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Evaluation of Pear (*Pyrus communis* L.) Hybrid Combinations for the Transmission of Fire Blight Resistance and Fruit Characteristics

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Abstract

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Fire blight is one of the most destructive diseases of pome fruits, especially pears. In current conditions under increasing demand for organic products, improvement of resistant rootstock and varieties is becoming important due to the lack of an effective disease management method against fire blight caused by *Erwinia amy-lovora* as well as the harmful effects of chemicals on environment, human and animal health. The findings of the researchers as to which variety performs well in terms of the transmission of fire blight resistance are important for new breeding programs. In the study, three varieties with high commercial value (Magness, Santa Maria and Williams) were identified as the maternal parents and 21 hybrid combinations were made with seven varieties (Akça, Ankara, Conference, Güz, Kaiser Alexandre, Kieffer and Taş) as pollinators. The maternal parent Magness was found superior when compared with the other maternal parents for the susceptibility level to fire blight. Apart from the differences in the susceptibility level of 21 different hybrid combinations to fire blight, the commercially superior ten hybrids (I-15-24, II-13-1, II-13-19, II-13-34, II-13-73, II-27-21, II-27-55, II -32-44, III-22-638, III-27-590) were selected by the weighted ranking of disease resistance and different fruit characteristics based on commercial preferences such as fruit quality, attractiveness, size, firmness and rustiness.

Keywords: *Erwinia amylovora*; fruit quality; pear breeding; weighted ranking method

Pear (*Pyrus communis* L.) is one of the important fruit species of the world as for production and export quantity. Pear production of the world was 25 798.644 tonnes in 2014. China, Argentina, United States of America, Italy, Turkey, Spain, South Africa, Belgium, Netherlands, and India were top ten pear producers in 2014 (FAO 2014). Pear export quantity of the world in 2013 was 2 488.364 tonnes (FAO 2013).

In order to optimize the pear production, great importance should be devoted to fighting with the most devastating disease, the fire blight. The facts that no definitive chemical management has been found, the proposed chemicals are expensive and harmful to human health, residues are found during the customs controls and organic farming is becoming more important highlighted the need to use resistant varieties, rootstocks and intermediate rootstocks in the control of the disease. For all these reasons, attention is drawn to the need to emphasize the improvement of varieties resistant to fire blight caused by *Erwinia amylovora* (Layne & Quamme 1975; Lombard & Westwood 1987).

Due to the polygenic nature of fire blight resistance and the complexity of its mechanism, controlled hybridization is generally used in breeding programs (Layne *et al*. 1968; Bell *et al*. 2005). For this reason, studies have been carried out in many countries primarily on determining the susceptibility level of

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genetic resources (Layne & Quamme 1975; Hasler & Kellerhals 1995; Aysan *et al*. 1999; Saygili *et al*. 1999; Sestras *et al*. 2008). Aurora, Bartlett, Bosc, Clapp's Favorite, Red Bartlett, Reimer Red and Starkrimson varieties were classified as very susceptible; Maxine and Seckel varieties were middle susceptible; Kieffer, Magness, Moonglow and Old Home varieties were determined as middle resistant to *E. amylovora* (Ellis 2010). In another study, Bartlett, Clapp's Favorite, Flemish Beauty, Gorham, Hardy, Sheldon and Winter Nelis varieties were found as very susceptible, d'Anjou, Comice, Douglas, Duchess, Ewart, Garber, Lincoln and Seckel were susceptible, and Kieffer, LeConte, Magness, Maxine, Moonglow, Starking Delicious, Tyson and Waite varieties were middle resistant (Yoder & Biggs 2010). Dr. Jules Guyot, Conference and Eldorado varieties were found very susceptible; Williams was found susceptible; Magness and Kaiser Alexandre were found middle susceptible; Kieffer, Harrow Delight and Moonglow were found less susceptible in terms of shoot blight (Honty *et al*. 2006).

As mentioned before, controlled hybridization with donors of resistance is a common method to obtain disease resistant varieties. The susceptibility or resistance of pear genotypes to *E. amylovora* obtained by hybridization and free pollination is determined by artificial inoculations (Dondini *et al.* 2002; Bergamaschi *et al*. 2006). AC Harrow Delicious and HW606 pear varieties were obtained from breeding programs, and compared to Williams and Dr. Jules Guyot varieties, it was mentioned that these varieties have improved tolerance to fire blight disease (Hunter & Layne 2004). In another program, Harrow Sweet, US309 and Michigan 437 varieties were chosen for fire blight resistance as donors of resistance genes, and Williams and Pierre Corneille were used for high fruit quality, and many cross combinations were made between these parents. Then, obtained hybrids were inoculated to determine their resistance to *E. amylovora* (Durel *et al.* 2004). The right choice of parents at the beginning of the study appears to be the most important criterion for obtaining the genotypes of the desired characteristics in the later stages of the study.

In this study, 21 hybridization combinations of crosses were carried out between three maternal parents and seven pollinators with different susceptibility to *E. amylovora*. All obtained hybrid individuals were analysed for susceptibility to *E. amylovora*. In addition, the fruits of the hybrid combinations were assessed by weighted ranking method to determine the ones with the superior commercial value. Thus, both the resistance to fire blight and the quality of fruits were investigated.

MATERIAL AND METHODS

The material consisted of 7834 hybrid plants obtained from 21 cross combinations where varieties Magness, Santa Maria and Williams were used as maternal component and varieties Akça, Ankara, Conference, Güz, Kaiser Alexandre, Kieffer and Taş were used as pollinators.

The susceptibility of hybrid plants was defined by artificial inoculations with seven *E. amylovora* isolates of very high virulence (Aysan *et al*. 2004; Saygili *et al*. 2004; Yilmaz & Aysan 2009). The hybrids were tested twice with a suspension of equal volumes of these isolates when their shoots reached at least 15–20 cm height.

Susceptibility of the shoots to the pathogen was calculated according to the formula shown below (Thompson *et al*. 1962) and the variety susceptibility (VS) value was calculated for each hybrid.

$$VS = (\text{length of the infected part (cm)}/\text{total length of shoot (cm)}) \times 100$$

In the study, a modified weighted ranking method was used to identify superior genotypes (Jaeger *et al*. 2003). Some of the hybrids, which start to bear, were evaluated by this method and the total scores of each genotype were determined. In the study, four commercially superior varieties (Ankara, Kieffer, Santa Maria, Williams) were included in the weighted ranking as a reference.

The parameters used in the modified weighted ranking method and the relative scores of the parameters and the class values and scores of these parameters are given in Table 1. International pear identification documents were used in selection of the criteria applied in the weighted ranking table and in setting the reference values of the criteria (UPOV 2000).

The study was based on the completely randomized factorial experimental design (3 × 7). In the statistical model of the study, the maternal parent effect is based on 3 varieties and the pollinator effect is based on 7 varieties while the interactions of the parents are also included in the model. It was investigated whether there is a statistical difference in the rate of fire blight susceptibility between the averages of the hybrids. A statistically significant ($P < 0.05$) dif-

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ference in the interaction averages is shown by the Tukey multiple comparison test. In the statistical analysis, the R software is used (Ver. 2.12.1, 2010).

RESULTS AND DISCUSSION

Susceptibility of hybrid combinations to fire blight disease. VS values to the fire blight disease were calculated in the F_1 offspring (totally 7834 hybrids) derived from controlled hybridization. The results are given in Table 2.

According to the results, the susceptibility of hybridization combinations to fire blight varied from 19.69% (Magness × Kieffer) to 92.31% (Williams × Taş).

All of the combinations in which the Magness variety was used as the maternal parent were found to be less susceptible to fire blight than the combinations of Santa Maria and Williams varieties. It is clear that the Magness variety as the maternal parent is more effective than the Santa Maria and Williams varieties in terms of the transmission of resistance to the disease.

It was found that when the Magness variety was used as the maternal parent, the most appropriate pollinators were Kieffer and Kaiser Alexandre for the transmission of resistance to fire blight; when

Table 1. A modified weighted ranking method for evaluation of pear genotypes

Parameter	Relative scores	Class values and scores of the characteristics	
Fire blight resistance	25	slightly susceptible	10
		less susceptible	8
		mid-susceptible	5
		susceptible	3
		very susceptible	1
Eating quality	15	very good	10
		good	7
		middle	4
		bad	1
Fruit attractiveness	15	very good	10
		good	7
		middle	4
		bad	1
Fruit size	10	very big	10
		big	8
		middle	5
		small	3
		very small	1
Length/diameter	10	very long	10
		long	8
		middle	5
		short	3
		very short	1
Soluble solids content	10	high	10
		middle	7
		low	3
Stone cell status of fruit	5	few	10
		middle	5
		lot	1
Fruit firmness	5	very firm	1
		firm	10
		middle	7
		soft	4
Rustiness	5	very few	10
		few	7
		middle	4
		lot	1

Table 2. Results of the Tukey HSD multiple comparison tests for the variety susceptibility (VS) means of pear genotypes obtained from different maternal parents and pollinator pear varieties

Maternal parent	Pollinator	No. of hybrids	VS mean (%) ± SD
Magness	Kiefer	326	19.69 ± 1.24^{a}
Magness	Kaiser Alexandre	122	21.21 ± 2.17^{a}
Magness	Ankara	407	36.92 ± 1.37^{b}
Magness	Conference	166	38.34 ± 2.11^{b}
Magness	Tas	265	38.85 ± 1.80^{b}
Magness	Guz	27	48.59 ± 6.38^{bcd}
Magness	Akca	280	50.01 ± 1.86^{c}
Willams	Kaiser Alexandre	417	67.41 ± 1.69^{de}
SantaMaria	Akca	696	67.80 ± 1.17^{e}
Willams	Akca	176	69.91 ± 1.96^{e}
SantaMaria	Conference	165	70.01 ± 2.43^{defgh}
Willams	Conference	657	70.05 ± 1.21^{e}
Willams	Kiefer	242	71.67 ± 2.01^{e}
Willams	Guz	42	75.19 ± 3.69^{ef}
SantaMaria	Ankara	949	77.29 ± 0.99^{fg}
SantaMaria	Kiefer	161	81.38 ± 2.42^{ghi}
SantaMaria	Kaiser Alexandre	630	82.21 ± 1.08^{ghij}
Willams	Ankara	1134	85.73 ± 0.77^{i}
SantaMaria	Guz	64	86.41 ± 2.94^{ghijk}
SantaMaria	Tas	453	90.33 ± 1.01^{jk}
Willams	Tas	455	92.31 ± 0.85^{k}

SD – standard deviation

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Table 3. The scores of the hybrid pear genotypes according to the weighted ranking method

Genotype	Disease resistance	Eating quality	Fruit attractiveness	Fruit size	Length/diameter	Soluble solids content	Stone cell status of fruit	Fruit firmness	Rustiness	Total
II-13-1 (Magness × Akça)	250	150	105	50	80	100	50	35	35	855
II-27-55 (Williams × Akça)	125	150	150	50	80	70	50	50	35	760
III-27-590 (Santa Maria × KA*)	75	150	150	100	80	70	50	50	35	760
II-27-21 (Williams × Akça)	125	150	150	50	80	70	50	35	35	745
II-32-44 (Williams × Taş)	75	150	150	80	80	70	50	50	35	740
I-15-24 (Magness × Akça)	250	105	105	50	50	70	25	35	35	725
II-13-73 (Magness × Akça)	75	150	150	30	100	100	50	35	35	725
III-22-638 (Santa Maria × Akça)	125	150	105	50	100	70	50	35	35	720
II-13-19 (Magness × Akça)	75	150	105	50	100	100	50	50	35	715
II-13-34 (Magness × Akça)	75	150	105	100	80	70	50	50	35	715
S. Maria	75	150	105	100	80	70	50	50	35	715
II-13-12 (Magness × Akça)	75	150	105	100	100	70	25	50	35	710
II-22-17 (Santa Maria × Kieffer)	75	105	105	100	100	100	25	50	35	695
II-31-144 (Williams × Kieffer)	25	150	150	50	80	100	50	50	35	690
III-34-292 (Williams × Conference)	125	105	60	100	80	100	50	35	20	675
II-21-7 (Santa Maria × Güz)	25	105	150	80	100	100	25	50	35	670
III-34-170 (Williams × Conference)	75	150	105	30	80	100	50	50	20	660
II-27-84 (Williams × Akça)	25	150	150	30	80	70	50	50	50	655
Kiefer	125	105	105	100	50	100	5	35	20	645
I-42-1 (Williams × KA)	125	105	105	80	50	100	25	35	20	645
II-31-137 (Williams × Kieffer)	75	105	150	80	50	100	25	35	20	640
III-35-249 (Williams × KA)	75	105	150	50	80	70	25	50	35	640
III-27-580 (Santa Maria × KA)	75	105	105	100	80	70	25	50	20	635
II-13-75 (Magness × Akça)	25	150	105	100	50	70	50	50	35	635
Williams	75	105	105	50	80	100	50	50	20	635
I-15-34 (Magness × Akça)	200	105	60	50	50	70	25	50	20	630
III-25-49 (Santa Maria × Conference)	75	105	105	80	80	70	25	50	35	625
II-22-542 (Santa Maria × Kieffer)	75	105	105	80	80	100	25	35	20	625
I-38-2 (Williams × Conference)	25	105	105	100	100	100	25	35	20	615
II-16-4 (Magness × Taş)	200	105	60	50	50	70	25	35	20	615
II-14-19 (Magness × Kieffer)	125	105	60	50	80	100	25	50	20	615
II-31-148 (Williams × Kieffer)	75	105	105	80	80	70	25	50	20	610
II-22-6 (Santa Maria × Kieffer)	25	105	150	80	80	70	25	35	35	605
II-24-1 (Santa Maria × Taş)	25	105	105	100	80	100	25	35	20	595
II-31-137 (Williams × Kieffer)	75	105	150	50	50	70	25	35	35	595
III-23-901 (Santa Maria × Ankara)	75	105	150	50	50	70	25	35	35	595
II-30-18 (Williams × Güz)	75	105	105	50	50	100	50	35	20	590
III-25-73 (Santa Maria × Conference)	75	105	105	30	80	70	50	35	35	585
II-21-29 (Santa Maria × Güz)	25	150	150	30	50	70	50	20	35	580

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Table 3 to be continuied

Genotype	Disease resistance	Eating quality	Fruit attractiveness	Fruit size	Length/diameter	Soluble solids content	Stone cell status of fruit	Fruit firmness	Rustiness	Total
II-19-7 (Santa Maria × Akça)	25	150	105	30	80	70	50	35	35	580
II-13-27 (Magness × Akça)	75	105	105	50	80	100	25	20	20	580
II-24-6 (Santa Maria × Taş)	75	105	60	80	50	100	25	35	35	565
I-15-20 (Magness × Akça)	200	60	60	30	50	70	25	35	35	565
Ankara	125	105	60	50	50	100	25	20	20	555
III-35-93 (Williams × KA)	75	105	60	80	30	100	50	35	20	555
II-28-405 (Williams × Ankara)	75	105	60	80	30	100	25	35	20	530
II-30-13 (Williams × Güz)	75	60	105	30	80	100	25	5	35	515
II-32-28 (Williams × Taş)	125	105	60	30	30	100	25	35	5	515
II-32-2 (Williams × Taş)	25	105	60	80	50	100	25	35	20	500
II-21-8 (Santa Maria × Güz)	75	105	60	30	50	70	25	50	35	500
II-13-40 (Magness × Akça)	25	60	105	80	80	70	25	20	35	500
III-27-513 (Santa Maria × KA)	25	105	105	50	50	70	25	35	20	485
II-24-13 (Santa Maria × Taş)	25	105	60	50	50	100	25	35	20	470
II-13-35 (Magness × Akça)	75	60	60	50	50	100	5	35	35	470
II-28-273 (Williams × Ankara)	75	105	60	50	30	100	25	35	20	470
II-28-249 (Williams × Ankara)	25	105	60	50	30	100	25	35	20	450
II-30-28 (Williams × Güz)	25	60	60	50	80	100	25	20	20	440
III-23-399 (Santa Maria × Ankara)	25	60	60	80	30	70	25	35	35	420
III-23-520 (Santa Maria × Ankara)	25	60	105	50	80	10	25	35	20	410
II-13-44 (Magness × Akça)	25	105	60	50	30	30	25	50	35	410

*Kaiser Alexandre

the Santa Maria variety was used as the maternal parent, the most appropriate pollinators were Akça and Conference; and when the Williams variety was used as the maternal parent, the most appropriate pollinators were Kaiser Alexandre, Akça, Conference and Kieffer varieties.

Momol *et al*. (1992) applied bacterial exudates isolated from the thin branches of pear trees in Bucak and Korkuteli to Ankara, Williams and Santa Maria pears. The result of their study was that the Ankara variety is the most resistant, Williams is moderately resistant and Santa Maria is the most susceptible. Çıtır and Mirik (1999) investigated the susceptibility levels to *E. amylovora* of apple and pear which are pome fruit species in Tokat and Amasya. As a result of the study, it was found that Ankara, Kieffer, Keklik Ayağı, Taş and Çiçek pears are resistant varieties while Santa Maria, Williams, Mustafa Bey and Akça varieties are very susceptible. In another similar study (Van Der Zwet & Beer 1991), Conference and Kaiser Alexandre were included in the moderately resistant group, while the Williams variety was in the most susceptible group to the pathogen. Kieffer, one of the important pear varieties, was resistant to blossom blight but moderately resistant to shoot blight. As to the Conference variety, it was found to be moderately resistant to shoot blight and very susceptible to blossom blight. Santa Maria and Williams varieties were susceptible to shoot blight and moderately resistant to blossom blight (Sobiczewski *et al*. 1997). In a study evaluating 13 pear species (Aysan *et al*. 1999),

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it was reported that Akça, Williams, Santa Maria, Laleliye, Deveci and Moonglow are very susceptible, Ankara, Mustafa Bey, Çermai and Hacı Hamza are moderately susceptible, Limon, Kieffer and Mıgırık varieties are less susceptible.

As it can be understood from the literature, the varieties Ankara, Conference, Kaiser Alexandre, Kieffer and Magness were found to be relatively more resistant to the disease while Akça, Santa Maria and Williams varieties were found to be susceptible. In our study as well, the average varietal susceptibility values of hybrids obtained from disease-resistant parents were found to be more resistant to disease than other combination averages.

Evaluation of the fruits of hybrid plants. The results of the weighted ranking method evaluating the nine criteria of hybrid fruits are given in Table 3.

Totally 56 hybrids, which belong to different combinations, were evaluated in terms of fruit characteristics and 15 of them belonged to Magness, 19 of them belonged to Santa Maria, and 22 of them belonged to Williams maternal parents.

As a result of the modified weighted ranking method, the total score varied between 410 and 855. Among the commercial varieties used as a reference and included in the weighted ranking, Santa Maria (715 points) got the highest score among varieties. In this study, hybrids with the score higher than that of Santa Maria variety were candidates for a new variety (Figure 1).

When the Magness variety was used as the maternal parent, 33.3% of the hybrids were found to have a registry potential. As for Williams and Santa Maria maternal parents, the registry potential of the hybrids was 13.6%, and 10.5%, respectively. It was seen that the Akça variety, as a pollinator, is very superior to other varieties. It was found that the pollinator variety Akça was used for 8 out of 10 hybrids with a high variety potential (II-13-1, II-27-55, II-27-21, I-15-24, II-13-73, III-22- 638, II-13-19, II-13-34) (Table 3).

In the combinations in which the Magness variety was used as the maternal parent, the hybrids were generally tolerant to the pathogen, the fruits were superior in terms of attractiveness and eating quality, while the size of the fruit and the content of soluble solids were moderate. The hybrids in which the Williams variety was used as the maternal parent were generally susceptible to the pathogen and the fruit was irregularly shaped and rusty. Although the fruits were good in terms of eating quality, they fall behind

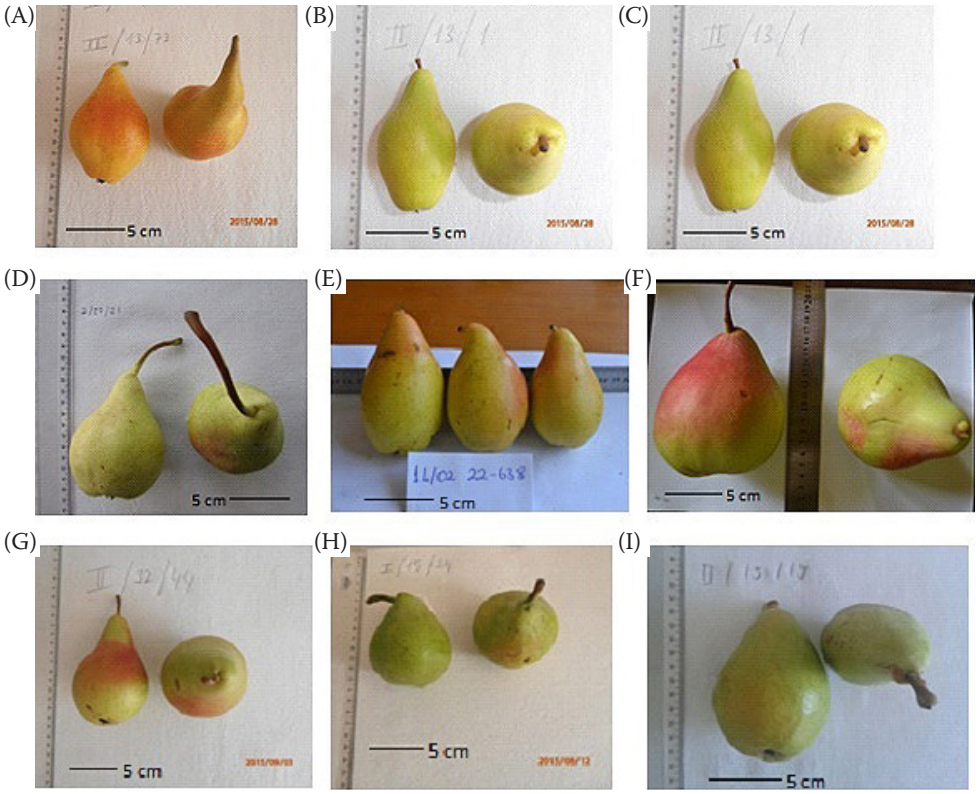

Figure 1. Fruits of some pear hybrids with registration potential: II-13-73 (A), II-13-1 (B), II-27-55 (C), II-27-21 (D), III-22-638 (E), III-27-590 (F), II-32-44(G), I-15-24 (H), II-13-19 (I)

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in other parameters. When the Santa Maria variety was used as the maternal parent, the hybrids were generally susceptible to the pathogen while the length/diameter ratio, the size and eating quality were good, and they had soluble solids content, attractiveness and fruit firmness on the moderate level.

Five of the ten hybrids (I-15-24, II-13-1, II-13-19, II-13-34, II-13-73) useful for registration as new varieties were from the combinations where the Magness variety was the maternal parent. Two (I-15-24 and II-13-1) hybrids belonged to the most resistant group to the pathogen and both were obtained from the Magness variety. Three of the hybrids belonging to Williams maternal parent (II-27-21, II-27-55, II-32-44) and two of the hybrids belonging to Santa Maria maternal parent (III-22-638 and III-27-590) were found to be useful for registration (Table 3).

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