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SCREENING OF APRICOT ELITES REACTION TO *PSEUDOMONAS* SPP.

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Abstract

Apricot decline remains an important threat to the apricot industry. Pathogens like: fungi, bacteria, viruses and mycoplasma are able to destroy the trees after several years of good growth. Diseases caused by Pseudomonas spp. in apricot has widely spread in fruit-producing areas worldwide. During the 2018 growing period, isolations were taken from apricot trees showing "blossom blast" symptom, grown in an apricot breeding orchard at the Fruit Growing Institute of Plovdiv, Bulgaria. It was found that in this area the species causing the observed symptom belong to Pseudomonas spp. In terms of integrated pest management genetical resistance of the cultivars is the most reliable way for reducing infection. In 2019, thirty-one apricot elites, selected from the hybrid families 'Harlayne' x 'Harcot', 'Lito' x 'Silistrenska ranna' and 'Harcot' x 'SEO' and all parental cultivars, were tested for their response to Pseudomonas spp. infection after artificial inoculation. The aim of this experiment was to study the reaction of all elites to the pathogen and to select the perspective ones for future breeding purposes. The laboratory tests were conducted in 2019 by artificial inoculation of one-year-old shoots in 'full flowering' phenophase with the obtained in 2018 isolate of the phytopathogen. In 48% of the studied elites, first symptoms were observed on the second day after the inoculation. Twenty-nine percent of the elites showed the first symptoms on the 5th day after inoculation. The parental cultivar Silistrenska ranna was the only genotype that showed symptoms on the 6th day. The lowest disease severity index was recorded for elite LS 14-30 – 3,45%, followed by HH 13-45 13,68%, HH 9-2- 15%, HH 13-20 - 15,43% and HH 9-1 - 17,50%. which makes them valuable for future breeding purposes.

Key words*: apricot, artificial inoculation, blossom blast, breeding.*

INTRODUCTION

Diseases of fruit trees caused by *Pseudomonas* spp. are of major concern in fruit-producing areas worldwide, because they need precise control, and result in significant economic losses. Disease symptoms include blossom blast and spur dieback, leaf and fruit lesions, cankers with associated gummosis of woody tissue, and decreased fruit yields (Kennelly et al., 2007). *Pseudomonas syringae* pv. *syringae* van Hall 1902, is causing a bacterial blast of apricot and cherry (Young, 1987). During periods of cool, wet weather or after frost events, blossom blast symptoms can occur. Early in the growing season, pathogen populations develop on apparently healthy blossoms. Blossom blast symptoms on stone fruits are similar to those of blossom blast on pear, except the damage to pear is not accompanied by further wood infection. Frost damage is recognized as an important predisposing injury for bacterial canker infection, and cankers initiated from blossom infections are a common symptom. The bacteria overwinter in cankers, in buds and within the tree (Kennelly et al., 2007). During wet patches in spring, the bacteria multiply, ooze from the branch cankers and are spread by rain. Spring is the time of year when infections of the bacterial blast can be most conspicuous. The most susceptible growth stages of the trees are during flowering and leaf buds opening. The spreading of the pathogen is also facilitated by cold winters and humid climates (Zhebentyayeva, 2012).

The apricot flowering stage in Bulgaria takes place in early spring when the weather is often cool and wet, accompanied by spring frosts. These environmental conditions are the most conducive for disease development (Marshall, 2015). On apricot trees, grown in commercial orchards and home gardens in the Silistra region are observed untimely tree decline antecedent by canker symptoms. Initial characteristic symptoms observed are mainly on trunk and scaffold branches as small or larger bark cankers with sap flow and dark-amber gumming at bud unions, crotches, pruning wounds or at the base of affected spurs.

The subsequent development of the disease is expressed as blossom blast, dried leaves attached to trees and twig dieback (Ivanova, 2007).

According to Kennelly et al. (2007) the management of diseases caused by *Pseudomonas* spp. is almost unattainable, due to the lack of effective chemical or biological control measures, lack of host resistance, and the endophytic nature of the pathogen during some phases of the disease cycle. Thus, the use of apricot genotypes resistant to *Pseudomonas* spp. is economically and technically the most reliable method for effective management (Bassi, 1999). The objective of this study was to evaluate the reaction of apricot elites after artificial inoculation with *Pseudomonas* spp. and to select the least sensitive ones for future breeding schemes.

MATERIALS AND METHODS

This study was conducted in 2018 and 2019 at the Fruit Growing Institute in Plovdiv, Bulgaria. In the spring of 2018 during the flowering phenophase of the apricots, the weather was rainy and cool. Thus, blossom blast symptom was observed on a big number of apricot hybrids in a breeding orchard. The observed disease symptoms included necrosis flower buds. Bark below the dead buds on severely affected trees showed black streaking. After bark shaving, dark xylem tissue was visible along the branch length, in contrast to the color of the healthy tissue. A fluorescent, gram-negative bacteria were consistently isolated onto King's B medium from twigs showing symptoms. *Pseudomonas* spp. was isolated onto a standard nutrient medium by generally accepted phytopathological methods (Kiraly et al., 1974). The pathogenicity of the bacterial isolates was tested on tobacco plants (*Nicotiana tabacum*) according to Klement's method (1963).

In the spring of 2019 in the laboratory of phytopathology at the Fruit Growing Institute, an artificial inoculation was done. For this purpose 1-year-old shoots, from trees showing no symptoms, were cut in BBCH 57 phenophase and placed in water containers. The reaction of 30 apricot elites obtained from tree hybrid families 'Harlayne' x 'Harcot', 'Harcot' x 'SEO', 'Lito' x 'Silistrenska ranna' and all 5 parental cultivars was evaluated. The artificial inoculation was done by spraying the 1-year-old apricot twigs in full flowering phenophase (BBCH 63-65) with 3 x 10^8 CFU mL bacterial suspensions in sterile water. Sprayed shoots were covered with moist plastic bags and maintained in favourable conditions - t = 23°C and 80-90% air humidity for 6 days. Symptom development was monitored daily. On the basis of first symptoms observed the incubation period was recorded. After 5 days the degree of attack was determined for each flower using a 5-grade scale (Figure 1): 0 - no symptoms; 1 - symptoms of necrosis on petals; 2 - symptoms of necrosis on pistil and receptacle; 3 - symptoms of necrosis observed on sepals; 4 - symptoms of necrosis observed on all flower parts. An average degree of attack was calculated.

Figure 1. Scale for determining the degree of attack

Five days after the artificial inoculation the disease severity index was calculated on the basis of the damaged flowers.

The twigs were left in water containers for further development and twenty days after the artificial inoculation the percentage of infected leaf buds was recorded.

For statistical data processing were used Duncan's test (Steele and Torrie, 1980) and hierarchical cluster analyses using between-groups linkage method of the IBM SPSS Statistics 19 statistical software.

To prove that the observed damages were caused by *Pseudomonas* spp. reisolations of the bacteria were done using two selective media – King's B and Aesculin hydrolysis (Kaluzna et al., 2012).

RESULTS AND DISCUSSIONS

Knowledge of the incubation period of infectious diseases (time between host infection and expression of disease symptoms) is crucial to our epidemiological understanding and the design of appropriate prevention and control policies. Plant diseases cause substantial damage to agricultural systems, but there is still very little information about how the incubation period varies within host populations (Leclerc et al., 2014). The duration of the incubation period is strongly influenced by environmental factors, especially the temperature, and the host sensitivity (Karov, 2006).

In our case, the hosts were artificially inoculated in a sensitive phenophase and maintained in suitable for the pathogen development conditions. Thus, the first symptoms occurred very fast (Table 1). The cultivars 'Harcot' and 'Lito' showed necrosis on the petals 2 days after the inoculation. Symptoms on 'SEO' and 'Harlayne' occurred on the 3rd and 4th day resp. After 5 days, symptoms with different severity were observed on all of the genotypes tested except cv. Silistrenska ranna. The incubation period for that cultivar was the longest and first symptoms were observed on the 6th day after the inoculation.

The degree of attack for the elites obtained by the 'Harlayne' x 'Harcot' cross and both parental cultivars ranged from 0.56 to 2.13 (Table 2). Statistically, non-significant difference was observed between both cultivars. The least affected by the pathogen were three of the elites - HH 13-45, HH 9-2 and HH 13-20. The difference between them and the parental cultivars was statistically proven. Although HH 9-2 showed first symptoms on the 2nd day after the spray inoculation, five days later the degree of attack was still low. These three genotypes are valuable because the degree of attack is low and they are better than both parental cultivars and 65% of the tested elites obtained by this parental combination.

The elites originating from 'Harcot' x 'SEO' combination were compared to their parental cultivars. The average degree of attack for these genotypes ranged from 1.37 to 2.85. The most severe damages were observed on the twigs of 'SEO' cv. A statistically significant difference was observed between 'SEO' and the other genotypes (Table 3). All elites tested had lower values of degree of attack than both parental cultivars but the difference with 'Harcot' was non-significant.

Five days after the artificial inoculation on the 'Silistrenska ranna' cut shoots were not observed any symptoms and its calculated average degree of attack was 0 (Table 4). For one of the tested elites were observed mild symptoms - LS 14-30. Although the first symptoms occurred on the second day, the pathogen did not spread in the next 4 days and for this elite, the calculated avarage degree of attack was 0.1.

Table 1. Duration of the incubation period

Incubation period (days)						
Day	**1**	**2**	**3**	**4**	**5**	**6**
Cultivar / Elite №		'Harcot'	'SEO'	'Harlayne'	HH 13-83	Silistrenska ranna
		'Lito'	HH 13-45	HH 12-19	HH 12-38	
		HH 9-1	HH 12-33	HH 13-20	HH 12-42	
		HH 12-60	HH 13-51	HH 12-22	HH 12-36	
		HH 9-2	LS 14-30	HS 12-20	HH 12-58	
		HH 13-1	HS 12-12		HH 12-47	
		HH 12-50			HS 12-16	
		HH 12-53			HS 12-19	
		HH 13-67				
		HH 13-24				
		HH 12-66				
		HH 12-30				
		HH 12-70				
		LS 14-18				
		HS 12-8				

Table 2. Degree of attack recorded for 'Harlayne' x 'Harcot' hybrid family

Cultivar/Elite №	Number of flowers inspected	Degree of attack (average)
HH 13-45	52	0.56 l
HH 9-2	20	0.60 l
HH 13-20	47	0.62 l
HH 9-1	20	0.70 kl
HH 12-22	28	0.82 jkl
HH 13-83	35	0.89 ijkl
HH 13-67	26	0.92 hijkl
HH 12-38	17	1.06 ghijkl
HH 12-58	28	1.21 fghijk
HH 13-3	31	1.29 efghij
HH 12-42	35	1.40 defghi
'Harlayne'	18	1.44 cdefgh
HH 13-51	26	1.54 bcdefg
HH 12-60	31	1.55 bcdefg
HH 12-50	18	1.56 bcdefg
HH 12-30	31	1.58 abcdefg
HH 12-66	34	1.68 abcdef
HH 12-36	44	1.75 abcdef
HH 12-70	21	1.81 abcde
HH 12-19	13	1.85 abcde
'Harcot'	29	1.93 abcd
HH 13-24	16	1.94 abcd
HH 12-33	17	2.00 abc
HH 12-53	55	2.07 ab
HH 12-47	31	2.13 a

Table 3. Degree of attack recorded for 'Harcot' x 'SEO' hybrid family

Elite №	Number of flowers inspected	Degree of attack (avarage)
HS 12-12	30	1.37 b
HS 12-16	17	1.65 b
HS 12-8	25	1.68 b
HS 12-19	19	1.84 b
'Harcot'	29	1.93 b
'SEO'	20	2.85 a

Table 4. Degree of attack recorded for 'Lito' x 'Silistrenska ranna' hybrid family

Elite №	Number of flowers inspected	Degree of attack (average)
'Silistrenska ranna'	30	0.00 b
LS 14-30	29	0.10 b
LS 14-18	29	2.34 a
LS 12-20	14	2.43 a
'Lito'	17	2.47 a

The highest disease sevirity index was recorded for 'SEO' cv. - 71.25% and the lowest for 'Silistrenska ranna' - 0%. Low severity index was recorded also for the LS 14-30 elite (3.45%), obtained by a controlled hybridization with the presence of 'Silistrenska ranna' as a parent (Figure 1).

As the least susceptible, on the basis of flower infection, could be defined 'Silistrenska ranna' cv. and the elites - LS 14-30, HH 9-2, HH 13-20, HH 13-45, HH 9-1, HH 13-83, HH 13-47, HH 12-22 and HH 12-38. The disease severity index for these genotypes ranged from 3.45 to 26.47%.

After the pathogen has infected the flower parts of the host it continues its development in the leaf buds. Some of the tested elites had low disease severity index but after 20 days a high percentage of the leaf buds were damaged - some were necrotic and did not develop at all for others necrosis on the young leaves was observed. For example - HH 13-45 and HH 13-20. Elite LS 12-30 and 'Silistrenska ranna' cv. had low disease severity index and a low percentage of leaf buds showing symptoms. Some of the elites obtained by the parental combination 'Harlayne' and 'Harcot' had a medium value of the disease severity index but the disease did not damage a big percentage of their leaf buds. For example HH 12-19, HH 12-30, HH 12-50. A very interesting genotype from this hybrid family was HH 12-22. Its disease severity index was 20.54 and the percentage leaf buds with symptoms for this elite was the lowest recorded - 12.12%.

For better comparison of all genotypes, hierarchical cluster analyses using between-groups linkage method was used. It divided all tested cultivars and elites according to the disease severity index and the percentage of damaged leaf buds together (Figure 2). On the dendrogram could be seen that the genotypes were grouped in 3 main clusters. The 'SEO' cultivar could be evaluated as the most susceptible to *Pseudomonas* spp. with disease severity index = 71.25% and 100% damaged buds. This cultivar was not grouped with any other genotypes which shows us that none of the tested elites and cultivars had such an intensive reaction to the pathogen. Our field observations also confirm the susceptibility of 'SEO'. All of the elites obtained by the parental combination 'Harcot' x 'SEO' show a disease severity index above 35% and less damaged leaf buds than 'SEO'.

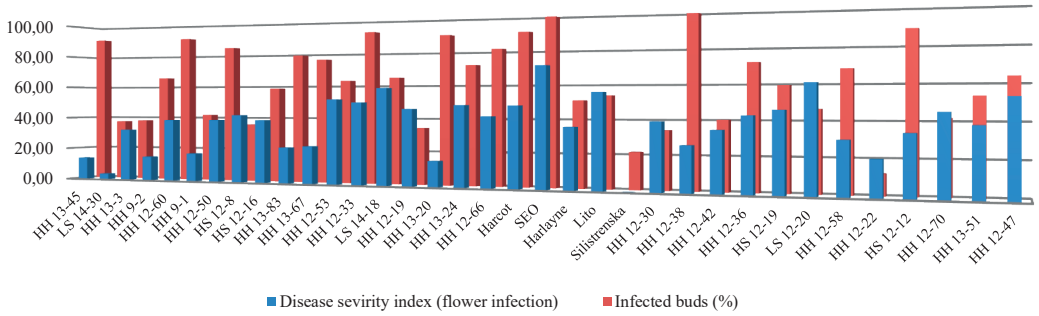

Figure 1. Disease severity index of flower infection and percentage of infected leaf buds 20 days after artificial inoculation

After the hierarchical cluster analyses as the least susceptible to the pathogen could be defined the grouped in one cluster genotypes - LS 14-30, HH 9-1, 'Silistrenska ranna' and HH 12-22.

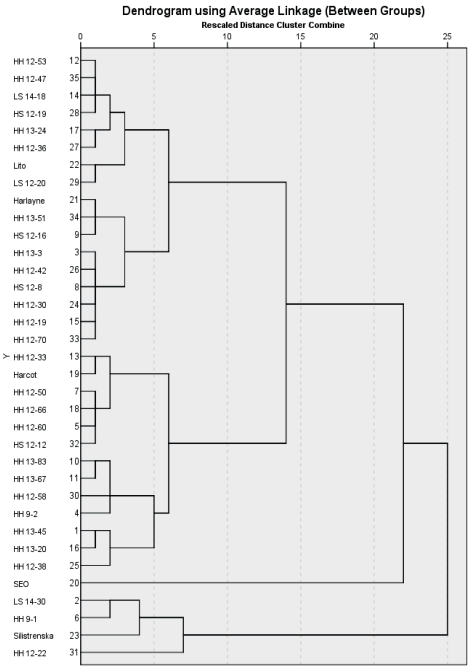

Figure 2. Dendrogram of hierarchical cluster analyses

In other previous our studies the elites were genotyped for *Plum Pox Virus* resistance by MAS (Milusheva et al., 2016). This gives us the opportunity, on the basis of this and our previous studies, to selected elites that combine low susceptibility to flower infection caused by *Pseudomonas* spp. and resistance to PPV for future breeding purposes.

Table 5. Promising elites

Elite №	Severity index to *Pseudomonas* spp. (%)	Percentage of infected leaf buds (%)	PPV resistance factor
LS 14-30	3.45	37.50	Resistant allele
HH 12-22	20.54	12.12	Resistant allele
HH 9-1	17.50	42	No data

The reisolated bacteria grown for 24-48 h on Aesculin hydrolysis gave a brown color of the medium. Grown onto King's B was observed a fluorescence reaction (Figure 3).

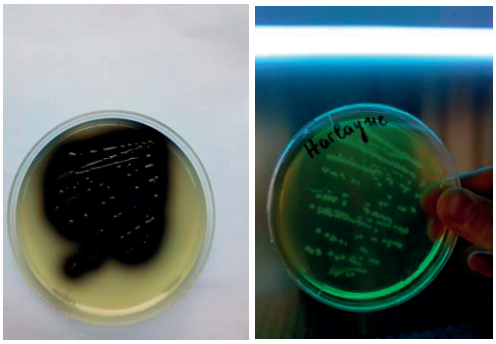

Figure 3. Reaction of the reisolated bacteria on to selective media

CONCLUSIONS

This study gives us the opportunity for conducting a very fast laboratory screening of breeding materials.

The obtained results allow us to evaluate the reliability of the tested parental combinations when used in breeding schemes aiming to low susceptibility to *Pseudomonas* spp.

The cultivar 'SEO' is proven and often used as a donor of *Plum Pox Virus* resistance but unreliable for obtaining genotypes with resistance to *Pseudomonas* spp. This indicates that when it is used in controlled crosses should be combined with cultivars showing low susceptibility to the bacterial disease.

'Silistrenska ranna' is promising cultivar for this purpose. Because it has some serious disadvantages, concerning the fruit quality, F2 and F3 generations could be obtained for combining a complex of valuable traits.

REFERENCES

Bassi, D. (1999). Apricot culture: present and future. *Acta Horticulturae*, 488, 35-40.

Ivanova, L. (2007). First occurrence of apricot blast disease caused by Pseudomonas syringae in the north-eastern part of Bulgaria. In *I Balkan Symposium on Fruit Growing*, *825*, 149-152.

Kałużna, M., Janse, J. D., Young, J. M. (2012). Detection and identification methods and new tests as used and developed in the framework of COST 873 for bacteria pathogenic to stone fruits and nuts *Pseudomonas syringae* pathovars. *Journal of Plant Pathology*, 94, 117-126

Karov S, Nakov B, Popov A, Neshev G. (2006). Phytopathology, *Academic textbook of Agricultural University*, Plovdiv.

Kennelly, M. M., Cazorla, F. M., de Vincente, A., Ramos, C., Sundin, G. W. (2007). *Pseudomonas syringae* diseases of fruit trees, progress toward understanding and control. *Plant Disease*, 91, 4-17.

Kiraly, Z., Klement, Z., Solymosy, F. and Voros, J. (1974). Methodi Phytopathologii, *Moskva*, 82-159

Klement, Z. 1963. Rapid detection of the pathogenecity of phytopathogenic *Pseudomonas*. *Nature*, 199: 299-300.

Leclerc, M., Doré, T., Gilligan, C. A., Lucas, P., Filipe, J. A. (2014). Estimating the delay between host infection and disease (incubation period) and assessing its significance to the epidemiology of plant diseases. *PloS one*, 9(1).

Marshall, K. (2015). The facts about bacterial blast: How to spot it, prevent & treat it. *NZ Gardener* https://www.stuff.co.nz/life-style/home-property/nz-gardener/74109095/null.

Milusheva, S., Bozhkova, V., Moreau, F., Evangelou, V., & Nesheva, M. (2016). Screening of apricot hybrids for resistance to Plum pox virus. In *III International Symposium on Plum Pox Virus*, *1163*, 19-24.

Steele, R. G. D., Torrie, J. H. (1980). Principles and procedures of statistics a biometrical approach.

Young, J. M. (1987). Orchard management and bacterial diseases of stone fruit. *New Zealand Journal of Experimental Agriculture*, 15(2), 257-266.

Zhebentyayeva, T., Ledbetter, C., Burgos, L., Llácer, G. (2012). Apricot. In *Fruit Breeding*. Springer, Boston, MA, pp. 415-458.