

# Determination of Resistance Levels of Some Onion Cultivars or Inbred Lines with Fusarium Testing at Seedling Stage

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## Abstract

This study was carried out to determine the resistance levels of some onion genotypes in Yalova onion gene pool by Fusarium testing during seedling stage. The isolate used in the study was *Fusarium oxysporum* f. sp. *cepae*, which causes damping off during the seedling stage and later basal rot in onion bulbs. The variance analysis for the onion seedling test and the mean differences against control were analyzed by using General Linear Model of the Tukey test. The germination rate of control seeds varied between 72% to 98%, while the germination rate of inoculated seeds varied between 39% to 93%. Texas Early Grano 502 showed the highest level of resistance with a survival rate of 83.8%, and resistance levels of 19Y07 and 19Y142 genotypes were higher than other genotypes. Resistance levels of 19Y51, 19Y15 and 19Y73 genotypes were lower than other genotypes. Akgün 12 showed moderate resistance with a survival rate of 59.6%. Determining the resistance levels of these onion genotypes during the seedling stage may be a preliminary step towards further studies.

## 1. Introduction

Onion (*Allium cepa* L.), belonging to the Alliaceae family is a significant vegetable crop worldwide. Onion is the herbaceous biennial crop, and it has a wide range of landraces and cultivars with edible bulbs (Nasr Esfahani, 2018; Singh et al., 2018). The onion plant can be infected by soil- and seed-borne fungal pathogens, resulting in significant yield and quality losses.

*Fusarium oxysporum* Schlechtend.: Fr. f. sp. *cepae* (Hans) Snyder and Hans (FOC) is caused seedborne and soilborne diseases such as damping off and basal rot (Köycü and Özer, 1997; Özer et al., 2003). This pathogen may be seen and noticed in different growth stages of onion and can cause serious loss in the field and during storage (Fantino and Schiavi, 1987; Özer et al., 2003). Symptoms of Fusarium basal rot (FBR) appear on

leaves, roots, basal plates, on the bulb scales of small seedlings, on the mature and dormant plants (Cramer, 2000; Özer et al., 2003). Symptoms are pre- and post-emergence damping-off of seedling in the field, root rot in older plants, discoloration in onion stem plate, basal rot in bulbs during storage (Abawi and Lorbeer, 1972; Cramer, 2000).

*Fusarium oxysporum* f. sp. *cepae* (FOC) exist in many countries such as the United States, Brazil, South Africa, the Netherlands, India, England, Iran, Sweden, Japan, and Uruguay, where onions are grown around the world (Cramer, 2000; Galvan et al., 2008; Dissanayake et al., 2009; Lager, 2011; Ghanbarzadeh et al., 2014; Ünsal et al., 2019) and also FOC exist in Turkey in onion production areas (Türkkan and Karaca, 2006; Bayraktar and Dolar, 2011).

*Fusarium oxysporum* f. sp. *cepae* can lead to losses reaching up to 50% in the field and 75% in

the greenhouse (Brayford, 1996; Stadnik and Dhingra, 1997; Ünsal et al., 2019). For this reason, the development and application of the most effective control methods of this destructive pathogen are vital. This pathogen can be controlled with some control methods such as resistance of host plant, crop rotation, solarization, various biological applications, and fungicide applications (Cramer, 2000; Ünsal et al., 2019). However, the using of resistant varieties is economic, applicable on a large scale, and stated as the best option (Cramer, 2000; Nasr Esfahani et al., 2012; Özer et al., 2003; Özer et al., 2004).

Many management strategies have been developed in the world for the detection and control of FOC. But over the time, control methods have become limited and inadequate. Therefore, it has become very important to identify and develop varieties that are tolerant or resistant to this pathogen. Resistance studies against FOC in onion have been performed before (Özer et al., 2003; Özer et al., 2004; Saxena and Cramer, 2009; Nasr Esfahani et al., 2012; Taylor et al., 2013). However, in this study, no previous study was conducted to define the resistance levels of 13 onion genotypes that were taken from the Yalova gene pool with Akgün 12 and grown in the same environment.

The goal of this study is to determine onion genotypes tolerance levels to develop tolerant varieties for commercial production. Developing resistance cultivars will prevent the loss of crops in the field and storehouse and will contribute to the national economy. This study aims to determine the tolerance/resistance level of some onion genotypes at the seedling stage. In this study, resistance levels of some onion genotypes to this pathogen were determined.

## 2. Materials and Methods

The plant materials were formed from 13 onion genotypes and 2 onion varieties. The onion genotypes were labeled 19Y01, 19Y06, 19Y07, 19Y15, 19Y16, 19Y17, 19Y18, 19Y19, 19Y34, 19Y46, 19Y51, 19Y73 and 19Y142. Akgün 12 and Texas Early Grano 502 have used as onion varieties. The thirteen genotypes and Akgün 12 were provided from Yalova onion gene pool and Texas Early Grano (TEG) 502 was provided by Bayram Seed Company. Whereas Akgün 12 onion variety was used as tolerant, TEG 502 variety was used as susceptible against *F. oxysporum* f. sp. *cepae* as reported according to previous research (Ko et al., 2002; Özer et al., 2003). The susceptibility levels of other onion genotypes were not known. As fungal material, one pathogenic and virulence isolate to be used in this study was provided from Ankara University, Agriculture Faculty, Plant Protection Department.

Onion seedling test was carried out by the method of inoculation of onion seeds. Onion seedling test was mainly included sterilization of

onion seeds, preparation of spore suspension, inoculation of onion seeds, sowing of onion seeds in the soil, and counting of seeds and seedlings. Initially, surface sterilization of onion seeds from each genotype was performed in the flow cabinet in the Mycology Laboratory, Plant Protection Department, Atatürk Horticultural Crops Central Research Institute. The onion seeds were kept in 1000 µl 1% sodium hypochlorite for 3 minutes in the Eppendorf tubes to disinfect the surface, then seeds were rinsed in the sterile distilled water 3 times and left to get dry on sterile filter papers. Then *Fusarium oxysporum* f. sp. *cepae* was cultured on PDA medium at 20°C for 10 days. Sterile water was added into the petri dish to allow the conidia to pass into the water onto the developing culture and filtered through the sterile cheesecloth by gentle mixing and the intensity of the spore was adjusted to a density of  $1 \times 10^6$  ml<sup>-1</sup> by the hemocytometer.

After preparation of spore suspension, each onion genotype was inoculated by standing in 1 ml of spore suspension for 1 hour and the seeds used for control were kept in 1 ml sterile purified water for 1 hour. After 1 hour, seeds were rinsed and put back into petri dishes.

Before sowing the seeds, the 1/3 garden soil + 1/3 farm manure + 1/3 stream sand mixture was filled in the fireproof bag and placed in the autoclave machine (60 min, 121°C) to be a sterile soil mixture. For each treatment, 100 seeds were planted for each genotype with independent 4 replications in each replication with 25 seeds.

The seeds planted in the seedling trays (10×12 cells per tray) were placed in the climate chamber and the climate chamber was adjusted to be 25 °C day / 18°C night and 16-hour light / 8-hour dark and 60% relative humidity. The counting of survival seedlings was performed twice a week after 10 days of planting and continued for 3 weeks (Özer et al., 2004; Taylor et al., 2013). The survival percentage of the genotypes was calculated according to control genotypes to ensure a variation of natural in the seed germination.

The data obtained before and after the seedling emergence were compared with the control. The variance analysis for the onion seedling test was analyzed by using the General Linear Model of the Tukey test. Variance analyses were performed with Minitab® 16.2.4 (e-academy version). Paired Sample T-Test for comparison of control and inoculated alive seedlings were analyzed by using Microsoft Excel. Counting of dead and alive seeds and seedlings were taken after two weeks and four weeks and the percentage of emergence and survival were calculated with the formula below (Saxena and Cramer, 2009).

$$\%Emergenc y = \frac{Emerged\ seedlings}{Total\ planted\ seed} \times 100$$

$$\%Survival = \frac{Survival\ seedlings}{Total\ emerged\ seedling} \times 100$$

### 3. Results and Discussion

#### 3.1. Observation of disease symptoms

FOC caused germination, plant death and adverse effects on the length and weight of the root and stem in plant disease studies (Behrani et al., 2015). On that account, significant differences were noted between plant growths of inoculated and control plants (Figure 1). Ten days after inoculation, germination almost completed for inoculated and control seeds.

Twelve days after inoculation, the initial disease symptoms began to seem such as the appearance of white mycelium on the soil and the death of several small seedlings (Figure 2). Before germination, it either germinated later than controls or death occurred. After germination, inoculated seedlings developed later than control. Yellowing occurred on the onion leaves (Figure 3). Cramer (2000) noticed that white mycelium may be on the basal plate of external bulb scales. Like to this work, white mycelium was noticed on the soil of the onion seedling during seedling stage in this study (Figure 2). It was noteworthy that when the root part of the seedlings that looked healthy in the soil was removed from the soil, it was already brown and broken. If the study had continued, seedlings would continue to die (Figure 4).

#### 3.2. Onion seedling test

A total of 13 onion genotypes and 2 onion varieties inoculated by the seed inoculation method were evaluated about resistance against FOC. There were highly significant differences among onion genotypes due to infection caused by FOC.

In this study, the percentage of germinated seeds, the percentage of alive seedlings and the percentage of dead seedlings in the inoculated onion seeds were calculated by comparing them with the control seedlings. At the end of the 4-week counts, the obtained data were evaluated, analyzed and the resistance levels against damping-off were determined among the onion genotypes (Table 1).

Dead seeds shown in the control seeds were non-germinated seeds. These dead seeds were percentage of seeds that were not germinated by their nature. In the case of inoculated seeds, it was showed the percentage of seedlings that died after germination. It was observed that germinated seeds and alive seedlings of onion genotypes had similar values, while their dead seedlings had significant differences. While the mean percentage of germinated seeds was found to be 70.26, the mean percentage of alive seedlings was found to be 57.00. The mean percentage of dead seedlings was found to be 21.40. In addition, when looking at dead seedlings, significant differences were observed

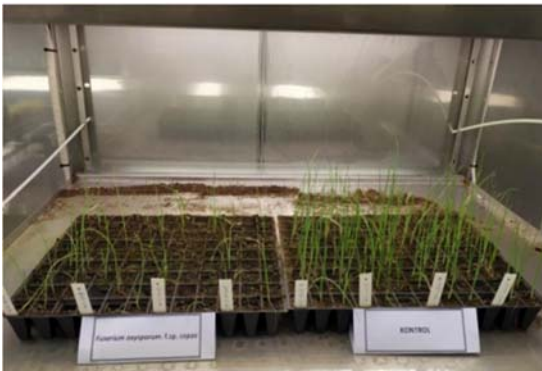


Figure 1. Final comparison between inoculated and control seedlings



Figure 2. View of dead seed and seedling and the formation of white mycelium (a, b)

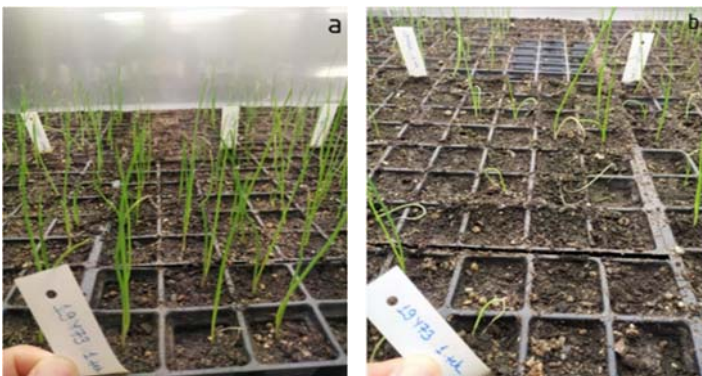


Figure 3. Comparison of control (a) and inoculated seedlings (b) of 19Y73



Figure 4. Comparing of the alive and dead seedling in the same inoculated genotype

between genotypes both according to control genotypes and among themselves (Table 1).

The mean of control dead seedlings was unexpectedly higher than inoculated dead seedlings in some genotypes (Table 1). These genotypes can be listed as follows: 19Y51, 19Y15, Akgün 12, 19Y46, 19Y34, 19Y01, and 19Y07. In the 19Y16 genotype, the mean of control and inoculated dead seedlings were equal. Other onion genotypes had higher average dead seedlings in inoculated genotypes as expected in the Table 1. Another remarkable point was that TEG 502 onion variety has more average germinated seeds than other genotypes in both control and inoculated seeds in the Table 1. Then the mean of control and inoculated alive seedlings at the end of the experiment were given comparatively in the Table 2.

Significant difference was not observed between control and inoculated live seedlings of some genotypes. Examples of these genotypes were 19Y06, 19Y19, 19Y142, Akgün 12 and TEG 502 ( $P > 0.05$ ). Significant differences ( $P < 0.05$ ) were seen in other onion genotypes (Table 2). When mean of the control and inoculated alive seedlings were compared, there were no statistically significant

differences for some onion genotypes such as TEG 502 ( $P = 0.253$ ), Akgün 12 ( $P = 0.110$ ), 19Y142 ( $P = 0.223$ ), 19Y19 ( $P = 0.063$ ) and 19Y06 ( $P = 0.427$ ).

Two onion varieties and 13 onion genotypes were inoculated by a FOC isolate. Then, seedling emergence rate and survival rate of onion seedlings at 2<sup>nd</sup> and 4<sup>th</sup> week were calculated and compared with each other in the Table 3. This evaluation was done with using the formula given by Saxena and Cramer (2009).

The survival seedling rates among onion genotypes ranged from 89.7% to 100% in the 2<sup>nd</sup> week, while survival seedling rates ranged from 39.02% to 83.87% in the 4<sup>th</sup> week (Table 3). If the study had continued, it would have estimated that the survival seedling rates, would continue to decrease in the following weeks.

Each onion genotype did not differ significantly at 2<sup>nd</sup> and 4<sup>th</sup> weeks. For example, FOC isolate caused a little change of pre- and post-emergence damping-off, by a decrease from 100% survival rate after 2 weeks to 83.87% survival rate after 4 weeks in TEG 502. However, FOC isolate caused a significant amount effect of pre- and post-emergence damping off, with a decrease from 90.24% survival rate after 2 weeks to 39.02%

Table 1. Genotypes, experiment, mean percentages of the germinated seeds, alive and dead seedlings of control and inoculated seeds.

Genotypes	Experiment	N	Germinated seeds		Alive seedlings		Dead seedlings	
			Mean	G	Mean	G	Mean	G
TEG 502	Control	4	98.00	a*	98.00	a	2.00	c
TEG 502	FOC	4	93.00	ab	78.00	a	15.00	ac
19Y18	Control	4	88.00	ac	88.00	a	12.00	bc
19Y18	FOC	4	64.00	bh	38.00	cd	26.00	ab
19Y142	Control	4	85.00	ad	85.00	a	15.00	ac
19Y142	FOC	4	71.00	ag	50.00	bc	21.00	ac
19Y17	Control	4	84.00	ad	84.00	a	16.00	ac
19Y17	FOC	4	62.00	ch	37.00	cd	25.00	ac
19Y19	Control	4	83.00	ae	83.00	a	17.00	ac
19Y19	FOC	4	60.00	ch	31.00	cd	29.00	ab
19Y16	Control	4	80.00	ae	80.00	a	20.00	ac
19Y16	FOC	4	60.00	ch	40.00	cd	20.00	ac
19Y07	Control	4	79.00	ae	79.00	a	21.00	ac
19Y07	FOC	4	43.00	gh	32.00	cd	11.00	b
19Y06	Control	4	79.00	ae	79.00	a	21.00	ac
19Y06	FOC	4	72.00	ag	36.00	cd	36.00	a
19Y01	Control	4	77.00	ae	77.00	a	23.00	ac
19Y01	FOC	4	44.00	gh	28.00	cd	16.00	ac
19Y73	Control	4	77.00	ae	77.00	a	23.00	ac
19Y73	FOC	4	45.00	fh	21.00	d	24.00	ac
19Y34	Control	4	74.00	af	74.00	ab	26.00	ab
19Y34	FOC	4	54.00	eh	30.00	cd	24.00	ac
19Y46	Control	4	74.00	af	74.00	ab	26.00	ab
19Y46	FOC	4	47.00	fh	26.00	cd	21.00	ac
Akgün 12	Control	4	74.00	af	74.00	ab	26.00	ab
Akgün 12	FOC	4	57.00	dh	34.00	cd	23.00	ac
19Y15	Control	4	72.00	ag	72.00	ab	28.00	ab
19Y15	FOC	4	39.00	h	17.00	d	22.00	ac
19Y51	Control	4	72.00	ag	72.00	ab	28.00	ab
19Y51	FOC	4	41.00	h	16.00	d	25.00	ac
General Mean			70.26		57.00		21.40	
General SE Mean			5.45		4.89		4.26	

\*Different letters following the mean in the same column signify that the mean is statistically significant difference. (ANOVA  $p = 0.05$ , Tukey test). N: Number of replication. G: Grouping.

Table 2. Genotypes, comparison of control and inoculated alive seedlings.

Genotypes	Control alive seedling		Inoculated alive seedling		p-value*
	Mean	Std Dev	Mean	Std Dev	
19Y01	0.77	0.09	0.44	0.12	0.005
19Y06	0.79	0.14	0.72	0.09	0.427
19Y07	0.79	0.02	0.43	0.23	0.021
19Y15	0.72	0.03	0.39	0.07	0.000
19Y16	0.80	0.09	0.60	0.05	0.012
19Y17	0.84	0.09	0.62	0.10	0.016
19Y18	0.88	0.09	0.64	0.09	0.008
19Y19	0.83	0.11	0.60	0.16	0.063
19Y34	0.74	0.11	0.54	0.07	0.043
19Y46	0.74	0.07	0.47	0.07	0.002
19Y51	0.72	0.09	0.41	0.13	0.009
19Y73	0.77	0.04	0.45	0.12	0.003
19Y142	0.85	0.10	0.71	0.10	0.223
TEG 502	0.98	0.04	0.93	0.04	0.253
Akgün 12	0.74	0.16	0.57	0.04	0.110

\*(Paired Sample T-Test, p = 0.05).

Table 3. Genotypes, percentages of emergence and survival rate of onion seedlings.

Genotypes	Inoculated seeds		
	% Emergence*	% Survival (2 <sup>nd</sup> week)	% Survival (4 <sup>th</sup> week)
19Y51	41.00	90.24	39.02
19Y15	39.00	89.70	43.58
19Y73	45.00	93.33	46.66
19Y06	72.00	93.05	50.00
19Y19	60.00	90.00	51.66
19Y46	47.00	97.87	55.31
19Y34	54.00	96.29	55.55
19Y18	64.00	93.75	59.37
Akgün 12	57.00	91.22	59.64
19Y17	62.00	90.32	59.67
19Y01	44.00	95.45	63.63
19Y16	60.00	98.33	66.66
19Y142	71.00	98.59	70.42
19Y07	43.00	97.67	74.41
TEG 502	93.00	100.00	83.87
Mean	56.80	94.38	58.63

\*Emergence rate and survival rate of onion seedlings at 2<sup>nd</sup> and 4<sup>th</sup> week was calculated using the formula given by Saxena and Cramer (2009).

survival rate after 4 weeks in 19Y51. As another example, although 19Y07 onion genotype has more loss than 19Y142 in terms of damping off before germination, 19Y07 onion genotype has less loss in terms of damping-off after germination than 19Y142 (Table 3). Moreover, among genotypes, while the survival rate of genotypes 19Y15 and 19Y51, where the first symptoms were seen, was the lowest compared to others, TEG 502 onion variety showing the last symptom had the highest survival rate (Table 3).

Özer et al. (2003) measured disease severity of onion varieties 7 days after inoculation and they observed that the disease severity of TEG 502 was higher than the disease severity of Akgün 12. Ko et al. (2002) remarked that TEG 502 was the most susceptible to FOC. For another study, Galvan et al. (2008) noted that the TEG 502 onion variety was

less resistant in their study. Nasr-Esfahani et al. (2013) announced that TEG was one of the susceptible onion genotypes in field and greenhouse conditions. In contrast, according to the data obtained in this study, at the end of the 4th week, in the inoculated seeds, TEG 502 showed 83.87% survival rate and 93.0% emergence rate in the Table 3. Thus, unlike other studies, it was observed that the resistance level of TEG 502 was highest against damping-off before and after germination (Figure 5).

According to a study by Özer et al. (2004), while Akgün 12 variety was found to be resistant in all bulb stages in their all experiments, in this study, the survival rate of Akgün 12 was showed 59.64% (Table 3). In this case, it was observed that Akgün 12 showed a moderate level of resistance against damping-off during the seedling stage. Akgün 12

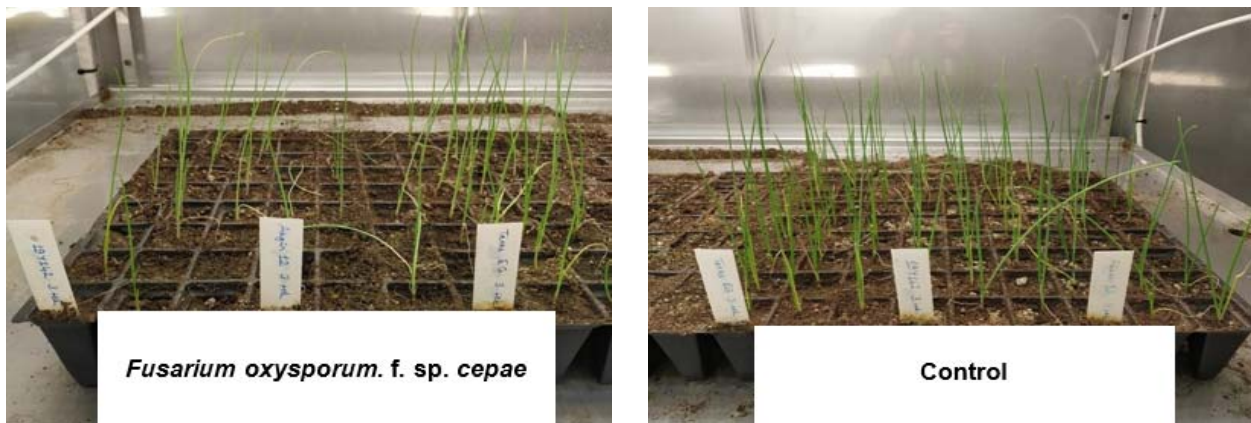


Figure 5. Comparison of inoculated and control seedlings of 19Y142, Akgün 12, and TEG 502.

may not appear more resistant compared to TEG 502. However, regarding the germination of control seeds, it should be considered that Akgün 12 has a 74.0% germination rate and TEG 502 has a 98.0% germination rate.

It has been thought that differences in resistance levels changed over time and the result obtained in the 4<sup>th</sup> week gave a more accurate result. According to observation, pre-damping off refers to the percentage of seeds that die before germination, while post-damping off refers to the percentage of seedlings that die after germination. The rate of dead seeds before germination were significantly higher than the rate of dead seedlings after germination.

#### 4. Conclusion

These 13 onion genotypes have never been used in a disease study. The results of Akgün 12 used in this study were like to previous studies. Accordingly, one can have an idea about the resistance level of 13 other onion genotypes grown under the same conditions as Akgün 12. However, the result of the Texas Early Grano 502 variety used in the study was not compatible with previous studies (Özer, 1998; Ko et al., 2002; Özer et al., 2004; Galvan et al., 2008; Nasr-Esfahani et al., 2013).

When the obtained results were analyzed, according to the survival percentages at the end of the 4<sup>th</sup> week, it is possible to specify the resistance levels of onion genotypes as follows: Texas Early Grano 502, 19Y07, 19Y142, 19Y16, 19Y01, 19Y17, Akgün 12, 19Y18, 19Y34, 19Y46, 19Y19, 19Y06, 19Y73, 19Y15 and 19Y51. Since it was observed that the pre-damping-off severity was higher than the post-damping-off severity, the production of onion at the seed stage should be done more carefully and controlled. Since the aggressiveness of pathogens changes from region to region and onion varieties found as resistant will not always show the same performance in time.

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