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Original scientific paper

SUSCEPTIBILITY, HETEROSIS AND HETEROBELTIOSIS EVALUATIONS FOR FIRE BLIGHT RESISTANCE IN PEAR

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Fire blight is a devastating disease of Pomaceous trees that can cause death of the plants and leading to substantial crop losses. Controlled hybridization is widely used in breeding programs, due to the polygenic nature of fire blight resistance and the complexity of its mechanism. Right choice of parents appears to be the most significant criterion for obtaining genotypes that have desired characteristics. In this study 13 parental pear cultivars and 9749 F_1 pear hybrids that were obtained from 36 different hybridization combinations were evaluated for fire blight resistance. Manifestation of heterosis and heterobeltiosis were also determined. As results of the study, 'Magness' is foremost as the most resistant variety, and followed by 'Ankara', 'Moonglow', 'Kiefer' and 'Kaiser Alexandre', respectively. 'Conference', 'Williams' and 'Santa Maria' were found susceptible to disease, while 'Guz', 'Limon', 'Bursa', 'Akca' and 'Tas' were highly susceptible to disease. Maternal parent was more effective for the transmission of fire blight resistance, when compared to pollinator parent. For this reason, it is suggested that disease-resistant varieties should be used as maternal parent. Heterosis was detected in 12 hybridization combinations. However, heterobeltiosis-focused approach was more effective for resistance breeding. For fire blight resistance, 'Magness × Kaiser Alexandre' and 'Magness × Kiefer' hybridization combinations that heterobeltiosis was determined are recommended.

Keywords: Erwinia amylovora, maternal effect, genetic, resistance breeding, hybridization

INTRODUCTION

Fire blight, caused by pathogenic bacterium *Erwinia amylovora*, is a devastating disease of Pomaceous trees that can cause death of the plants and leading to significant crop losses

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(GAALICHE *et al.,* 2018). Although the pathogen is considered to be a quarantine organism and its introduction is prohibited by almost all countries, fire blight is common in all regions where members of *Rosaceae* family cultivation takes place (BONN and VAN DER ZWET, 2000). All plant organs except seeds are considered to be potential source of dissemination of this pathogen (VAN DER ZWET *et al.,* 2012).

E. amylovora has polyphagous nature but it causes biggest damage to pear species (PULAWSKA and SOBICZEWSKI, 2012). Most of the cultivated pear varieties worldwide are highly susceptible to fire blight. Susceptibility of these varieties to the disease is threatening pear cultivation.

In order to optimize pear production, great importance should be implemented against fire blight (REININGER *et al.,* 2017). Chemical management is not a definite solution. It causes negative effects on human and environmental health and reduces export level of pear because of residue problem in customs. Also consumers' demand for organic products is increasing (CEYLAN *et al.,* 2018). Use of resistant varieties, rootstocks and intermediate rootstocks are prominent ways in the control of the disease. Therefore, the importance of breeding fire blight-resistant varieties is emphasized (FAZIO *et al.,* 2015; HUNTER, 2016; KELLERHALS *et al.,* 2017; MERTOGLU and EVRENOSOGLU, 2017).

Hybridization is generally used in breeding studies, due to the polygenic control of resistance to fire blight and complexity of its mechanism (BOKSZCZANIN *et al.,* 2012). For this reason, studies have been carried out in many countries primarily on determining the susceptibility level of genetic resources for parent selection (HEVESI *et al.,* 2004; HONTY *et al.,* 2006; SESTRAS *et al.,* 2008; OZRENK *et al.,* 2012; KELLERHALS *et al.,* 2012; TOTH *et al.,* 2013; SOBICZEWSKI *et al.,* 2015; CALIS *et al.,* 2017; HARSHMAN *et al.,* 2017; ŞAHIN *et al*., 2020).

In this study 13 pear varieties and 9749 F_1 pear hybrids that were obtained from 36 different hybridization combinations formed with these 13 parental varieties were evaluated for fire blight resistance. As results of the study, susceptibility characteristics of parental varieties and manifestation of heterosis and heterobeltiosis were determined.

MATERIAL AND METHODS

F_1 hybrid plants

In this study, 13 parental varieties ('Santa Maria', 'Williams', 'Magness', 'Conference', 'Kaiser Alexandre', 'Kieffer', 'Moonglow', 'Akca', 'Ankara', 'Bursa', 'Guz', 'Limon' and 'Tas') and 9749 F_1 pear hybrids were used as material. These hybrids were obtained from 36 different hybridization combinations.

Pathogenic bacteria

Seven highly virulent *E. amylovora* strains, that were chosen according to their pathogenicity levels among 75 *E. amylovora* strains, isolated by AYSAN *et al.* (2004), SAYGILI *et al.* (2004) and YILMAZ and AYSAN (2009) from different locations in Turkey (Adana, Amasya, Bursa, Eskisehir, Karaman and Konya), were used in the study.

Evaluation for fire blight susceptibility

The susceptibility levels of hybrid plants and parental varieties were defined by artificial inoculations. Bacterial suspension (10^8 cell/milliliter density prepared from the 48-hour bacterial culture developed in King B medium) was injected to the top of the shoots by using syringe when their shoots reached approximately 15-30 cm height. Inoculations were made to two branches of each plant. Length of the infected part of the shoots was measured at the end of 8 weeks according to the formula (1) and then the average of two values was taken and the genotype susceptibility (GS) value was calculated for each hybrid and variety (MERTOGLU and EVRENOSOGLU, 2017).

$$\text{Genotype Susceptibility (GS)} = \frac{\text{Length of the Infected Part (cm)}}{\text{Total Length of Shoot (cm)}} \times 100 \qquad (1)$$

Statistical analysis

Difference between the susceptibility levels of varieties used as parents was determined using one-way ANOVA. The differences between the groups were determined by Tukey multiple comprasion test. Relative Heterosis was estimated as percent deviation of hybrid value from its mid-parental value using formula (2). Heterobeltiosis was calculated at the superiority of hybrid value from the better parent value using formula (3). Significance of the estimates of heterosis and heterobeltiosis were tested by using t-test (MISTRY *et al.*, 2018). In the formulas, abbreviations can be explained as, $\bar{F}_1$ = Mean of F_1 hybrids performance; $MeanP$ = Mean of mid parental and $BetterP$ = Mean performance of better parent.

$$\%\ Relative\ Heterosis\ (MPH) = \frac{\bar{F}_1 - MeanP}{MeanP} \times 100 \qquad (2)$$

$$\%\ \text{Heterobeltiosis (BP)} = \frac{\bar{F}_1 - BetterP}{BetterP} \times 100 \qquad (3)$$

RESULTS AND DISCUSSION

Susceptibility levels of parental varieties to fire blight varied between 33.0% (Magness) and 100.0 % (Tas). ‘Magness’ came forward as the most resistant variety, followed by ‘Ankara’, ‘Moonglow’, ‘Kiefer’ and ‘Kaiser Alexandre’, respectively. ‘Conference’, ‘Williams’ and ‘Santa Maria’ were found susceptible to disease, while ‘Guz’, ‘Limon’, ‘Bursa’, ‘Akca’ and ‘Tas’ were highly susceptible to disease (Table 1).

Table 1. Fire blight susceptibility of parental cultivars

Cultivars	Susceptibility to disease
Magness	33.0 ± 3.61a
Ankara	41.0 ± 4.00ab
Moonglow	45.7 ± 2.08bc
Kiefer	51.0 ± 1.73cd
Kaiser Alexandre	55.3 ± 3.51de
Conference	60.3 ±2.52e
Williams	60.7 ± 3.06e
Santa Maria	61.7 ± 2.08e
Guz	74.7 ± 4.73f
Limon	75.0 ±4.36f
Bursa	75.3 ± 2.52f
Akca	80.0 ± 2.65f
Tas	100.0 ± 0.0g

In previous studies, it was stated that 'Kiefer' variety was resistant to blossom blight while moderately resistant to shoot blight; 'Conference' was moderately resistant to shoot blight but very susceptible to blossom blight; 'Santa Maria' and 'Williams' were susceptible to shoot blight and moderately susceptible to blossom blight (SOBICZEWSKI *et al.,* 1997). As a result of similar researches, pears 'Williams' and 'Santa Maria' were determined to be susceptible to the disease, whereas 'Ankara', 'Mustafa Bey', 'Conference', and 'Kaiser Alexandre' were moderately susceptible, and 'Limon', 'Kiefer', and 'Magness' had low susceptibility (VAN DER ZWET and BEER, 1992; AYSAN *et al.,* 1999; HEVESI *et al.,* 2004; SESTRAS *et al.,* 2008; OZRENK *et al.,* 2012). As it can be understood from former studies in which the susceptibility of parental varieties to fire blight was investigated, s 'Ankara', 'Conference', 'Kaiser Alexandre', 'Kiefer' and 'Magness' were found to be relatively more resistant to the disease while 'Akca', 'Santa Maria' and 'Williams' were found to be susceptible. The results of the study agree with previous findings.

Susceptibility levels of hybridization combinations to fire blight varied between 'Magness × Kiefer' (19.79%) and 'Williams × Tas' (92.31%) (Table 2). Because susceptibility levels of hybrids were calculated, negative values indicate a contribution towards resistance while positive values illustrate increase in susceptibility for heterosis and heterobeltiosis. As a results of the study, there were heterosis in thirteen hybridization combinations. Seven ('Magness × Kiefer', 'Magness × Kaiser Alexandre', 'Magness × Conference', 'Magness × Tas', 'Magness × Akca', 'Magness × Limon' and 'Santa Maria × Akca') of which were statistically significant compared to parents' average (mid-parent) in terms of fire blight resistance. Heterobeltiosis was found in two hybridization combinations and the significance test of these combinations on better parent were statistically significant (Table 2).

In pears, fire blight resistance is transmitted to the next generation by both parents (DONDINI *et al.,* 2005). This aspect was put forward in raspberries and apples, too (STEWART *et al.,* 2005; VAN DE WEG *et al.,* 2018). But, because of the genetic variance related to maternity is

much higher than pollinator, maternal parent has greater effect on disease resistance (NYADANU *et al.*, 2017).

Table 2. Heterosis (%MPH) and heterobeltiosis (%BP) for fire blight resistance in thirtysix hybridization combinations in pear

Hybridization combinations	Number of Hybrids	%MPH	%BP
Magness × Kiefer	327	-52,88**	-40,03**
Magness × Kaiser Alexandre	122	-51,96**	-35,72**
Magness × Ankara	407	-0,2	11,89
Magness × Conference	166	-17,82**	16,18
Magness × Tas	265	-41,58**	17,72
Magness × Limon	27	-17,13**	35,6
Magness × Santa Maria	106	-0,89	42,2
Magness × Guz	27	-9,8	47,2
Magness × Akca	280	-11,49**	51,54
Akca × Kiefer	16	-13,9	10,5
Williams × Moonglow	558	18,77	38,26
Kiefer × Santa Maria	374	13,61	25,53
Williams × Kaiser Alexandre	417	16,23	21,91
Santa Maria × Akca	696	-4,3**	9,89
Williams × Akca	176	-0,63	15,17
Santa Maria × Conference	165	14,78	16,11
Williams × Conference	657	15,79	16,17
Williams × Kiefer	242	28,32	40,52
Santa Maria × Moonglow	400	35,61	59,35
Williams × Guz	42	11,06	23,87
Santa Maria × Bursa	70	11,49	23,77
Williams × Santa Maria	184	25,44	26,47
Santa Maria × Ankara	949	50,52	88,51
Akca × Santa Maria	14	11,97	28,6
Akca × Kaiser Alexandre	20	19,22	45,8
Santa Maria × Kiefer	161	44,41	59,56
Santa Maria × Kaiser Alexandre	630	40,52	48,66
Akca × Conference	52	18,52	37,88
Santa Maria × Williams	134	37,31	38,44
Akca × Tas	12	-5.42	6,4
Akca × Williams	17	21,13	40,39
Williams × Ankara	1134	68,59	109,09
Santa Maria × Guz	64	26,7	40,05
Williams × Bursa	44	30,59	46,3
Santa Maria × Tas	453	11,73	46,41
Williams × Tas	455	14,89	52,08

Combinations that the 'Magness' variety, which is the most resistant one among the investigated varieties, was used as maternal plant, has better results with regard to fire blight resistance than other combinations, and this situation confirms the knowledge above. Both heterobeltiosis detected hybridization combinations belong to 'Magness'. In addition, the fruits of hybrid plants obtained from 'Magness' were reported to have positive features in terms of attractiveness and organoleptic quality (MERTOGLU and EVRENOSOGLU, 2019).

Considering the hybrids of 'Santa Maria' and 'Kiefer' varieties in the study, it was revealed that the level of resistance to the disease where 'Kiefer' variety is the maternal-parent, and 'Santa Maria' is the pollinator is higher than its reciprocal combination. Similar results were obtained in 'Williams' - 'Akca' and 'Santa Maria' - 'Williams' hybrids, too. STEWART *et al.* (2005) found out significant differences in reciprocal hybridization combinations that made in order to determine the effect of maternal parent in blackberries, in terms of fire blight resistance. According to these results, it is possible to say that cytoplasmic inheritance may be effective in resistance to the disease. In most plant species, two-thirds of the endosperm genetic material and the extranuclear DNA in mitochondria and plastids of the embryo come from maternal parent including Rosaceous plants (VOLK *et al.,* 2015). Effects of cytoplasmic inheritance were shown on desired traits with the use of high breeding value varieties as female parent (NYADANU *et al.,* 2017; MASNY *et al.,* 2018).

CONCLUSIONS

Heterosis and heterobeltiosis focused aproach is effective method on transferring fire blight resistance to the next generation. By this purpose, disease reactions of parental varieties should be known, primarily. Among parental varietiess used in this study, 'Magness' is the most resistant one, and followed by 'Ankara', 'Moonglow', 'Kiefer' and 'Kaiser Alexandre'. 'Conference', 'Williams' and 'Santa Maria' were detected as susceptible, and 'Guz', 'Limon', 'Bursa', 'Akca' and 'Tas' were highly susceptile to fire blight disease.

Knowing how the heredity mechanism realizes, allows effectively planning of breeding program. Superior results of 'Magness', that is the most resistant variety, as maternal parent in cross combinations and reciprocal combinations, reveals maternal parent is more effective on transferring of the disease resistance. Using resistant varieties as maternal parents in cross breeding programs will enable obtaining more efficient results.

As a result of the study, it was put forward that, heterobeltiosis is the most suitable method for fire blight resistant hybrid breeding. Heterobeltiosis was detected on 'Magness × Kaiser Alexandre' and 'Magness × Kiefer' combinations among 36 investigated cross combinations, and were described as promising for future breeding study.

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OCENA OSETLJIVOSTI, HETEROZISA I HETEROBELTIOZISA NA PLAMENJAČU KRUŠKE

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Izvod

Seme četiri vrste mung pasulja (V1 = NM-98, V2 = KM1, V3 = Inkilab mung i V4 = Sona mung) tretirano gama zracima analizirano je na molekularnom i biohemijskom nivou. Randomiziran kompletan blok dizajn (RCBD) korišćten je za eksperiment sa četiri tretmana I tri ponavljanja (T0 = kontrola, T1 = 5 krad, T2 = 10 krad, T3 = 15 krad i T4 = 20 Krad). Zabeležena je maksimalna biohemijska i molekularna varijabilnost među genotipima. Utvrđena je i veća količina ukupnog sadržaja šećera i prolina u biljkama izraslim iz semena tretiranog T1, T2, T3 i T4 u odnosu na kontrolne (T0) biljke. S druge strane, uticaj na sadržaj proteina bio je obrnuto proporcionalan rastućem opsegu zračenja, tj. u biljkama tretiranim T4 sadržaj belančevina bio je nizak u poređenju sa T3, T2, T1 i T0. Međutim, zabeležen je niži sadržaj proteina u svim tretiranim uzorcima sa povećanjem doza gama zračenja. Random amplificirani polimorfni DNK (RAPD) prajmeri su korišćeni za proveru varijabilnosti na molekularnom nivou pri različitim tretmanima gama zračenja. Tri prajmera (OPS-03, OPB-1 i B-11) amplifikovala su četiri dodatna alela u tretiranim biljkama. Ove dodatne tarke evidentirane su kod NM-98 (posle T1, T2 i T3 tretmana), KM-1 (posle T2, T3 i T4 tretmana), Inkilabmung (posle T1 tretmana) i Sonamung (posle T2, T3 i T4 tretmana). Varijacije na fiziološkom, biohemijskom i, što je još interesantnije, molekularnom (amplifikacija dodatnih alela / traka) nivou kod tretiranih biljaka mogu biti posledica promena u genomu ovih sorti. Ova studija može poslužiti kao model za proveru uticaja gama zraka na druge ekonomski važne vrste useva.

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