N, 27º13'728"E	82	Bergama	Aegean
	Fc6	38º49'995"N, 27º42'685"E	276	Saruhanlı	Aegean
	Fc7	38º49'995"N, 27º42'685"E	276	Saruhanlı	Aegean
	Fc8	38º6'93"N, 27º24'485"E	74	Pamukyazı	Aegean
	Fc9	38º6'93"N, 27º24'485"E	74	Pamukyazı	Aegean
	Fc10	38º6'93"N, 27º24'485"E	74	Pamukyazı	Aegean
	Fc12	37º58'784"N, 27º24'6"E	31	Pamukyazı	Aegean
	Fc13	37º51'652"N, 27º41'135"E	206	Germencik	Aegean
	Fc14	37º21'641"N, 39º26'950"E	2240	Urfa, Viranşehir-Siverek road	Southeast Anatolia
	Fc15	37º21'641"N, 39º26'950"E	2240	Urfa, Viranşehir-Siverek road	Southeast Anatolia
	Fc16	37º44'547"N, 38º18'731"E	1935	Adıyaman	Southeast Anatolia
	Fc17	37º44'547"N, 38º18'731"E	1935	Adıyaman	Southeast Anatolia
	Fc18	37º44'547"N, 38º18'731"E	1935	Adıyaman	Southeast Anatolia
	Fc19	37º44'547"N, 38º18'731"E	1935	Adıyaman	Southeast Anatolia

Appendix 1. Continued.

Fc20	37º44'547"N, 38º18'731"E	1935	Adıyaman	Southeast Anatolia
Fc21	37º44'547"N, 38º18'731"E	1935	Adıyaman	Southeast Anatolia
Fc22	40º47'208"N, 35º27'263"E	1789	Çorum	Black Sea
Fc23	40º47'208"N, 35º27'263"E	1789	Çorum	Black Sea
Fc24	40º51'510"N, 35º28'685"E	2157	Merzifon	Black Sea
Fc25	41º1'958"N, 35º33'28"E	2114	Havza	Black Sea
Fc26	41º37'124"N, 35º35'144"E	23	Yakakent	Black Sea
Fc27	41º34'641"N, 35º51'215"E	80	Yakakent	Black Sea
Fc28	41º24'721"N, 36º10'512"E	117	Bafra-Samsun	Black Sea
Fc29	40º53'982"N,35º50'385"E	3083	Samsun	Black Sea
Fc30	40º50'785"N,45º35'42"E	1500	Suluova-Amasya	Black Sea
Fc31	40º50'785"N,45º35'42"E	1500	Suluova-Amasya	Black Sea
Fc32	40º32'943"N,35º40'555"E	1348	Göynücek	Black Sea
Fc34	37º42'783"N, 33º23'769"E	3295	Karapınar-Konya	Central Anatolia
Fc35	-		Alpu-Eskişehir	Central Anatolia
Fc36	-		Alpu-Eskişehir	Central Anatolia
Fc37	-		Alpu-Eskişehir	Central Anatolia
Fc38	-		Yusuflar-Eskişehir	Central Anatolia
Fc39	-		Konuklar-Tim-Konya	Central Anatolia
Fc40	-		Ankara Polatlı	Central Anatolia
Fc41	-		Ankara Polatlı	Central Anatolia
Fc42	-		Ankara Polatlı	Central Anatolia
Fc43	-		Ankara Polatlı	Central Anatolia
Fc44	39º39'709"N, 34º25'515"E	2572	ILCI, Yozgat (High area)	Central Anatolia

Appendix 1. Continued.

	Fc45	39º39'709"N, 34º25'515"E	2572	ILCI, Yozgat (High area)	Central Anatolia
	Fc46	39º39'709"N, 34º25'515"E	2572	ILCI, Yozgat (High area)	Central Anatolia
	Fc47	39º39'709"N, 34º25'515"E	2572	ILCI, Yozgat (High area)	Central Anatolia
	Fc48	39º39'60"N, 34º25'874"E	2617	ILCI, Yozgat (Low area)	Central Anatolia
F.pseudograminearum	**Fpg1**	39º39'60"N, 34º25'874"E	2617	ILCI, Yozgat (Low area)	Central Anatolia
	Fpg2	39º39'60"N, 34º25'874"E	2617	ILCI, Yozgat (Low area)	Central Anatolia
	Fpg3	39º40'903"N, 34º23'901"E	2463	Yerköy, Yozgat	Central Anatolia
F. graminearum	**Fg1**	41º10'845"N, 35º18'778"E	790	Vezirköprü	Black Sea
F. equiseti	**Feq1**	38º30'669"N, 28º18'740"E	495	Mersinli-Salihli	Aegean
	Feq2	38º30'669"N, 28º18'740"E	495	Mersinli-Salihli	Aegean
	Feq3	38º30'669"N, 28º18'740"E	495	Mersinli-Salihli	Aegean
	Feq4	38º33'826"N, 27º2'701"E	38	İzmir (Ege TAE)	Aegean
	Feq5	38º34'152"N, 27º2'202"E	33	İzmir (Ege TAE)	Aegean
	Feq6	38º34'152"N, 27º2'202"E	33	İzmir (Ege TAE)	Aegean
	Feq7	38º34'152"N, 27º2'202"E	33	İzmir (Ege TAE)	Aegean
	Feq8	38º34'152"N, 27º2'202"E	33	İzmir (Ege TAE)	Aegean
	Feq9	38º42'399"N, 26º59'537"E	88	Menemen	Aegean
	Feq10	38º42'399"N, 26º59'537"E	88	Menemen	Aegean
	Feq11	38º42'399"N, 26º59'537"E	88	Menemen	Aegean
	Feq12	38º59'579"N, 27º3'375"E	69	Aliağa	Aegean
	Feq13	38º59'579"N, 27º3'375"E	69	Aliağa	Aegean
	Feq14	39º4'824"N, 27º7'109"E	80	Bergama	Aegean
	Feq15	39º4'824"N, 27º7'109"E	80	Bergama	Aegean
	Feq16	39º4'824"N, 27º7'109"E	80	Bergama	Aegean

Appendix 1. Continued.

Feq17	39º4'824"N, 27º7'109"E	80	Bergama	Aegean
Feq18	39º6'432"N, 27º24'663"E	160	Kınık-İzmir	Aegean
Feq19	39º6'432"N, 27º24'663"E	160	Kınık-İzmir	Aegean
Feq20	39º6'432"N, 27º24'663"E	160	Kınık-İzmir	Aegean
Feq21	39º7'212"N, 27º26'554"E	245	Kınık-İzmir	Aegean
Feq22	38º57'408"N, 27º48'880"E	518	Soma	Aegean
Feq23	38º57'408"N, 27º48'880"E	518	Soma	Aegean
Feq24	38º49'995"N, 27º42'685"E	276	Saruhanlı	Aegean
Feq25	38º49'995"N, 27º42'685"E	276	Saruhanlı	Aegean
Feq26	38º13'756"N, 27º17'987"E	268	Ayrancılar	Aegean
Feq27	38º6'93"N, 27º24'485"E	74	Pamukyazı	Aegean
Feq28	38º6'93"N, 27º24'485"E	74	Pamukyazı	Aegean
Feq29	37º52'582"N, 27º33'418"E	200	Kızılcapınar-Aydın	Aegean
Feq30	37º54'190"N, 28º32'743"E	335	Pamukören	Aegean
Feq31	37º54'190"N, 28º32'743"E	335	Pamukören	Aegean
Feq32	37º54'190"N, 28º32'743"E	335	Pamukören	Aegean
Feq33	37º54'190"N, 28º32'743"E	335	Pamukören	Aegean
Feq34	37º52'745"N, 29º1'543"E	703	Denizli-Ankara road	Aegean
Feq35	37º52'745"N, 29º1'543"E	703	Denizli-Ankara road	Aegean
Feq36	37º52'745"N, 29º1'543"E	703	Denizli-Ankara road	Aegean
Feq37	37º48'426"N, 40º23'952"E	1867	Diyarbakır, Yuvacık	Southeast Anatolia
Feq38	37º10'20"N, 40º36'344"E	1905	Mardin, Kızıltepe-Şenyurt road	Southeast Anatolia
Feq39	37º7'241"N, 40º38'590"E	1638	Mardin, Kızıltepe	Southeast Anatolia
Feq40	37º6'915"N, 40º40'226"E	1554	Kızıltepe, Şenyurt road	Southeast Anatolia

Appendix 1. Continued.

Feq41	37º6'915"N, 40º40'226"E	1554	Kızıltepe, Şenyurt road	Southeast Anatolia
Feq42	37º6'915"N, 40º40'226"E	1554	Kızıltepe, Şenyurt road	Southeast Anatolia
Feq43	37º6'915"N, 40º40'226"E	1554	Kızıltepe, Şenyurt road	Southeast Anatolia
Feq44	37º3'495"N, 40º18'456"E	1506	Kızıltepe-Ceylanpınar road	Southeast Anatolia
Feq45	37º3'495"N, 40º18'456"E	1506	Kızıltepe-Ceylanpınar road	Southeast Anatolia
Feq46	37º3'495"N, 40º18'456"E	1509	Kızıltepe-Ceylanpınar road	Southeast Anatolia
Feq47	37º3'495"N, 40º18'456"E	1509	Kızıltepe-Ceylanpınar road	Southeast Anatolia
Feq48	36º55'184"N, 40º3'786"E	1334	Kızıltepe-Ceylanpınar road	Southeast Anatolia
Feq49	36º50'687"N, 40º0'167"E	1187	Urfa, Ceylanpınar-TIGEM	Southeast Anatolia
Feq50	36º50'687"N, 40º0'167"E	1187	Urfa, Ceylanpınar-TIGEM	Southeast Anatolia
Feq51	36º52'892"N, 39º56'215"E	1366	Urfa, Ceylanpınar-TIGEM	Southeast Anatolia
Feq52	36º52'892"N, 39º56'215"E	1366	Urfa, Ceylanpınar-TIGEM	Southeast Anatolia
Feq53	37º1'975"N, 39º56'10"E	1322	Urfa, Ceylanpınar-Viranşehir road	Southeast Anatolia
Feq54	37º1'975"N, 39º56'10"E	1322	Urfa, Ceylanpınar-Viranşehir road	Southeast Anatolia
Feq55	37º1'975"N, 39º56'10"E	1322	Urfa, Ceylanpınar-Viranşehir road	Southeast Anatolia
Feq56	37º1'975"N, 39º56'10"E	1322	Urfa, Ceylanpınar-Viranşehir road	Southeast Anatolia
Feq57	37º1'975"N, 39º56'10"E	1322	Urfa, Ceylanpınar-Viranşehir road	Southeast Anatolia
Feq58	37º7'933"N, 39º50'232"E	1584	Urfa, Ceylanpınar-Viranşehir road	Southeast Anatolia
Feq59	37º13'283"N, 39º30'859"E	1975	Urfa, Viranşehir-Siverek road	Southeast Anatolia
Feq60	37º13'283"N, 39º30'859"E	1975	Urfa, Viranşehir-Siverek road	Southeast Anatolia
Feq61	37º13'283"N, 39º30'859"E	1986	Urfa, Viranşehir-Siverek road	Southeast Anatolia
Feq62	37º53'738"N, 39º58'682"E	2785	Diyarbakır	Southeast Anatolia
Feq63	37º40'803"N, 39º15'66"E	2326	Adıyaman	Southeast Anatolia
Feq64	37º38'254"N, 39º12'174"E	2100	Adıyaman	Southeast Anatolia

Appendix 1. Continued.

Feq65	37º38'254"N, 39º12'174"E	2100	Adıyaman	Southeast Anatolia
Feq66	37º36'393"N, 39º2'646"E	2003	Adıyaman	Southeast Anatolia
Feq67	37º36'393"N, 39º2'646"E	2003	Adıyaman	Southeast Anatolia
Feq68	37º36'393"N, 39º2'646"E	2003	Adıyaman	Southeast Anatolia
Feq69	37º36'393"N, 39º2'646"E	2003	Adıyaman	Southeast Anatolia
Feq70	37º26'961"N, 38º19'760"E	1578	Adıyaman	Southeast Anatolia
Feq71	37º26'961"N, 38º19'760"E	1578	Adıyaman	Southeast Anatolia
Feq72	37º26'961"N, 38º19'760"E	1578	Adıyaman	Southeast Anatolia
Feq73	37º26'961"N, 38º19'760"E	1578	Adıyaman	Southeast Anatolia
Feq74	37º33'546"N, 38º12'888"E	2001	Adıyaman	Southeast Anatolia
Feq75	37º33'546"N, 38º12'888"E	2001	Adıyaman	Southeast Anatolia
Feq76	37º33'546"N, 38º12'888"E	2001	Adıyaman	Southeast Anatolia
Feq77	37º39'624"N, 38º10'497"E	2045	Adıyaman	Southeast Anatolia
Feq78	37º44'547"N, 38º18'731"E	1935	Adıyaman	Southeast Anatolia
Feq79	37º44'547"N, 38º18'731"E	1935	Adıyaman	Southeast Anatolia
Feq80	37º42'960"N, 38º21'232"E	1882	Adıyaman	Southeast Anatolia
Feq81	37º42'960"N, 38º21'232"E	1882	Adıyaman	Southeast Anatolia
Feq82	40º51'510"N, 35º28'685"E	2157	Merzifon	Black Sea
Feq83	41º27'144"N, 34º553'911"E	833	Durağan	Black Sea
Feq84	41º32'403"N, 34º46'683"E	1155	Boyabat	Black Sea
Feq85	41º49'240"N, 35º4'378"E	548	Gerze, Sinop	Black Sea
Feq86	41º34'641"N, 35º51'215"E	80	Yakakent	Black Sea

Appendix 1. Continued.

Feq87	40º53'702"N,35º48'791"E	3037	Samsun	Black Sea
Feq88	40º53'402"N,35º45'463"E	3155	Samsun	Black Sea
Feq89	40º52'727"N,35º41'460"E	2705	Samsun	Black Sea
Feq90	40º52'727"N,35º41'460"E	2706	Samsun	Black Sea
Feq91	40º52'727"N,35º41'460"E	2706	Samsun	Black Sea
Feq92	40º50'740"N,35º40'272"E	2088	Samsun	Black Sea
Feq93	40º34'162"N,35º44'92"E	1312	Göynücek, Amasya	Black Sea
Feq94	40º16'638"N,35º16'867"E	2276	Göynücek	Black Sea
Feq95	40º16'638"N,35º16'867"E	2276	Göynücek	Black Sea
Feq96	40º12'56"N, 34º54'187"E	3129	Akpınar	Black Sea
Feq97	40º7'661"N, 34º54'654"E	3096	Alaca	Central Anatolia
Feq98	39º41'521"N, 34º37'458"E	3145	Yerköy, Yozgat	Central Anatolia
Feq99	39º39'60"N, 34º25'874"E	2617	ILCI, Yozgat (Low area)	Central Anatolia
Feq100	39º39'709"N, 34º25'515"E	2572	ILCI, Yozgat (High area)	Central Anatolia
Feq101	39º43'539"N, 34º17'440"E	2402	Yerköy	Central Anatolia
Feq102	39º4'710"N, 34º17'387"E	3850	Kırşehir	Central Anatolia
Feq103	38º18'279"N, 34º45'329"E	4330	Nevşehir	Central Anatolia
Feq104	38º18'279"N, 34º45'329"E	4330	Nevşehir	Central Anatolia
Feq105	38º18'279"N, 34º45'329"E	4330	Nevşehir	Central Anatolia
Feq106	37º47'340"N, 34º34'749"E	3561	Kemerhisar	Central Anatolia
Feq107	37º37'198"N, 32º37'677"E	3344	İçeri Çumra	Central Anatolia
Feq108	37º37'198"N, 32º37'677"E	3344	İçeri Çumra	Central Anatolia
Feq109	37º37'198"N, 32º37'677"E	3344	İçeri Çumra	Central Anatolia

Appendix 1. Continued.

	Feq110	38º22'179"N, 32º47'297"E	3144	Konya	Central Anatolia
	Feq111	38º50'376'281'N, 32º57'184"E	3006	Konya-Ankara	Central Anatolia
	Feq112	38º50'376'281'N, 32º57'184"E	3006	Konya-Ankara	Central Anatolia
	Feq113	38º50'376'281'N, 32º57'184"E	3006	Konya-Ankara	Central Anatolia
	Feq114	-		Alpu-Eskişehir	Central Anatolia
	Feq115	-		Alpu-Eskişehir	Central Anatolia
	Feq116	-		Yusuflar-Eskişehir	Central Anatolia
	Feq117	-		Yusuflar-Eskişehir	Central Anatolia
	Feq118	-		Yusuflar-Eskişehir	Central Anatolia
	Feq119	-		Kuşçular village-Kastamonu	Black Sea
	Feq120	-		İçeri Çumra-Konya	Central Anatolia
	Feq121	-		Haymana	Central Anatolia
	Feq122	39º39'60"N, 34º25'874"E	2617	ILCI, Yozgat (Low area)	Central Anatolia
	Feq123	39º39'60"N, 34º25'874"E	2617	ILCI, Yozgat (Low area)	Central Anatolia
F. acuminatum	**Fac1**	38º37'376"N, 28º55'544"E	1519	After Uşak	Aegean
	Fac2	38º37'376"N, 28º55'544"E	1519	After Uşak	Aegean
	Fac3	38º31'602"N, 28º23'208"E	1231	Mersinli entrance	Aegean
	Fac4	38º34'152"N, 27º2'202"E	33	İzmir (Ege TAE)	Aegean
	Fac5	39º4'824"N, 27º7'109"E	80	Bergama	Aegean
	Fac6	39º4'824"N, 27º7'109"E	80	Bergama	Aegean
	Fac7	39º4'824"N, 27º7'109"E	80	Bergama	Aegean
	Fac8	39º7'70"N, 27º13'728"E	82	Bergama	Aegean
	Fac9	38º57'408"N, 27º48'880"E	518	Soma	Aegean
	Fac10	37º52'582"N, 27º33'418"E	200	Kızılcapınar-Aydın	Aegean

Appendix 1. Continued.

Fac11	37º52'582"N, 27º33'418"E	200	Kızılcapınar-Aydın	Aegean
Fac12	37º57'146"N, 28º53'745"E	493	Sarayköy-Denizli	Aegean
Fac13	37º50'927"N, 29º4'660"E	953	Denizli-Ankara road	Aegean
Fac14	37º50'927"N, 29º4'660"E	953	Denizli-Ankara road	Aegean
Fac15	37º7'241"N, 40º38'590"E	1638	Mardin, Kızıltepe	Southeast Anatolia
Fac16	37º8'80"N, 40º29'923"E	1479	Kızıltepe-Ceylanpınar road	Southeast Anatolia
Fac17	36º57'964"N, 40º12'366"E	1288	Kızıltepe-Ceylanpınar road	Southeast Anatolia
Fac18	36º55'184"N, 40º3'787"E	1325	Kızıltepe-Ceylanpınar road	Southeast Anatolia
Fac19	36º50'687"N, 40º0'167"E	1187	Urfa, Ceylanpınar-TIGEM	Southeast Anatolia
Fac20	36º50'687"N, 40º0'167"E	1187	Urfa, Ceylanpınar-TIGEM	Southeast Anatolia
Fac21	37º13'283"N, 39º30'859"E	1986	Urfa, Viranşehir-Siverek road	Southeast Anatolia
Fac22	37º33'546"N, 38º12'888"E	2001	Adıyaman	Southeast Anatolia
Fac23	37º42'960"N, 38º21'232"E	1882	Adıyaman	Southeast Anatolia
Fac24	37º42'960"N, 38º21'232"E	1882	Adıyaman	Southeast Anatolia
Fac25	40º47'208"N, 35º27'263"E	1789	Çorum	Black Sea
Fac26	41º49'240"N, 35º4'378"E	548	Gerze, Sinop	Black Sea
Fac27	41º3'540"N, 35º58'296"E	1990	Samsun	Black Sea
Fac28	41º3'540"N, 35º58'296"E	1990	Samsun	Black Sea
Fac29	40º53'402"N,35º45'463"E	3155	Samsun	Black Sea
Fac30	40º53'402"N,35º45'463"E	3155	Samsun	Black Sea
Fac31	40º52'727"N,35º41'460"E	2705	Samsun	Black Sea
Fac32	40º52'727"N,35º41'460"E	2706	Samsun	Black Sea
Fac33	40º50'740"N,35º40'272"E	2088	Samsun	Black Sea
Fac34	40º50'740"N,35º40'272"E	2088	Samsun	Black Sea

Appendix 1. Continued.

	Fac35	40º50'740"N,35º40'272"E	2088	Samsun	Black Sea
	Fac36	40º50'740"N,35º40'272"E	2088	Samsun	Black Sea
	Fac37	40º34'162"N,35º44'92"E	1312	Göynücek, Amasya	Black Sea
	Fac38	40º14'827"N, 34º58'656"E	3490	Akpınar village, Çorum	Black Sea
	Fac39	40º14'827"N, 34º58'656"E	3490	Akpınar village, Çorum	Black Sea
	Fac40	40º14'827"N, 34º58'656"E	3490	Akpınar village, Çorum	Black Sea
	Fac41	40º2'673"N, 34º56'40"E	3288	Alaca	Central Anatolia
	Fac42	37º9'13"N, 35º32'200"E	835	Adana	Mediterrenaian
	Fac43	37º9'13"N, 35º32'200"E	835	Adana	Mediterrenaian
	Fac44	37º51'149"N, 32º40'397"E	3283	Konya	Central Anatolia
	Fac45	37º51'149"N, 32º40'397"E	3283	Konya	Central Anatolia
	Fac46	37º11'945"N, 33º7'245"E	3414	Karaman-Kazımkarabekir	Central Anatolia
	Fac47	37º11'945"N, 33º7'245"E	3414	Karaman-Kazımkarabekir	Central Anatolia
	Fac48	-		Kastamonu	Black Sea
	Fac49	-		Haymana	Central Anatolia
	Fac50	39º39'709"N, 34º25'515"E	2572	ILCI, Yozgat (High area)	Central Anatolia
F. brachygibbosum	**Fb1**	38º37'376"N, 28º55'544"E	1519	After Uşak	Aegean
	Fb2	38º31'602"N, 28º23'208"E	1231	Mersinli entrance	Aegean
	Fb3	38º42'399"N, 26º59'537"E	88	Menemen	Aegean
	Fb4	38º59'579"N, 27º3'375"E	69	Aliağa	Aegean
	Fb5	39º4'824"N, 27º7'109"E	80	Bergama	Aegean
	Fb6	38º16'248"N, 27º10'541"E	361	Torbalı	Aegean
	Fb7	37º52'582"N, 27º33'418"E	200	Kızılcapınar-Aydın	Aegean
	Fb8	37º56'315"N, 40º17'236"E	2308	Diyarbakır-Silvan road	Southeast Anatolia

Appendix 1. Continued.

	Fb9	37º54'562"N, 40º38'174"E	2206	Diyarbakır, Silvan-Bismil	Southeast Anatolia
	Fb10	37º48'426"N, 40º23'952"E	1867	Diyarbakır, Yuvacık	Southeast Anatolia
	Fb11	37º48'426"N, 40º23'952"E	1867	Diyarbakır, Yuvacık	Southeast Anatolia
	Fb12	37º3'495"N, 40º18'456"E	1506	Kızıltepe-Ceylanpınar road	Southeast Anatolia
	Fb13	37º3'495"N, 40º18'456"E	1506	Kızıltepe-Ceylanpınar road	Southeast Anatolia
	Fb14	36º50'687"N, 40º0'167"E	1187	Urfa, Ceylanpınar-TIGEM	Southeast Anatolia
	Fb15	36º50'687"N, 40º0'167"E	1187	Urfa, Ceylanpınar-TIGEM	Southeast Anatolia
	Fb16	36º52'892"N, 39º56'215"E	1366	Urfa, Ceylanpınar-TIGEM	Southeast Anatolia
	Fb17	37º40'803"N, 39º15'66"E	2326	Adıyaman	Southeast Anatolia
	Fb18	37º38'254"N, 39º12'174"E	2100	Adıyaman	Southeast Anatolia
	Fb19	37º33'546"N, 38º12'888"E	2001	Adıyaman	Southeast Anatolia
	Fb20	37º39'624"N, 38º10'497"E	2045	Adıyaman	Southeast Anatolia
	Fb21	37º39'624"N, 38º10'497"E	2045	Adıyaman	Southeast Anatolia
	Fb22	37º42'960"N, 38º21'232"E	1882	Adıyaman	Southeast Anatolia
	Fb23	37º42'960"N, 38º21'232"E	1882	Adıyaman	Southeast Anatolia
	Fb24	37º42'960"N, 38º21'232"E	1882	Adıyaman	Southeast Anatolia
	Fb25	37º42'211"N, 38º20'901"E	1825	Adıyaman, (GAP Institute)	Southeast Anatolia
F. hostae	**Fh1**	38º30'669"N, 28º18'740"E	495	Mersinli	Aegean
	Fh2	38º29'665"N, 28º2'11"E	363	Salihli	Aegean
	Fh3	38º50'676"N, 27º0'809"E	42	Aliağa (İzmir)	Aegean
	Fh4	38º51'380"N, 27º44'8"E	300	Gölmarmara	Aegean
	Fh5	38º13'756"N, 27º17'987"E	268	Ayrancılar	Aegean
	Fh6	37º26'46"N, 38º21'917"E	1656	Adıyaman	Southeast Anatolia
	Fh7	37º42'960"N, 38º21'232"E	1882	Adıyaman	Southeast Anatolia

Appendix 1. Continued.

	Fh8	37º42'960"N, 38º21'232"E	1882	Adıyaman	Southeast Anatolia
	Fh9	37º42'960"N, 38º21'232"E	1882	Adıyaman	Southeast Anatolia
	Fh10	37º42'960"N, 38º21'232"E	1882	Adıyaman	Southeast Anatolia
	Fh11	37º42'960"N, 38º21'232"E	1882	Adıyaman	Southeast Anatolia
	Fh12	40º47'208"N, 35º27'263"E	1789	Çorum	Black Sea
	Fh13	41º10'845"N, 35º18'778"E	790	Vezirköprü	Black Sea
	Fh14	41º36'573"N, 35º41'682"E	56	Yakakent	Black Sea
	Fh15	41º8'762"N, 36º42'553"E	95	Tekkeköy	Black Sea
	Fh16		.	Samsun	Black Sea
	Fh17	41º3'540"N, 35º58'296"E	1990	Samsun	Black Sea
	Fh18	40º53'402"N,35º45'463"E	3155	Samsun	Black Sea
	Fh19	40º52'727"N,35º41'460"E	2706	Samsun	Black Sea
	Fh20	37º15'86"N, 33º3'800"E	3373	Mesudiye-Konya	Central Anatolia
F. redolens	**Fred1**	38º37'376"N, 28º55'544"E	1519	After Uşak	Aegean
	Fred2	38º37'376"N, 28º55'544"E	1519	After Uşak	Aegean
	Fred3	38º37'376"N, 28º55'544"E	1519	After Uşak	Aegean
	Fred4	38º37'376"N, 28º55'544"E	1519	Uşak	Aegean
	Fred5	38º31'602"N, 28º23'208"E	1231	Mersinli entrance	Aegean
	Fred6	40º2'349"N, 40º31'824"E	2426	Diyarbakır-Silvan road	Southeast Anatolia
	Fred7	37º6'915"N, 40º40'232"E	1523	Kızıltepe, Şenyurt	Southeast Anatolia
	Fred8	37º3'495"N, 40º18'456"E	1506	Kızıltepe-Ceylanpınar road	Southeast Anatolia
	Fred9	37º1'975"N, 39º56'10"E	1322	Urfa, Ceylanpınar-Viranşehir road	Southeast Anatolia
	Fred10	37º13'283"N, 39º30'859"E	1986	Urfa, Viranşehir-Siverek road	Southeast Anatolia
	Fred11	40º35'716"N, 35º3'470"E	3875	Çorum	Black Sea

Appendix 1. Continued.

	Fred12	40º53'402"N,35º45'463"E	3155	Samsun	Black Sea
	Fred13	40º52'727"N,35º41'460"E	2706	Samsun	Black Sea
	Fred14	39º20'147"N, 34º1'397"E	3933	Kırıkkale	Central Anatolia
	Fred15	37º29'987"N, 32º41'834"E	3380	İçeri Çumra	Central Anatolia
	Fred16	37º29'987"N, 32º41'834"E	3380	İçeri Çumra	Central Anatolia
	Fred17	37º11'945"N, 33º7'245"E	3414	Karaman-Kazımkarabekir	Central Anatolia
	Fred18	-		Domaniç-Kütahya	Aegean
	Fred19	-		Domaniç-Kütahya	Aegean
F. avenaceum	**Fav1**	38º59'579"N, 27º3'375"E	69	Aliağa	Aegean
	Fav2	38º16'838"N, 27º8'11"E	529	Gaziemir-İzmir	Aegean
	Fav3	37º52'582"N, 27º33'418"E	200	Kızılcapınar-Aydın	Aegean
	Fav4	37º52'582"N, 27º33'418"E	200	Kızılcapınar-Aydın	Aegean
	Fav5	-		Urfa	Southeast Anatolia
	Fav6	-		Urfa	Southeast Anatolia
	Fav7	-		Urfa	Southeast Anatolia
	Fav8	41º1'958"N, 35º33'28"E	2114	Havza	Black Sea
	Fav9	41º27'144"N, 34º553'911"E	833	Durağan	Black Sea
	Fav10	41º37'124"N, 35º35'144"E	23	Yakakent	Black Sea
	Fav11	41º37'124"N, 35º35'144"E	23	Yakakent	Black Sea
	Fav12	41º34'641"N, 35º51'215"E	80	Yakakent	Black Sea
F. oxysporum	**Fox1**	38º37'376"N, 28º55'544"E	1519	Uşak	Aegean
	Fox2	38º37'376"N, 28º55'544"E	1519	Uşak	Aegean
	Fox3	38º37'376"N, 28º55'544"E	1519	Uşak	Aegean
	Fox4	38º30'669"N, 28º18'740"E	495	Mersinli	Aegean

Appendix 1. Continued.

	Fox5	38°16'838"N, 27°8'11"E	529	Gaziemir-İzmir	Aegean
	Fox6	41°32'403"N, 34°46'683"E	1155	Boyabat	Black Sea
	Fox7	41°51'288"N, 35°6'867"E	439	Sinop	Black Sea
	Fox8	41°8'762"N, 36°42'553"E	95	Tekkeköy-Samsun	Black Sea
	Fox9	41°8'762"N, 36°42'553"E	95	Tekkeköy-Samsun	Black Sea
	Fox10	41°15'132"N, 36°46'309"E	31	Ömerli village-Samsun	Black Sea
	Fox11	39°43'539"N, 34°17'440"E	2402	Yerköy	Central Anatolia
	Fox12	38°22'179"N, 32°47'297"E	3144	Konya	Central Anatolia
F. torulosum	**Ftor1**	37°1'975"N, 39°56'10"E	1322	Urfa, Ceylanpınar-Viranşehir road	Southeast Anatolia
	Ftor2	37°53'738"N, 39°58'682"E	2785	Diyarbakır	Southeast Anatolia
	Ftor3	37°44'547"N, 38°18'731"E	1935	Adıyaman	Southeast Anatolia
	Ftor4	37°44'547"N, 38°18'731"E	1935	Adıyaman	Southeast Anatolia
	Ftor5	37°56'669"N, 40°15'115"E		Diyarbakır	Southeast Anatolia
	Ftor6	37°56'669"N, 40°15'115"E		Diyarbakır	Southeast Anatolia
	Ftor7	40°14'827"N, 34°58'656"E	3490	Akpınar village, Çorum	Black Sea
	Ftor8	-	.	Çerikli	Central Anatolia
	Ftor9	-		Digor-Kars	Eastern Anatolia
F. proliferatum	**Fpro1**	38°49'995"N, 27°42'685"E	276	Saruhanlı	Aegean
	Fpro2	37°8'80"N, 40°29'923"E	1479	Kızıltepe-Ceylanpınar road	Southeast Anatolia
	Fpro3	37°3'495"N, 40°18'456"E	1509	Kızıltepe-Ceylanpınar road	Southeast Anatolia
	Fpro4	36°50'687"N, 40°0'167"E	1187	Urfa, Ceylanpınar-TIGEM	Southeast Anatolia
	Fpro5	36°52'892"N, 39°56'215"E	1366	Urfa, Ceylanpınar-TIGEM	Southeast Anatolia
	Fpro6	37°13'283"N, 39°30'859"E	1975	Urfa, Viranşehir-Siverek road	Southeast Anatolia
	Fpro7	37°38'254"N, 39°12'174"E	2100	Adıyaman	Southeast Anatolia

Appendix 1. Continued.

	Fpro8	37º36'393"N, 39º2'646"E	2003	Adıyaman	Southeast Anatolia
F. flocciferum	**Fflo1**	38º51'380"N, 27º44'8"E	300	Gölmarmara	Aegean
	Fflo2	37º51'652"N, 27º41'135"E	206	Germencik-Aydın	Aegean
	Fflo3	37º58'937"N, 40º35'172"E	2309	Diyarbakır	Southeast Anatolia
	Fflo4	37º3'495"N, 40º18'456"E	1509	Kızıltepe-Ceylanpınar road	Southeast Anatolia
	Fflo5	40º51'510"N, 35º28'685"E	2157	Merzifon	Black Sea
	Fflo6	40º51'510"N, 35º28'685"E	2157	Merzifon	Black Sea
F. solani	**Fsol1**	38º33'826"N, 27º2'701"E	38	İzmir (Ege TAE)	Aegean
	Fsol2	37º38'950"N, 40º28'652"E	2540	Çınar-Mardin road	Southeast Anatolia
	Fsol3	40º20'88"N,35º23'877"E	1765	Göynücek	Black Sea
F. incarnatum	**Finc1**	37º8'80"N, 40º29'923"E	1479	Kızıltepe-Ceylanpınar road	Southeast Anatolia
	Finc2	37º8'80"N, 40º29'923"E	1479	Kızıltepe-Ceylanpınar road	Southeast Anatolia
	Finc3		.	Samsun	Black Sea
F. tricinctum	**Ftric1**	41º37'990"N, 35º31'282"E	130	Yakakent	Black Sea
F. reticulatum	**Fret1**	39º7'212"N, 27º26'554"E	245	Kınık-İzmir	Aegean

Appendix 2. Lists of wheat lines used for screening experiment and related information

15ENT	15 Nursery	14Nursery	14Entry	CROSS NAME
2	**15SWM**	34ESWYT	103	PRL/2*PASTOR
3	**15SWM**	34ESWYT	104	MUNAL #1
4	**15SWM**	34ESWYT	105	PFAU/SERI.1B//AMAD/3/WAXWING
5	**15SWM**	34ESWYT	106	SITE/MO//PASTOR/3/TILHI/4/WAXWING/KIRITATI
6	**15SWM**	34ESWYT	107	ATTILA*2/PBW65*2//KACHU
7	**15SWM**	34ESWYT	108	ROLF07/4/BOW/NKT//CBRD/3/CBRD/5/FRET2/TUKURU//FRET2
8	**15SWM**	34ESWYT	109	KACHU #1/4/CROC_1/AE.SQUARROSA(205)//BORL95/3/2*MILAN/5/KACHU
9	**15SWM**	34ESWYT	110	SAUAL/3/ACHTAR*3//KANZ/KS85-8-4/4/SAUAL
10	**15SWM**	34ESWYT	111	BECARD/KACHU
11	**15SWM**	34ESWYT	112	ALTAR 84/AE.SQUARROSA(221)//3*BORL95/3/URES/JUN//KAUZ/4/WBLL1/5/MILAN/S87230//BAV92
12	**15SWM**	34ESWYT	113	NAC/TH.AC//3*PVN/3/MIRLO/BUC/4/2*PASTOR/5/KACHU/6/KACHU
13	**15SWM**	34ESWYT	114	CHIBIA//PRLII/CM65531/3/SKAUZ/BAV92/4/MUNAL #1
14	**15SWM**	34ESWYT	115	KACHU//WBLL1*2/BRAMBLING
15	**15SWM**	34ESWYT	116	KACHU/KIRITATI
16	**15SWM**	34ESWYT	117	KACHU #1//WBLL1*2/KUKUNA
17	**15SWM**	34ESWYT	118	KIRITATI/WBLL1//FRANCOLIN #1
18	**15SWM**	34ESWYT	119	PFAU/SERI.1B//AMAD/3/WAXWING/4/BAJ #1
19	**15SWM**	34ESWYT	120	PFAU/SERI.1B//AMAD/3/WAXWING/4/WBLL1*2/BRAMBLING
20	**15SWM**	34ESWYT	121	PFAU/SERI.1B//AMAD/3/WAXWING/4/BECARD
21	**15SWM**	34ESWYT	122	BAJ #1/3/KIRITATI//ATTILA*2/PASTOR
22	**15SWM**	34ESWYT	123	WBLL4/KUKUNA//WBLL1/3/WBLL1*2/BRAMBLING
23	**15SWM**	34ESWYT	124	ITP40/AKURI
24	**15SWM**	34ESWYT	125	KIRITATI/WBLL1//MESIA/3/KIRITATI/WBLL1
25	**15SWM**	34ESWYT	126	KIRITATI/WBLL1/4/2*BABAX/LR42//BABAX*2/3/KURUKU
26	**15SWM**	34ESWYT	127	FRNCLN*2/TECUE #1
27	**15SWM**	34ESWYT	128	PFAU/SERI.1B//AMAD/3/WAXWING/4/AKURI/5/PFAU/SERI.1B//AMAD/3/WAXWING
28	**15SWM**	34ESWYT	129	MILAN/S87230//BAV92*2/3/TECUE #1
29	**15SWM**	34ESWYT	130	WBLL1*2/VIVITSI//AKURI/3/WBLL1*2/BRAMBLING
30	**15SWM**	34ESWYT	131	MILAN/S87230//BAV92*2/3/AKURI

Appendix 2 Continued.

31				
	15SWM	34ESWYT	132	BAJ #1*2/WHEAR
32	**15SWM**	34ESWYT	133	TACUPETO F2001*2/KIRITATI//VILLA JUAREZ F2009
33	**15SWM**	34ESWYT	134	KACHU/KINDE
34	**15SWM**	34ESWYT	135	PBW343*2/KUKUNA/3/PASTOR//CHIL/PRL/4/GRACK
35	**15SWM**	34ESWYT	136	VILLA JUAREZ F2009/CHYAK
36	**15SWM**	34ESWYT	137	WBLL1*2/BRAMBLING//QUAIU
37	**15SWM**	34ESWYT	138	BECARD/QUAIU #1
38	**15SWM**	34ESWYT	139	BECARD/QUAIU #1
39	**15SWM**	34ESWYT	140	BECARD/FRNCLN
40	**15SWM**	34ESWYT	141	WBLL1*2/BRAMBLING//CHYAK
41	**15SWM**	34ESWYT	142	BECARD//ND643/2*WBLL1
42	**15SWM**	34ESWYT	143	ATTILA/3*BCN*2//BAV92/3/KIRITATI/WBLL1/4/DANPHE
43	**15SWM**	34ESWYT	144	FRET2*2/BRAMBLING//BECARD/3/WBLL1*2/BRAMBLING
44	**15SWM**	34ESWYT	145	KAUZ*2/MNV//KAUZ/3/MILAN/4/BAV92/5/AKURI/6/MILAN/S87230//BAV92
45	**15SWM**	34ESWYT	146	KACHU/BECARD//WBLL1*2/BRAMBLING
46	**15SWM**	34ESWYT	147	KAUZ/PASTOR//PBW343/3/KIRITATI/4/FRNCLN
47	**15SWM**	34ESWYT	148	PFAU/SERI.1B//AMAD/3/WAXWING*2/4/TECUE #1
48	**15SWM**	34ESWYT	149	FRANCOLIN #1/AKURI #1//FRNCLN
49	**15SWM**	34ESWYT	150	ND643/2*TRCH/3/MILAN/S87230//BAV92/4/PFAU/SERI.1B//AMAD/3/WAXWING
51	**15SWM**	21HRWYT	202	VOROBEY
52	**15SWM**	21HRWYT	203	PROINTA FEDERAL
53	**15SWM**	21HRWYT	204	KLEIN CACIQUE
54	**15SWM**	21HRWYT	205	KENYA HEROE
55	**15SWM**	21HRWYT	206	REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA(213)//PGO/4/HUITES/5/T.DICOCCON PI94624/AE.SQUARROSA(409)//BCN/6/REH/HARE//2*BCN/3/CROC_1/AE.S
56	**15SWM**	21HRWYT	207	BABAX/LR42//BABAX*2/3/KURUKU/4/KINGBIRD #1
57	**15SWM**	21HRWYT	208	MUNAL//WBLL1*2/BRAMBLING
58	**15SWM**	21HRWYT	209	WBLL1*2/BRAMBLING//KINGBIRD #1
59	**15SWM**	21HRWYT	210	WBLL4/KUKUNA//WBLL1/3/WBLL1*2/BRAMBLING
60	**15SWM**	21HRWYT	211	ONIX/KBIRD
61	**15SWM**	21HRWYT	212	PFAU/WEAVER*2//TUKURU/4/BABAX/LR42//BABAX*2/3/KURUKU/5/QUAIU
62	**15SWM**	21HRWYT	213	VORB/FISCAL//WBLL1*2/KURUKU/3/QUAIU
63	**15SWM**	21HRWYT	214	MILAN/S87230//BAV92/3/WBLL1*2/BRAMBLING/4/WBLL1*2/BRAMBLING

Appendix 2 Continued.

64	**15SWM**	21HRWYT	215	MILAN/S87230//BAV92/3/WBLL1*2/BRAMBLING/4/WBLL1*2/BRAMBLING
65	**15SWM**	21HRWYT	216	TUKURU//BAV92/RAYON/3/WBLL1*2/BRAMBLING/4/WBLL1*2/BRAMBLING
66	**15SWM**	21HRWYT	217	WBLL1*2/BRAMBLING/5/WBLL1*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/6/WBLL1*2/BRAMBLING
67	**15SWM**	21HRWYT	218	PANDORA//WBLL1*2/BRAMBLING/3/WBLL1*2/BRAMBLING
68	**15SWM**	21HRWYT	219	BABAX/LR42//BABAX*2/3/KUKUNA/4/BACEU #1/5/BECARD
69	**15SWM**	21HRWYT	220	BABAX/LR42//BABAX*2/3/KUKUNA/4/CROSBILL #1/5/BECARD
70	**15SWM**	21HRWYT	221	BABAX/LR42//BABAX*2/3/KUKUNA/4/CROSBILL #1/5/BECARD
71	**15SWM**	21HRWYT	222	PFAU/MILAN//FISCAL/3/VORB/4/MILAN/S87230//BAV92
72	**15SWM**	21HRWYT	223	BABAX/LR42//BABAX*2/3/KURUKU/4/WBLL1*2/BRAMBLING
73	**15SWM**	21HRWYT	224	BECARD/3/PASTOR//MUNIA/ALTAR 84
74	**15SWM**	21HRWYT	225	PBW65/2*PASTOR/4/PFAU/SERI.1B//AMAD/3/WAXWING/5/CHYAK
75	**15SWM**	21HRWYT	226	REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA(213)//PGO/4/HUITES/5/T.DICOCCON PI94624/AE.SQUARROSA(409)//BCN/6/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA(213)//PGO/4/HUITES/7/MILAN/S87230//BAV92
76	**15SWM**	21HRWYT	227	MON/IMU//ALD/PVN/3/BORL95/4/OASIS/2*BORL95/5/SKAUZ/BAV92
77	**15SWM**	21HRWYT	228	1447/PASTOR//KRICHAUFF/3/VORB
78	**15SWM**	21HRWYT	229	CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA(TAUS)/4/WEAVER/5/2*JANZ/6/SERI*3//RL6010/4*YR/3/PASTOR/4/BAV92/7/VORB
79	**15SWM**	21HRWYT	230	VORB*2/5/CROC_1/AE.SQUARROSA(224)//OPATA/3/RAC655/4/SLVS/PASTOR
80	**15SWM**	21HRWYT	231	METSO/ER2000/3/EMB16/CBRD//CBRD
81	**15SWM**	21HRWYT	232	KA/NAC//TRCH/3/VORB
82	**15SWM**	21HRWYT	233	KA/NAC//TRCH/3/VORB
83	**15SWM**	21HRWYT	234	
84	**15SWM**	21HRWYT	235	KA/NAC//TRCH/3/VORB
85	**15SWM**	21HRWYT	236	KA/NAC//TRCH/3/VORB
86	**15SWM**	21HRWYT	237	C80.1/3*BATAVIA//2*WBLL1/3/EMB16/CBRD//CBRD/4/MILAN/KAUZ//DHARWAR DRY/3/BAV92
87	**15SWM**	21HRWYT	238	C80.1/3*BATAVIA//2*WBLL1/3/EMB16/CBRD//CBRD/4/MILAN/KAUZ//DHARWAR DRY/3/BAV92
88	**15SWM**	21HRWYT	239	C80.1/3*BATAVIA//2*WBLL1/3/EMB16/CBRD//CBRD/4/MILAN/KAUZ//DHARWAR DRY/3/BAV92
89	**15SWM**	21HRWYT	240	C80.1/3*BATAVIA//2*WBLL1/3/EMB16/CBRD//CBRD/4/MILAN/KAUZ//DHARWAR DRY/3/BAV92

Appendix 2 Continued.

91	**15SWM**	21SAWYT	302	HIDDAB
92	**15SWM**	21SAWYT	303	DHARWAR DRY
93	**15SWM**	21SAWYT	304	CHAM 6
94	**15SWM**	21SAWYT	305	SUNCO.6/FRAME//PASTOR/3/PAURAQ
95	**15SWM**	21SAWYT	306	1447/PASTOR//KRICHAUFF/3/PAURAQ
96	**15SWM**	21SAWYT	307	MILAN/KAUZ//DHARWAR DRY/3/BAV92/4/PAURAQ
97	**15SWM**	21SAWYT	308	METSO/ER2000/5/2*SERI*3//RL6010/4*YR/3/PASTOR/4/BAV92
98	**15SWM**	21SAWYT	309	WORRAKATTA/2*PASTOR//DANPHE #1
99	**15SWM**	21SAWYT	310	KA/NAC//TRCH/3/DANPHE #1
100	**15SWM**	21SAWYT	311	QING HAIBEI/WBLL1//BRBT2/3/PAURAQ
101	**15SWM**	21SAWYT	312	BERKUT/MUU//DANPHE #1
102	**15SWM**	21SAWYT	313	METSO/ER2000//MONARCA F2007/3/WBLL1*2/KKTS
103	**15SWM**	21SAWYT	314	1447/PASTOR//KRICHAUFF/5/2*SERI*3//RL6010/4*YR/3/PASTOR/4/BAV92
104	**15SWM**	21SAWYT	315	C80.1/3*BATAVIA//2*WBLL1/3/EMB16/CBRD//CBRD/4/CHEWINK #1
105	**15SWM**	21SAWYT	316	SLVS/3/CROC_1/AE.SQUARROSA(224)//OPATA/5/VEE/LIRA//BOW/3/BCN/4/KAUZ/6/2*KA/NAC//TRCH
106	**15SWM**	21SAWYT	317	SNLG/3/EMB16/CBRD//CBRD/4/KA/NAC//TRCH
107	**15SWM**	21SAWYT	318	SNLG/3/EMB16/CBRD//CBRD/4/KA/NAC//TRCH
108	**15SWM**	21SAWYT	319	CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA(TAUS)/4/WEAVER/5/2*JANZ/6/SKAUZ/BAV92
109	**15SWM**	21SAWYT	320	METSO/ER2000//MUU
110	**15SWM**	21SAWYT	321	TOB/ERA//TOB/CNO67/3/PLO/4/VEE#5/5/KAUZ/6/FRET2/7/MINO
111	**15SWM**	21SAWYT	322	TOB/ERA//TOB/CNO67/3/PLO/4/VEE#5/5/KAUZ/6/FRET2/7/PASTOR//MILAN/KAUZ/3/BAV92
112	**15SWM**	21SAWYT	323	TOB/ERA//TOB/CNO67/3/PLO/4/VEE#5/5/KAUZ/6/FRET2/7/PASTOR//MILAN/KAUZ/3/BAV92
113	**15SWM**	21SAWYT	324	KA/NAC//TRCH/3/VORB
114	**15SWM**	21SAWYT	325	KA/NAC//TRCH/4/MILAN/KAUZ//DHARWAR DRY/3/BAV92
115	**15SWM**	21SAWYT	326	KA/NAC//TRCH/3/DANPHE #1
116	**15SWM**	21SAWYT	327	KA/NAC//TRCH/3/DANPHE #1
117	**15SWM**	21SAWYT	328	EMB16/CBRD//CBRD/4/BETTY/3/CHEN/AE.SQ//2*OPATA
118	**15SWM**	21SAWYT	329	TILILA/JUCHI/4/SERI.1B//KAUZ/HEVO/3/AMAD
119	**15SWM**	21SAWYT	330	BAV92//IRENA/KAUZ/3/HUITES/4/2*ROLF07
120	**15SWM**	21SAWYT	331	FRET2/TUKURU//FRET2/3/MUNIA/CHTO//AMSEL/4/FRET2/TUKURU//FRET2
121	**15SWM**	21SAWYT	332	FRET2/TUKURU//FRET2/3/MUNIA/CHTO//AMSEL/4/FRET2/TUKURU//FRET2
122	**15SWM**	21SAWYT	333	WBLL1*2/4/BABAX/LR42//BABAX/3/BABAX/LR42//BABAX

Appendix 2 Continued.

123	**15SWM**	21SAWYT	334	ROLF07*2/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA(213)//PGO/4/HUITES
124	**15SWM**	21SAWYT	335	WBLL1/FRET2//PASTOR*2/3/MURGA
125	**15SWM**	21SAWYT	336	WBLL1/4/BOW/NKT//CBRD/3/CBRD/5/WBLL1*2/TUKURU
126	**15SWM**	21SAWYT	337	WBLL1*2/4/YACO/PBW65/3/KAUZ*2/TRAP//KAUZ*2/5/DEMAI 4
127	**15SWM**	21SAWYT	338	PFAU/SERI.1B//AMAD/3/WAXWING/4/BABAX/LR42//BABAX*2/3/KURUKU
128	**15SWM**	21SAWYT	339	MILAN/S87230//BAV92/3/ROLF07
129	**15SWM**	21SAWYT	340	WBLL1*2/BRAMBLING/5/BABAX/LR42//BABAX*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ
130	**15SWM**	21SAWYT	341	FRANCOLIN #1/WBLL1
131	**15SWM**	21SAWYT	342	FRANCOLIN #1//WBLL1*2/BRAMBLING
132	**15SWM**	21SAWYT	343	WBLL1*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/BAJ #1
133	**15SWM**	21SAWYT	344	BECARD/KACHU
134	**15SWM**	21SAWYT	345	FRANCOLIN #1//WBLL1*2/KURUKU
135	**15SWM**	21SAWYT	346	MILAN/S87230//BAV92/3/AKURI
136	**15SWM**	21SAWYT	347	BAJ #1/AKURI
137	**15SWM**	21SAWYT	348	BAJ #1/TECUE #1
138	**15SWM**	21SAWYT	349	NAC/TH.AC//3*PVN/3/MIRLO/BUC/4/2*PASTOR/5/KACHU/6/KACHU
139	**15SWM**	21SAWYT	350	MUU/5/WBLL1*2/4/YACO/PBW65/3/KAUZ*2/TRAP//KAUZ/6/WBLL1*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ
141	**15SWM**	8EBWYT	502	MUNAL #1
142	**15SWM**	8EBWYT	503	BECARD #1/5/KIRITATI/4/2*SERI.1B*2/3/KAUZ*2/BOW//KAUZ
143	**15SWM**	8EBWYT	504	PRL/2*PASTOR/3/PFAU/WEAVER*2//CHAPIO
144	**15SWM**	8EBWYT	505	TACUPETO F2001*2/BRAMBLING//KIRITATI/2*TRCH
145	**15SWM**	8EBWYT	506	KACHU//KIRITATI/2*TRCH
146	**15SWM**	8EBWYT	507	KIRITATI//HUW234+LR34/PRINIA/3/BAJ #1
147	**15SWM**	8EBWYT	508	KIRITATI//HUW234+LR34/PRINIA/3/BAJ #1
148	**15SWM**	8EBWYT	509	MUTUS//ND643/2*WBLL1
149	**15SWM**	8EBWYT	510	ND643/2*WBLL1/4/WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL1
150	**15SWM**	8EBWYT	511	BAJ #1/KISKADEE #1
151	**15SWM**	8EBWYT	512	WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL1/5/PRL/2*PASTOR/4/CHOIX/STAR/3/HE1/3*CNO79//2*SERI
152	**15SWM**	8EBWYT	513	WHEAR/VIVITSI//WHEAR/3/FRNCLN
153	**15SWM**	8EBWYT	514	QUAIU*2/KINDE
154	**15SWM**	8EBWYT	515	MUU/FRNCLN//FRANCOLIN #1
155	**15SWM**	8EBWYT	516	WAXWING*2/TUKURU/3/2*WHEAR/VIVITSI//WHEAR
156	**15SWM**	8EBWYT	517	DANPHE #1*2/CHYAK

Appendix 2 Continued.

157	**15SWM**	8EBWYT	518	MUTUS*2/HARIL #1
158	**15SWM**	8EBWYT	519	MUTUS*2//ND643/2*WBLL1
159	**15SWM**	8EBWYT	520	FRNCLN/NIINI #1//FRANCOLIN #1
160	**15SWM**	8EBWYT	521	FRNCLN/3/ND643//2*PRL/2*PASTOR/4/FRANCOLIN #1
161	**15SWM**	8EBWYT	522	FRNCLN/3/KIRITATI//HUW234+LR34/PRINIA/4/FRANCOLIN #1
162	**15SWM**	8EBWYT	523	WBLL1*2/BRAMBLING*2//BAVIS
163	**15SWM**	8EBWYT	524	SWSR22T.B./2*BLOUK #1//WBLL1*2/KURUKU
164	**15SWM**	8EBWYT	525	CROC_1/AE.SQUARROSA(205)//BORL95/3/PRL/SARA//TSI/VEE#5/4/FRET2/5/CHONTE/6/INQALAB 91*2/KUKUNA//KIRITATI
165	**15SWM**	8EBWYT	526	FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/KIRITATI/2*TRCH/6/BAJ #1
166	**15SWM**	8EBWYT	527	PRL/2*PASTOR/4/CHOIX/STAR/3/HE1/3*CNO79//2*SERI/5/KIRITATI/2*TRCH/6/PRL/2*PASTOR/4/CHOIX/STAR/3/HE1/3*CNO79//2*SERI
167	**15SWM**	8EBWYT	528	MUNAL*2/CHONTE
168	**15SWM**	8EBWYT	529	WAXWING*2/TUKURU//2*FRNCLN
169	**15SWM**	8EBWYT	530	FRANCOLIN #1/CHONTE//FRNCLN

Appendix 3. Analysis of Variance (ANOVA) for pathogenicity of *Fusarium culmorum* isolates

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	69.217[a]	46	1.505	3.752	.001
Intercept	1401.046	1	1401.046	3493.020	.001
Isolate	69.217	46	1.505	3.752	**.001**
Error	36.500	91	.401		
Total	1515.000	138			
Corrected Total	105.717	137			

Appendix 4. Analysis of Variance (ANOVA) for pathogenicity of *Fusarium pseudograminearum* isolates

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	14.917[a]	3	4.972	9.944	0.004
Intercept	102.083	1	102.083	204.167	0.001
Isolate	14.917	3	4.972	9.944	**0.004**
Error	4	8	0.5		
Total	121	12			
Corrected Total	18.917	11			

Appendix 5. Analysis of Variance (ANOVA) for pathogenicity of *Fusarium hostae* isolates

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	31.048[a]	20	1.552	6.113	.001
Intercept	289.286	1	289.286	1139.063	.001
Isolate	31.048	20	1.552	6.113	**.001**
Error	10.667	42	.254		
Total	331.000	63			
Corrected Total	41.714	62			

Appendix 6. Analysis of Variance (ANOVA) for pathogenicity of *Fusarium redolens* isolates

Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Corrected Model	27.650[a]	19	1.455	6.237	.001
Intercept	170.017	1	170.017	728.643	.001
Isolate	27.650	19	1.455	6.237	**.001**
Error	9.333	40	.233		
Total	207.000	60			
Corrected Total	36.983	59			

Appendix 7. Analysis of Variance (ANOVA) for pathogenicity of *Fusarium avenaceum* isolates

Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Corrected Model	11.077[a]	12	.923	1.636	.142
Intercept	246.256	1	246.256	436.545	.001
Isolate	11.077	12	.923	1.636	**.142**
Error	14.667	26	.564		
Total	272.000	39			
Corrected Total	25.744	38			

Apendix 8. Analysis of Variance (ANOVA) for aggressiveness of *Fusarium culmorum* isolates

Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Corrected Model	243.991[a]	46	5.304	10.497	.001
Intercept	3795.009	1	3795.009	7510.124	.001
Isolate	243.991	46	5.304	10.497	**.001**
Error	190.000	376	.505		
Total	4229.000	423			
Corrected Total	433.991	422			

Appendix 9. Analysis of Variance (ANOVA) for screening wheat lines against *Fusarium culmorum* isolate number 2 (FC2)

Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Corrected Model	315.641[a]	173	1.825	8.119	.001
Intercept	8453.959	1	8453.959	37621.197	.001
Germplasm	315.641	173	1.825	8.119	**.001**
Error	156.400	696	.225		
Total	8926.000	870			
Corrected Total	472.041	869			

BIOGRAPHY

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Dilla University: Lecturer (March 2008 to December 2009), Head of Gender office (November 2008 to December 2009)

Tinaw Flower Farm: Production Manager (2007-2008)

Summit Agro Industry, PLC: Pack house supervisor (July 2004 to October 2005)

Fellowship awarded: The Norman E. Borlaug Leadership Enhancement in Agriculture Program (Borlaug LEAP, USA) 2013.

Symposium/conference attended:

- The World Food Prize. October 2013, Des Moines, Iowa, USA.
- Borlaug Leadership Enhancement in Agriculture Program (Borlaug LEAP) Ethiopia Alumini Association Roundtable Discussion, March 19, 2015, Addis Ababa, Ethiopia.

- The 5th International Cereal Nematodes Initiative Workshop, 12-16 September 2015, Ankara, Turkey

Publications:

Gebremariam, E.S., Karakaya, A., Erginbas-Orakci, G., Dababat, A., Sharma-Poudyal, D., and Paulitz, T. C. 2015. First Report of *Fusarium redolens* causing crown rot of wheat (*Triticum* spp.) in Turkey. Plant Disease, 99(9), 1280.

Gebremariam, E.S., Dababat, A., Erginbas-Orakci, G., Karakaya, A., Sharma-Poudyal, D. and Paulitz, T. C. 2015. First Report of *Fusarium hostae* causing crown rot of wheat (*Triticu*m spp.) in Turkey. Plant Disease, (in press).