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In vitro and in vivo assessment of the sensitivity of some tangerine mutants to *Alternaria alternata* pv. *citri*

In vitro ve *in vivo* koşullarında bazı mandarin mutantlarının *Alternaria alternata* pv. *citri* etmenine duyarlılıklarının değerlendirilmesi

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ABSTRACT

Alternaria brown spot disease caused by the *Alternaria alternata* pv. *citri* factor affects the leaves, branches and fruits of many types of tangerines and their hybrids. This disease has caused serious declines in the plantation areas of Minneola tangelo which is widely produced in the Mediterranean Region while at the same time it has hindered the expansion of the area of cultivation of late maturing tangerines like Fortune. The isolate used in this study was obtained from a commercial orchard of 'Minneola tangelo' in the province of Adana, Turkey. The sequence analysis of the application of this isolates shows that a matchup of 99% was achieved with isolate AHS-467.6 on the scale of the IFS primer. The aim of the study was to identify tolerant genotypes from the mutants bred form the 'Fortune' tangerine which is known to be sensitive to the brown spot disease of citrus. The sensitivity of individual mutants was tested by using leaves under *in vitro* conditions. The disease was inoculated to tolerant mutant varieties under *in vivo* conditions and the results registered. The results of the study showed that 9 mutant varieties among the 110 individuals under study were found to be tolerant against the disease.

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ÖZ

Alternaria alternata pv. citri etmeninin neden olduğu Alternaria kahverengi leke hastalığı birçok mandarin ve melezlerinin yaprak, dal ve meyvelerini etkiler. Söz konusu hastalık Akdeniz bölgesinde yetiştiriciliği yapılan Minneola tanjelonun dikim alanlarında ciddi azalmalara neden olmuş ve ayrıca yetiştiricilik açısından geççi bir çeşit olan Fortune gibi mandarin çeşitlerinin gelişimini engellemiştir. Çalışmada kullanılan izolat Adana ilinde bulunan ticari 'Minneola tanjelo' bahçelerinden elde edilmiştir. Bu izolat sekans analizi sonucunda ITS1 primerine göre %99 oranında AHS-467-6 izolatı ile eşleşme sağlamıştır. Mutasyon ıslahı ile turunçgillerde kahverengi leke hastalığına hassas olduğu bilinen 'Fortune' mandarini mutantları arasından tolerant genotiplerin elde edilmesi hedeflenmiştir. *In vitro* koşullarda koparılmış yapraklar kullanılarak mutant bireylerin hastalığa karşı hassasiyetleri belirlenmiştir. Ayrıca, tolerant olarak bulunan mutantların fidanlarına *in vivo* koşullarda hastalık inokulasyonu yapılarak sonuçlar kontrol edilmiştir. Çalışma sonucunda 111 mutant birey arasından 9 adet mutant birey hastalığa karşı tolerant olarak bulunmuştur.

1. Introduction

Work on mutation breeding has generally been conducted to produce more colorful, tastier fruits or to preserve inherited material that may be lost through evolution. Mutations obtained by changing the structure of the genes artificially through the use of certain physical or chemical mutagens in citrus breeding is considered a successful method for the breeding of new varieties.

Though mutations in citrus, particularly mutations using the budwood are widely used in obtaining new varieties, the mechanisms of mutation itself are not yet fully clarified (Liu et al. 2009). Furthermore, in mutations it is still not possible to distinguish minor genetic variations from original varieties (Deng et al. 1995; Breto et al. 2001; Liu et al. 2009).

The tangerine pathotype of the factor *Alternaria alternata* is a fungal pathogen which produces brown spots on the leaves and fruit of the tangerine and its hybrids. Alternaria black spot disease was first discovered in Australia on the 'Emperor' variety of tangerines in 1903 (Pegg 1966). Subsequently the disease was spotted in South Africa (Schutte et al. 1992), Israel (Solel 1991), Cuba (Herrera 1992), Colombia (Castro Carcedo et al. 1994), Turkey (Canıhoş et al. 1997), Argentina (Peres et al. 2003) and Peru (Marin et al. 2006). Alternaria brown spot disease is an important disease as it affects the leaves, branches and unripe fruit of the tangerine and its hybrids. (Pegg 1996; Canıhoş at al. 1999). It has been reported that Alternaria brown spot disease affects the tangerine and its hybrids when the right conditions for the infection are materialized (Kiely 1964; Pegg 1966; Whiteside 1976; Gardner et al. 1986). Among tangerine varieties and their hybrids particularly 'Dancy' and to a lesser extent 'Fortune' are the most succeptible to the disease (Nemsa et al. 2012). Peever et al. (2000) report that 'Minneola', 'Orlando', 'Sunburst' and 'Nova' hybrids are also very sensitive to this pathogen. At the present time Alternaria brown spot disease is considered the most serious fungal disease affecting the tangerine and its hybrids. In our own country, Turkey, Alternaria brown spot disease is considered a serious problem for late maturing varieties like Minneola tangelo and Fortune.

The aim of this study is to determine the sensitivity of Fortune variants to Alternaria brown spot disease to develop new tangerine varieties.

2. Materials and Method

Acute gamma rays of 50 and 60 gray doses were administered on "Fortune" budwood and the radiated budwoods grafted onto common sour oranges (M_1V_1) in 2012. Following the grafting the plants were vegetatively brought to M_1V_2 and M_1V_3 stages. 111 mutant individuals (M_1V_3) and three commercial varieties (Fortune, Clementine and Okitsu Wase) were used in the study in 2015.

Alternaria brown spot disease isolates have been obtained from commercial 'Minneola tangelo' orchards in the Province of Adana, Turkey. In the sequence analysis of this isolate a matchup of 99% on the scale of ITS1 has been registered with the AHS-467-6 isolate.

Young leaves of 30-40 mm width were inoculated with a spore suspension containing 10^6 spores per ml (Kohmoto et al. 1991; Canıhoş et al. 1999; Dalkilic et al. 2005). The inoculation was administered in the form of spraying 2 drops (40 µl) of the spore suspension on the lower surface of each leaf. 4 leaves from each individual mutant were used. The control leaves were inoculated with distilled sterile water. The inoculated leaves were left for incubation at 27°C in a dark and humid environment. The leaves were examined 48 hours after the inoculation. To measure the intensity of the disease necrotic lesions on the leaves were marked with a positive (+) sign and those with no necrotic lesions were marked with a negative (-) sign. The procedure was conducted on a total of 114 genotypes using the randomized plot design method. The *in vivo* study was repeated for a second time to control and verify the results.

Following the *in vitro* analysis the individuals identified as being tolerant to the disease were grafted to develop into shoots. These shoots were tested for resistance to Alternaria brown spot disease. The mother stock genotypes on which the grafting was done were pruned in order to encourage the growth of new shoots and leaves to facilitate the assessment of the sensitivity of the plants to the disease. Following the pruning, when new leaves reached lengths of 1 to 3 cm. each leaf was inoculated with 2 ml spore suspension containing $5x10^5$ spores per ml of the suspension (Azevedo et al. 2010). Following the inoculation, the plants were transferred into polyethylene bags in order to preserve the humidity and prevent the drying of the leaves. Symptoms of the disease are usually appearing 24 hours after the inoculation (Dalkilic et al. 2005). For this reason, symptoms of the appearance of the disease were examined at this time.

3. Results and Discussion

The *in vivo* examination of 114 genotypes including 111 mutant individuals and 3 commercial varieties (Fortune, Clementine and Okitsu Wase) showed that 102 of the mutant individuals (91.89%) were found to be quite sensitive. Of the commercial varieties the Clementine and Okitsu Wase were found to be tolerant to the brown spot disease while Fortune proved to be quite sensitive (Table 1).

The pathogen produces host-selective ACT toxin, and several genes (named ACTT) responsible for ACT-toxin biosynthesis have been identified. A special toxin (ACT) which measures the extent of hosting by the *Alternaria alternata* pv. *citri* factor and at the same time determined the sensitivity of certain tangerine varieties and their hybrids has been produced. There are different studies on the sensitivity or resistance of citrus varieties to the ACT toxin with varying findings. Kohmoto et al. (1991) reported 28 citrus varieties sensitive to the citrus pathotype including tangelo, tangerine and tangors. Solel and Kimchi (1997) found 'Minneola tangelo', 'Dancy' 'Ellendale' 'Murcott', 'Nova', 'Satsuma', 'Orlando Tangelo' and 'Page' to be sensitive. Similarly, other studies showed 'Daisy', 'Temple x Dancy' and 'Satsuma x Murcott' to be sensitive (Stuart et al. 2009). While Solel and Kimchi (1997) reported Satsuma to be sensitive to the pathogen, the present study finds Okitsu Wase, which is a tangerine belonging to the Satsuma group to be tolerant to the pathogen.

The results of the *in vitro* and *in vivo* analyses in the present study confirm each other. Similar to the findings of Souza et al. (2009), our study finds the severity of the disease to be higher in *in vitro* experiments as compared to the *in vivo* experiments.

4. Conclusion

Our study has determined the sensitivity of some Fortune tangerine mutant individuals and some commercial varieties to the Alternaria brown spot disease. 99.91% of mutant individuals have been found to be sensitive to this disease. Our study has developed 9 individual mutants resistant to the Alternaria brown spot disease; 7 of these having received a dose of 50 (1-5-1; 5-3-2; 2B; 1A; 2A; 4-3-6; 7-4-1) and 2 individuals having received a dose of 60 gray (6B and 6D).

Table 1. *In vitro* study of the sensitivity of citrus genotypes to the Alternaria brown spot disease.

Mutation dose (gray)	Genotype	Lesion diameter (mm)*	Presence of lesions	Mutation dose (gray)	Genotype	Lesion diameter (mm)*	Presence of lesions
50	7-5-5	1.00 yz	+	50	2-3-4	4.50 nw	+
50	7-2-4	1.00 yz	+	50	1-3-4	6.50 fp	+
50	7-5-4	1.00 yz	+	50	4-2-2	4.00 px	+
50	6-5-1	5.50 js	+	50	2-2-4	5.25 kt	+
50	1-5-3	7.75 bk	+	50	3-3-5	3.25 sy	+
50	1-2-1	2.25 vz	+	50	4-5-2	2.50 uz	+
50	1C	2.75 ty	+	50	4-3-1	5.00 lu	+
50	7-5-6	3.25 sy	+	50	5-3-6	4.00 px	+
50	1-4-1	1.00 yz	+	50	3C	3.25 sy	+
50	1-4-2	1.50 xz	+	50	1	5.00 lu	+
50	7-1-1	3.75 qx	+	50	5-3-4	5.50 js	+
50	2-5-1	4.75 mv	+	50	4-5-1	3.50 ry	+
50	1-2-2	6.50 fp	+	50	4-2-1	7.50 cl	+
50	7-5-2	4.00 px	+	50	4-3-3	8.00 bj	+
50	1-2-3	3.25 sy	+	50	4-5-3	9.75 ac	+
50	2-3-2	3.75 qx	+	50	3-3-4	4.75 mv	+
50	7-2-1	5.00 lu	+	50	3-3-3	5.00 lu	+
50	5-4-2	7.50 cl	+	50	5-2-1	7.00 dn	+
50	4-4-2	5.50 js	+	50	4-4-1	5.50 js	+
50	1-5-2	3.75 qx	+	50	3-3-1	6.00 hr	+
50	7-5-3	5.00 lu	+	50	1-3-2	8.25 bı	+
50	4	9.00 bf	+	50	5-2-3	5.25 kt	+
50	3	1.00 yz	+	50	5-3-1	5.50 js	+
50	2	3.75 qx	+	50	6-2-3	3.25 sy	+
50	6-4-3	10.25 ab	+	50	5-1-4	3.50 ry	+
50	1B	5.25 kt	+	50	4-3-4	4.00 px	+
50	5-4-3	5.00 lu	+	50	6-2-1	4.25 ow	+
50	5	6.25 gq	+	50	6-2-4	5.00 lu	+
50	5-4-1	5.50 js	+	50	6-4-1	6.00 hr	+
50	3A	1.00 yz	+	50	5-3-3	11.75 a	+
50	2D	5.50 js	+	50	6-2-5	5.00 lu	+
60	6C	2.00 wz	+	50	4-4-3	5.75 ıs	+
50	2C	4.50 nw	+	50	5-3-5	10.25 ab	+
60	6A	2.75 ty	+	50	5-1-3	4.00 px	+
50	2-2-2	7.75 bk	+	50	6-2-6	5.25 kt	+
50	7A	2.75 ty	+	50	6-2-2	6.25 gq	+
50	3B	2.50 uz	+	50	5-1-2	6.25 gq	+
50	4-4-5	8.00 bj	+	50	1-3-1	9.25 ae	+
50	4-3-2	3.50 ry	+	50	3-4-1	5.75 ıs	+
50	5-1-1	6.00 hr	+	50	5-2-2	9.50 ad	+
50	4-3-7	2.00 wz	+	50	3-3-2	7.25 cm	+
50	6-4-2	4.75 mv	+	50	3-4-2	8.25 bı	+
50	4-3-5	7.50 cl	+	50	4-5-4	8.50 bh	+
50	2-2-3	6.00 hr	+	50	1-3-5	7.50 cl	+
50	4-4-4	7.25 cm	+	50	2-2-1	8.75 bg	+
50	7-2-3	6.25 gq	+	50	7-5-1	10.25 ab	+
50	7-4-2	9.50 ad	+	50	7-3-1	8.75 bg	+
50	2-3-1	8.50 bh	+	50	7-1-3	5.25 kt	+
50	7-2-2	9.00 bf	+	50	1-3-3	4.50 nw	+
50	7-1-2	1.00 yz	+	50	2-3-3	6.75 eo	+
50	2-5-2	7.25 cm	+	50	4-2-3	5.25 kt	+
50	**1-5-1**	0.00 z	-	60	**6B**	0.00 z	-
50	**5-3-2**	0.00 z	-	60	**6D**	0.00 z	-
50	**2B**	0.00 z	-	50	**2A**	0.00 z	-
50	**1A**	0.00 z	-	50	**4-3-6**	0.00 z	-
Clementine		0.00 z	-	50	**7-4-1**	0.00 z	-
Okitsu Wase		0.00 z	-	Fortune		4.75 mv	+

*The values with different letters are significantly different at p< 0.05.

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