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Evaluation of Fire Blight (*Erwinia amylovora*) Disease Reaction of Pear Hybrid Combinations

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ABSTRACT

Fire blight caused by pathogenic bacterium *Erwinia amylovora*, is the serious disease of pear. Since there is no effective chemical management to this disease except antibiotic-type compounds, it is very important to improve new fire blight resistant cultivars. In this research, it was aimed to select and develop fire blight resistant pear types and to determine fire blight susceptibility levels of pear hybrids, obtained from different projects. Hybrid plants were inoculated by shoot injections twice each year. Evaluations were made through necrotic shoot rate and susceptibility levels of hybrids were determined. During the experiments, 7036 hybrid pear seedlings inoculated, and 12.28% of them were found as "very low susceptibility" (A), 3.62% as "low susceptibility" (B) classes.

Keywords: Artificial inoculation; *Erwinia amylovora*; Hybrid; Pear

Farklı Armut Melezleme Kombinasyonlarında Ateş Yanıklığı Hastalığı (*Erwinia amylovora*) Reaksiyonunun Değerlendirilmesi

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ÖZET

Erwinia amylovora bakterisinin neden olduğu ateş yanıklığı hastalığı, armudun önemli hastalıklarından biridir. Hastalığa karşı, antibiyotikler dışında etkili bir kimyasal mücadele bulunmaması nedeni ile ateş yanıklığına dayanıklı yeni

çeşitlerin geliştirilmesi çok önemlidir. Bu araştırmada, ateş yanıklığına dayanıklı armut tiplerinin seçilmesi ve geliştirilmesinin hedeflenmesinin yanısıra farklı projelerden farklı melezleme kombinasyonlarıyla elde edilen melezlerin ateş yanıklığına hassasiyet seviyelerinin belirlenmesi amaçlanmıştır. Melez bitkiler sürgün enjeksiyonu yoluyla her yıl iki kez inokule edilmişlerdir. Nekroz oluşturan sürgün oranına göre değerlendirmeler gerçekleştirilmiş ve hassasiyet sınıfları belirlenmiştir. Denemeler boyunca, inokule edilen toplam 7036 melez bitkiden, % 12.28'i "çok az duyarlı" (A), % 3.62'si ise "az duyarlı" (B) sınıfta yer almışlardır.

Anahtar Kelimeler: Suni inokulasyon; *Erwinia amylovora*; Melez; Armut

1. Introduction

Pear is one of the most important pome fruits in the World and included in family *Rosaceae*, and genus *Pyrus*. Most of the cultivated high quality varieties are belong to *Pyrus communis* species. Fire blight disease caused by *Erwinia amylovora* is the most destructive bacterial disease of pear (Van Der Zwet & Beer 1995). The disease occurs extensively in several regions of the World where pear and species belong to *Rosaceae* family is grown. Fire blight disease brings out significant damages at nurseries and affects trade seriously. *Pyrus communis* species namely European pear is highly susceptible to fire blight. On the other hand, other major species as *P. ussuriensis, P. pyrifolia,* and *P. serotina* are resistant to the disease (Shoemaker & Teskey 1959).

E. amylovora affects all upper organs as flowers, shoots, branches of host plants and kills whole plant. Disease development is connected to environment, host and pathogen interaction, and intensity and damage changes through year (Van Der Zwet & Beer 1995).

Cultural practices, different chemical and biological management methods can be used as integrated management against fire blight, though there is no certain management technique (Aysan et al 1999). Using resistant cultivars, rootstocks and interstocks can be thought as the best way of controlling fire blight disease (Layne & Quamme 1975; Bergamaschi et al 2006).

Related to resistance to fire blight of *E. amylovora*, disease reaction of pear cultivars and cultigens was investigated in different countries (Layne & Quamme 1975; Hasler & Kellerhals 1997; Saygılı et al 1999; Honty et al 2006; Sestras et al 2008; Ellis 2010; Yoder & Biggs 2010). To obtain resistant cultivars and rootstocks, hybridization breeding is widely used because of polygenic inheritance of fire blight disease (Bell et al 2005). Besides, new resistant varieties were improved by selection (Saygılı et al 1999), hybridization (Ryugo 1982; Durel et al 2004; Hevesi et al 2004; Hunter & Layne 2004; Bergamaschi et al 2006), and molecular level studies as gene transfers (Reynoird et al 1999 a, b; Chevreau et al 2000; Aldwinckle et al 2003; Brown 2003). Susceptibility of the hybrids, obtained from inter- and intra-specific crosses, to *E. amylovora* is determined by artificial inoculations (Layne & Quamme 1975).

Turkey is one of the origins of pear plant. Most of the superior quality pear cultivars grown in Turkey are known as susceptible to fire blight. In Turkey, hybridization projects have been performed to obtain fire blight resistant and high quality cultivars (Evrenosoğlu et al 2011; Öztürk et al 2011). In this study, different crosses [resistant x resistant; resistant x susceptible; susceptible x resistant; susceptible x susceptible (as the fire blight resistance character known as recessive and poligenic (Layne & Quamme 1975))] have been made between susceptible and resistant cultivars and cultigens to obtain fire blight resistant hybrids. The susceptibility levels of hybrids were determined by artificial inoculations and "very low susceptibility" (A) and "low susceptibility" (B) F_1 hybrids were planted in Eskişehir province of Turkey.

2. Material and Methods

2.1. F_1 hybrid plants

In this study, totally 10751 F_1 pear hybrids were used as material. For this purpose, different crosses (resistant x resistant; resistant x susceptible; susceptible x resistant; susceptible x susceptible) (Rosati et al 2002) have been made through research projects supported by TUBITAK (project numbers TOVAG 106O719 and 110O938). As maternal parents, susceptible 'Akça', 'Santa Maria' and 'Williams', moderate susceptible 'Mustafa Bey', and resistant 'Magness' and 'Kieffer' cultivars were used, and as pollinators, different resistant or susceptible cultivars and cultigens ('Akça', 'Ankara', 'Bursa', 'Conference', 'Güz', 'Kaiser Alexandre', 'Kieffer', 'Limon', 'Moonglow', 'Santa Maria', 'Taş', 'Williams') were used (Momol et al 1992; Van Der Zwet & Beer 1995; Ünal et al 1998; Aysan et al 1999; Çıtır & Mirik 1999; Öden 1999; Bell et al 2005; Honty et al 2006).

2.2. Pathogenic bacteria

Highly virulent six *E. amylovora* strains, that were chosen according to their pathogenicity levels among 75 *E. amylovora* strains, isolated by Aysan et al (2004), Saygılı et al (2004), and Yılmaz & Aysan (2009) from different locations in Turkey (Adana, Amasya, Bursa, Eskisehir, Karaman and Konya), were used in the study.

2.3. Artificial inoculation

Inoculation of the pathogen was carried out twice each year, in May and August, between 2009 and 2011, on the same material. Equal amount of bacterial suspension was injected to the top of the shoots of each hybrid. As control, saplings of susceptible parents were inoculated by *E. amylovora* using the same procedure. After inoculation with pathogenic bacteria, plants were screened for eight weeks in greenhouse at 80-90% humidity and 27 ºC (Quamme et al 1976) and routinely fertilized and irrigated.

2.4. Evaluation

Disease development was examined through eight weeks and at the end of this period, necrotic parts of inoculated shoots were measured in proportion to the whole shoot length. Susceptibility level of the shoots was calculated as 'Variety Susceptibility Value' using the Equation 1 (Thompson et al 1962).

$$\text{Variety susceptibility value} = \frac{\text{Length of necrotic shoot (cm)}}{\text{Total shoot length (cm)}} \text{x100} \quad (1)$$

Susceptibility values were calculated according to the table performed by Thibault et al (1987) and susceptibility characters and classes of hybrids were detected by scoring "A"-"E" susceptibility levels (Table 1).

3. Results and Discussion

Distribution of hybrids to susceptibility classes in general evaluation of all combinations at the end of two different inoculation periods each year is as follows; 12.28% of inoculated hydrids were found as "very low susceptibility" (class "A"), 3.62% were "low susceptibility" (class "B"), 5.56% were "moderate susceptibility" (class "C"), 11.34% were

Table 1- Evaluation of susceptibility of pear tree against *E. amylovora* through artificial inoculation (Thibault et al 1987)

Çizelge 1- Suni inokulasyon sonucu armut ağacının E. amylovora'ya karşı hassasiyetinin değerlendirilmesi (Thibault et al 1987)

Variety susceptibility value	*0-10%*	*11-20%*	*21-40%*	*41-60%*	*61-100%*
Susceptibility class	A	B	C	D	E
Susceptibility character	Very low susceptibility	Low susceptibility	Moderate susceptibility	High susceptibility	Very high susceptibility

"high susceptibility" (class "D"), and 67.20% were "very high susceptibility" (class "E"). 32.65% of totaly inoculated 10751 hybrids were died after inoculations (Table 2 and 3).

Totaly 304 inoculated hybrids of susceptible 'Akça' combinations were distributed to susceptibility classes (14.47% were in class "A", 2.96% were in class "B", 2.30% were in class "C", 4.93% were in class "D", 75.33% were in class "E"). 15.79% of hybrids were killed by *Erwinia amylovora*. 'Akça' x 'Kieffer' (18.75%) and 'Akça' open pollination (21.39%) had the highest amount of class "A" hybrids among all 'Akça' combinations. On the other hand, in proportion of 62.50-94.12% class "E" hybrids were obtained from all 'Akça' combinations. The highest died hybrid rate was found in the combination of 'Akça' x 'Conference' (40.38%) and 'Akça' x 'Taş' (41.67%) (Table 2).

As for evaluation of all combinations of resistant 'Kieffer' parent, 5.30% of totally inoculated 604 hybrid plants were "very low susceptibility" character, 2.15% were "low susceptibility" character, 5.63% were "moderate susceptibility" character, 13.25% were "high susceptibility" and 73.68% were "very high susceptibility" character. Only 3.81% of hybrids were died after inoculations. Although maternal parent is resistant to *Erwinia amylovora*, hybrid number belong to class "A" (very low susceptibility) was quite low (4.01-7.39%) (Table 2).

On the combinations of the resistant parent 'Magness', there were much more hybrids on "very low susceptibility" character (Class "A"), when compared to susceptible parents. 'Magness' x 'Kieffer' (48.62%) combination was remarkable in terms of high rates of class "A" hybrids, compared to other combinations. It is stated that, 'Magness' x 'Kaiser Alexandre' combination had the same remarkable conclusion on high rates of class "A" hybrids (54.10%), same as this combination (Evrenosoğlu et al 2014). The reason of this high resistance rates can be explained as high fire blight resistance of 'Magness' maternal parent (Spotts & Mielke 1999; McGraw 2006), besides, high fire blight resistance of pollinator varieties (Momol et al 1992; Honty et al 2006). Magness cultivar is thought to be used as pollinator for several combinations, but as this cultivar is pollen sterile, it could not be used as pollinator for any combination. The lowest hybrid rates on class "A" were determined on 'Magness' x 'Santa Maria' and 'Magness' x 'Akça' combinations with rates of 13.33% and 17.86%, respectively. Distribution of the hybrids to classes for Magness combinations as follows; 29.22% of inoculated 1509 'Magness' hybrids were placed in class "A", 7.95% were in class "B", 14.45% were in class "C", 21.87% were in class "D" and only 26.51% were in class "E" (Table 2).

When it comes to the distribution of 73 hybrid plants of moderate susceptible 'Mustafa Bey' combinations, 39.73% of them were took place in class "A", on the other hand, 41.10% of them were in class "E". Class "A" hybrid rate of 'Mustafa Bey' x 'Moonglow' combination (56.10%) was higher than other combinations. No hybrid loss has been detected in all 'Mustafa Bey' combinations (Table 2).

As it was seen in Table 3, totally 1113 hybrid plants of 'Santa Maria' combinations were distributed to susceptibility classes as, 3.59% of hybrids were belong to "very low susceptibility" character (A), 1.35% were belong to "low susceptibility" character (B), 2.34% were belong to "moderate susceptibility" character (C), 7.46% were belong to "high susceptibility" character (D), and 85.27% were belong to "very high susceptibility" character (E). 'Santa Maria' open pollination (7.36%) and 'Santa Maria' x 'Kieffer' combination (6.83%) had the highest class "A" hybrids in 'Santa Maria' combinations. On the other hand, any class "A" hybrids have not obtained from 'Santa Maria' x 'Güz' combination (Table 3). It was found that, 'Santa Maria' x 'Moonglow' combination had the highest class "A" hybrids (14.85%), compared to other 'Santa Maria' combinations (Evrenosoğlu et al 2014).

Table 2- Distribution of hybrid plants to susceptibility classes

Çizelge 2- Melez bitkilerin hassasiyet sınıflarına dağılımı

Combinations	*Total hybrid number*	*Distribution of hybrid plants to susceptibility classes*										*Died hybrids*	
		A		*B*		*C*		*D*		*E*			
		No	*%*	*No*	*%*	*No*	*%*	*No*	*%*	*No*	*%*	*No*	*%*
'Akça'													
x 'Conference'	52	3	5.77	-	0.00	3	5.77	-	0.00	46	88.46	21	40.38
x 'Kaiser Alexandre'	20	1	5.00	2	10.00	-	0.00	1	5.00	16	80.00	5	25.00
x 'Kieffer'	16	3	18.75	2	12.50	-	0.00	1	6.25	10	62.50	1	6.25
x 'Santa Maria'	14	-	0.00	-	0.00	-	0.00	1	7.14	13	92.86	-	0.00
x 'Taş'	12	-	0.00	-	0.00	-	0.00	3	25.00	9	75.00	5	41.67
x 'Williams'	17	-	0.00	1	5.88	-	0.00	-	0.00	16	94.12	3	17.65
Open Pollination	173	37	21.39	4	2.31	4	2.31	9	5.20	119	68.79	13	7.51
'Akça' TOTAL	304	44	14.47	9	2.96	7	2.30	15	4.93	229	75.33	48	15.79
'Kieffer'													
x 'Santa Maria'	374	15	4.01	5	1.34	11	2.94	54	14.44	289	77.27	14	3.74
Open Pollination	230	17	7.39	8	3.48	23	10.00	26	11.30	156	67.83	9	3.91
'Kieffer' TOTAL	604	32	5.30	13	2.15	34	5.63	80	13.25	445	73.68	23	3.81
'Magness'													
x 'Akça'	280	50	17.86	13	4.64	30	10.71	75	26.79	112	40.00	39	13.93
x 'Ankara'	407	125	30.71	23	5.65	55	13.51	131	32.19	73	17.94	12	2.95
x 'Güz'	27	9	33.00	1	3.70	3	11.11	4	14.81	10	37.03	-	0.00
x 'Kieffer'	327	159	48.62	44	13.46	48	14.68	37	11.31	39	11.93	7	2.14
x 'Limon'	27	10	37.04	-	0.00	-	0.00	2	7.41	15	55.56	1	3.70
x 'Santa Maria'	105	14	13.33	8	7.62	12	11.43	6	5.71	65	61.90	2	1.90
x 'Taş'	265	55	20.75	29	10.94	59	22.26	64	24.15	58	21.89	23	8.68
Open Pollination	71	19	26.76	2	2.82	11	15.49	11	15.49	28	39.44	4	5.63
'Magness' TOTAL	1509	441	29.22	120	7.95	218	14.45	330	21.87	400	26.51	88	5.83
'Mustafa Bey'													
x 'Güz'	8	2	25.00	-	0.00	-	0.00	-	0.00	6	75.00	-	0.00
x 'Moonglow'	41	23	56.10	2	4.88	2	4.88	3	7.32	11	26.83	-	0.00
x 'Williams'	24	4	16.67	-	0.00	3	12.50	4	16.67	13	54.17	-	0.00
'Mustafa Bey' TOTAL	73	29	39.73	2	2.74	5	6.85	7	9.59	30	41.10	-	0.00

No, hybrid number; %, rate of hybrids to total hybrid number

Distribution rates of "A", "B", "C", "D", and "E" classes of 3433 hybrid plants in 'Williams' combinations were 8.09%, 2.80%, 2.94%, 8.24%, and 77.92%, respectively. 'Williams' x 'Moonglow' combination (22.57%) had the highest rate of "very low susceptibility" class "A" hybrids among all 'Williams' combinations. But, only 0.88% of hybrids of 'Williams' x 'Taş' combination was included in class "A" (Table 3).

4. Conclusions

In the current study, totaly 4739 hybrids of inoculated 7036 hybrids survived and distributed to different susceptibility classes. Totally, 32.65% of all inoculated hybrids were killed by fire blight disease (Table 3). Distribution of hybrid plants, obtained from 'Magness' parent, to susceptibility classes was quite different than the other parents

Table 3- Distribution of hybrid plants to susceptibility classes

Çizelge 3- Melez bitkilerin hassasiyet sınıflarına dağılımı

Combinations	*Total hybrid number*	*Distribution of hybrid plants to susceptibility classes*										*Died hybrids*	
		A		*B*		*C*		*D*		*E*			
		No	*%*	*No*	*%*	*No*	*%*	*No*	*%*	*No*	*%*	*No*	*%*
'Santa Maria'													
x 'Bursa'	70	4	5.71	4	5.71	-	0.00	11	15.71	51	72.86	36	51.43
x 'Güz'	64	-	0.00	2	3.13	1	1.56	3	4.69	58	90.63	27	42.19
x 'Kieffer'	161	11	6.83	3	1.86	9	5.59	15	9.31	123	76.40	97	60.25
x 'Taş'	453	6	1.32	2	0.44	11	2.43	44	9.71	390	86.09	360	79.47
x 'Williams'	134	2	1.49	-	0.00	1	0.75	3	2.24	128	95.52	14	10.45
Open Pollination	231	17	7.36	4	1.73	4	1.73	7	3.03	199	86.15	56	24.24
'Santa Maria' TOTAL	1113	40	3.59	15	1.35	26	2.34	83	7.46	949	85.27	590	53.01
'Williams'													
x 'Akça'	176	9	5.11	4	2.27	1	0.57	7	3.98	155	88.07	4	2.27
x 'Ankara'	1127	61	5.41	16	1.42	19	1.69	47	4.17	984	87.31	577	51.20
x 'Bursa'	44	1	2.27	1	2.27	-	0.00	2	4.55	40	90.91	18	40.91
x 'Güz'	42	1	2.38	1	2.38	1	2.38	3	7.14	36	85.71	3	7.14
x 'Kaiser Alexandre'	417	35	8.39	31	7.43	33	7.91	65	15.59	253	60.67	174	41.73
x 'Kieffer'	242	21	8.68	3	1.24	5	2.07	29	11.98	184	76.03	70	28.93
x 'Moonglow'	514	116	22.57	28	5.45	24	4.67	64	12.45	282	54.86	260	50.58
x 'Santa Maria'	184	19	10.33	4	2.17	7	3.80	21	11.41	133	72.28	110	59.78
x 'Taş'	454	4	0.88	3	0.66	3	0.66	31	6.83	413	90.97	279	61.45
Open Pollination	233	11	4.72	5	2.15	8	3.43	14	6.01	195	83.69	53	22.75
'Williams' TOTAL	3433	278	8.09	96	2.80	101	2.94	283	8.24	2675	77.92	1548	45.09
GENERAL TOTAL	7036	864	12.28	255	3.62	391	5.56	798	11.34	4728	67.20	2297	32.65

No, hybrid number; %, rate of hybrids to total hybrid number

such as 'Santa Maria', 'Williams' and 'Akça'. Hybrid rates in "A", "B", "C", and "D" classes were much higher, but hybrid rate in class "E" was lower in 'Magness' combinations, when compared to other parents. Another parent that had significantly high rate of class "A" hybrids was 'Mustafa Bey' (39.73%) (Table 2). Furthermore, died hybrid rate after inoculations was 53.01% and 45.09% in 'Santa Maria' and 'Williams' parents respectively, and this rate was only 5.83% for 'Magness' combinations (Table 2 and 3). Other parents that had low rates of died hybrids were 'Mustafa Bey' (0.00%) and 'Kieffer' (3.81%) (Table 2). On the combinations of the resistant parent 'Magness', much more hybrids on "very low susceptibility" character were detected (Class "A"). Additionally, high rates of class "A" hybrids were obtained from 'Magness' x 'Kieffer' combinations (approximately 50%) (Table 2).

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