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Resistance of pepper types to *Xanthomonas euvesicatoria* and *Xanthomonas vesicatoria* – impact of the species, races and hosts specialization

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Abstract. *An economically important disease of the traditional vegetable pepper crop in Bulgaria is bacterial spot (Xanthomonas euvesicatoria and Xanthomonas vesicatoria). The resistance of pepper varietal types (Shipka, Pumpkin, Kapia) was determined by the species of causative agent of bacterial spot, pepper host specialization and structure of races. Shipka, Bulgarski ratund, Calabre and Ivaylovska kapia were classified in defined groups with a proven statistical difference depending on reaction to artificial inoculation with heterogeneous species X. euvesicatoria and X. vesicatoria. The cultivars from different types were medium sensitive to X. vesicatoria, independently of race P (P2), pathotype P3T2, P1T2 and the host from the strains was isolated. The races P3, P4 (P4T2p, t) to X. euvesicatoria were more virulent and aggressive than X. vesicatoria races P1 (P1T2t), P2, P3 (P3T2p). The genetic diversity between X. euvesicatoria and X. vesicatoria, their phylogenetic positions and composition of races, suggest the use of separate statistical analysis groups to determine the resistance of pepper cultivars.*

Keywords: bacterial spot, species, pathotype, races, host specialization, pepper types

Introduction

Bacerial spot (BS) of pepper caused by *X. euvesicatoria* and *X. vesicatoria*, is one of the most destructive diseases in the field when environmental conditions are favorable for the pathogens. High summer temperatures and frequent rains stimulate the development and spread of BS and cause damage to upper ground part of the plants, leaves defoliation and scab on the fruit, which degrade the quantity and quality of the production. The gram-negative plant pathogenic bacteria *X. vesicatoria* and *X. euvesicatoria* are causative agents of the disease (Karov, 1965; Kizheva et al., 2013). The natural population of pathogens are heterogeneous by symptoms, species, phenotypic and genotypic characteristics, pathotype and races (Stoyanova et al., 2014; Vancheva, 2015; Vancheva et al., 2018; 2021; Vasileva and Bogatzevska, 2019; Bogatzevska et al., 2021). *X. vesicatoria* form large, single, brown, water-soaked lesions, cell death and tissue necrosis on the leaves, while *X. euvesicatoria* are characterized by small, irregular, water-soaked, greasy-appearing spots with a necrotic centre. A typical symptom of the BS on pepper is leaf defoliation. Pathogens develop symptoms or are symptomless in hosts, become residents of weeds, are stored and spread by seeds and during the growing season by water drops, such as from rainfall or heavy dew, or aerosols containing the bacteria (Bogatzevska, 2002; Bogatzevska et al., 2007;

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Bogatzevska and Pandeva, 2009; Stall et al., 2009; Potnis et al., 2015; Dutta et al., 2016; An et al., 2020).

The Bulgarian population of *X. euvesicatoria* is more diverse and prevalent than the population of *X. vesicatoria* on pepper. *X. euvesicatoria* P is a host specific pathogen and it causes disease in species from *Capsicum* genus with *AvrBs3* and *AvrBs4* genes only (Vancheva, 2015). *AvrBs3* and *AvrBs4* are recognized specifically by the corresponding pepper Bs3 and tomato Bs4 R genes, respectively (Potnis et al., 2015). *AvrBsT* has been shown to be a virulence factor by suppressing defence responses in tomato (Kim et al., 2010), possibly conferring a competitive advantage to pathogens in tomato fields.

Among the Bulgarian strains of *X. euvesicatoria* isolated after 2008, dominated P pathotype and the presence of *AvrBs4* gene. The presented strains had *AvrBs3* and/or *AvrBs4* genes. The absence of these genes in 6.5% of the strains of *X. euvesicatoria* suggests the involvement of other pathogenicity factors (Vancheva, 2015).

X. vesicatoria and *X. euvesicatoria* are phenotypically and genetically heterogeneous species with phylogenetic position (Hamza et al., 2010; Stoyanova et al., 2014; Vancheva, 2015; Vancheva et al., 2018, 2021). Greater genetic diversity and private allelic richness was observed in Bulgarian populations of *X. euvesicatoria*, absence of genetic differentiation between the regions and the sharing of haplotypes or clonal complex by strains (Vancheva et al., 2018; Vancheva et al., 2021). The genetic diversity among *X. euvesicatoria* strains was consistent with the worldwide movement of clonal populations in seeds, whereas geographic isolation appeared to be shaping the population structure of *X. vesicatoria* (Dahkal et al., 2019; Timilsina et al., 2020; Vancheva et al., 2021).

Races P3 and P6 to *X. euvesicatoria* are the distributed cultivars of pepper in the country. The dominant race in PT is P4 in combination with T2. The population of *X. vesicatoria* PT races P1 and P3 prevailed in combination with tomato race T2, in P - dominated race P2 (Vasileva and Bogatzevska, 2019; Bogatzevska et al., 2021).

Dynamic changes in the natural populations of *X. vesicatoria* and *X. euvesicatoria* in recent years, the introduced new Bulgarian accessions, exchange of seed transmission, led to the emergence and spread of new virulent races and combinations; hence, all these characteristics in the life cycle of BS on pepper and tomato require in-depth studies of the relationship in the host-pathogen system.

The aim of the research was to investigate the impact of the species, pathotype and races of *X. vesicatoria* and *X. euvesicatoria* resistance of pepper varietal types and the role of the host from which they were isolated.

Material and methods

Plant material – varietal type peppers:

Small-fruited– (var. Shipka) cultivar (cv.) Shipka; Large-fruited bell pepper (var. Pumpkin) – cv. Bulgarski ratund, pungent small fruit bell – cv. Calabre; Large-fruited kapia- (var. Kapia) – cv. Ivaylovska kapia.

Bacterial pathogens: *X. euvesicatoria* P3, XeuvP3 -№74b; *X. euvesicatoria* P4T2p, Xeuv№109b – isolate of pepper (p); *X. euvesicatoria* P4T2t, Xeuv№94t – isolate of tomato (t); *X. vesicatoria* P2, Xv №14b; *X. vesicatoria* P3T2p, Xv №36b – isolate of pepper (p); *X. vesicatoria* P1T2t, Xv №98t – isolate of tomato (t).

The strains were identified with Biolog™ (GN microplate) and characterized by different molecular methods (Stoyanova et al., 2014; Kizheva et al., 2016; Vancheva et al., 2018; Kizheva et al., 2020). The race composition of pepper and tomato isolates was determined by Vasileva and Bogatzevska (2019) and Bogatzevska et al. (2021).

Inoculation procedure

Plants in phase first true leaf were vacuum infiltrated with bacterial suspension (10^8 cfu/ml) prepared from 36h culture of *X. vesicatoria* and *X. euvesicatoria*. Infiltration was carried out with vacuum pump 55-60 кPa (1at=101.3 кPa). Inoculated plants (20-25 plants) were grown in Knop solution under laboratory conditions at temperature 25°C (Bogatzevska et al., 2006).

Evaluation of disease

Leaf symptoms and number of fallen leaves with ring shape necrosis of the leaf stalk were reported 4 - 5 days after infiltration on a 5-disease rating scale (0 to 4) (Bogatzevska et al., 2006). The limit values of resistance groups were established by mean score (ms) based on conducted statistical analysis: I -immune; R- resistant; MS - medium sensitive; S - sensitive; SS - strongly sensitive (Table1).

Table 1. The grouping by mean score of infection

Groups	I	R	MS	S	SS
X. vesicatoria	0	0.01 - 0.36	0.37 - 0.79	0.80 - 1.18	1.19 - over
X. euvesicatoria	0	0.01 - 0.44	0.45 - 0.79	0.80 - 1.12	1.13 - over

*immune (I); resistant (R); medium sensitive (MS); sensitive (S); strongly sensitive (SS) (Vasileva and Bogatzevska, 2021).

The grouping was performed for each bacterium separately. The mean score of disease was estimated by the scale of Vasileva and Bogatzevska (2021). Index of defoliation (Di%) was calculated by Pesti et al. (1990).

Statistical methods

The data were mathematically analysed using Multiple Range Test (Duncan, 1955). The standard deviation for grouping of accession was applied. The software products were MS Excel Analysis Tool Pak Add-Ins 2019 (https://support.office.com) and R-4.0.3 in combination with RStudio-0.98 and installed package Agricole 1.2-2 (Mendiburu, 2015).

Results and discussion

Investigated impact of the species, pathotype and races *X. euvesicatoria* and *X. vesicatoria* on resistance of varietal types: *Shipka*, *Pumpkin*, *Kapia* and role of the host specialization are presented in Table 2.

Pathogens form brown spots on the leaves with a necrotic centre surrounded by a chlorotic halo, single or merged in the necrotic zones. A ring shape necrosis forms at the base of the leaf stalk and the leaves fall off. The typical symptom of the causes of bacterial spot is leaf fall, which is determined by the defoliation index (Di) (Figure 1).

1 2 3

Figure 1. Symptoms of *X. euvesicatoria* and *X. vesicatoria* to pepper varietal types: *Shipka*, *Pumpkin*, *Kapia*. *1 - Ring shape necrosis caused by *X. euvesicatoria* and *X. vesicatoria*; 2 - Defoliation of cv. Calabre infected with X. euvesicatoria P3; 3 - Defoliation lack of cv. Bulgarski ratund infected with X. vesicatoria P1T2-t;

With infestation by artificial inoculation with *X. euvesicatoria* (P3, P4T2 p, t,) and *X. vesicatoria* (P2, P3T2p, P1T2t) the studied varieties were characterized by different mean score. The grouping of resistance varieties was performed for each bacterium separately (Table 2).

Table 2. Impact of the species, pathotype and races of BS (*X. euvesicatoria* and *X.vesicatoria*) to resistance pepper varietal types: *Shipka*, *Pumpkin*, *Kapia*

Cultivars	Total number of leaves	0	1	2	3	4	ms	Di%	Xeu	Total number of leaves	0	1	2	3	4	ms	Di%	Xv
	***X. euvesicatoria* P3**									***X. vesicatoria* P2**								
1. Shipka	54	21	31	2	0	0	**0.65**	**0**	**MS**	50	30	18	2	0	0	**0.55**	**11.1**	**MS**
2. Bulgarski ratund	89	30	38	21	0	0	**0.89**	**9.2**	**S**	91	50	33	6	2	0	**0.56**	**6.19**	**MS**
3. Calabre	84	66	13	5	0	0	**0.27**	**18.4**	**R**	98	44	40	8	6	0	**0.75**	**13**	**MS**
4. Ivaylovska kapia	97	63	23	7	4	0	**0.5**	**4.9**	**MS**	93	59	23	8	3	0	**0.52**	**2.1**	**MS**
	***X. euvesicatoria* P4T2-p**									***X. vesicatoria* P3T2-p**								
1. Shipka	51	29	21	1	0	0	**0.45**	**1.92**	**MS**	63	38	22	3	0	0	**0.44**	**7.4**	**MS**
2. Bulgarski ratund	82	40	32	5	4	1	**0.71**	**25**	**MS**	61	27	27	7	0	0	**0.67**	**10.3**	**MS**
3. Calabre	79	44	27	5	3	0	**0.58**	**13.2**	**MS**	81	34	44	3	0	0	**0.6**	**13**	**MS**
4. Ivaylovska kapia	99	62	24	13	0	0	**0.51**	**3.9**	**MS**	106	54	51	1	0	0	**0.5**	**0**	**MS**
	***X. euvesicatoria* P4T2-t**									***X. vesicatoria* P1T2-t**								
1. Shipka	50	46	4	0	0	0	**0.08**	**0**	**R**	88	61	27	0	0	0	**0.3**	**18**	**R**
2. Bulgarski ratund	96	68	28	0	0	0	**0.29**	**9.43**	**R**	71	33	36	2	0	0	**0.56**	**0**	**MS**
3. Calabre	106	81	25	0	0	0	**0.24**	**2.75**	**R**	79	42	32	5	0	0	**0.5**	**17**	**MS**
4. Ivaylovska kapia	98	74	24	0	0	0	**0.25**	**2.97**	**R**	89	55	34	0	0	0	**0.38**	**8.3**	**MS**

ms – mean score of infestation; Di% - index of defoliation; **Xeu-** *X. euvesicatoria;* **Xv** *X. vesicatoria*

Varietal type Shipka – cv. Shipka

P pathotype – *X. euvesicatoria* P3 and *X. vesicatoria* P2

Cultivar Shipka is medium sensitive to *X. euvesicatoria* P3 (ms 0.65) and *X. vesicatoria* P2 (ms 0.55). There were not statistically proven differences for the two pathogens. The ratio between healthy leaves and those rated with diagnostic scale 1 and 2 infected with *X. euvesicatoria* P3 was (39:57:4), respectively. *X. vesicatoria* P2 formed ring shape necrosis at the base of the leaf stalks, the leaves were symptomless or with brown spots with chlorotic halo, wilted and fallen (Di 11%).

Interaction plant-host with *X. vesicatoria* P2, Shipka reacted to the tissue's penetration of the non-specific pathogen by restricting the bacteria at the base of the leaf stalk with the formation of a brown ring shape necrosis, which led to drying of the leaves.

PT pathotype - *X. euvesicatoria* P4T2p and *X. vesicatoria* P3T2p

Shipka was medium sensitive to *X. euvesicatoria* P4T2p (ms 0.45) and *X. vesicatoria* P3T2p (ms 0.44). Healthy leaves with diagnostic scale 1 predominate the ones infected with separate species. Single leaves were rated with a diagnostic scale 2. A higher percentage of defoliation was reported in the infected plants with *X. vesicatoria* P3T2p - Di-7.4%.

PT pathotype - *X. euvesicatoria* P4T2t and *X. vesicatoria* P1T2t

Resistant to *X. euvesicatoria* P4T2t (ms 0.08) and *X. vesicatoria* P1T2t (ms 0.3) was Shipka. Healthy leaves

(92%) and these with single spots (31%) predominate the ones infected with *X. vesicatoria* P1T2t compared to *X. euvesicatoria* P4T2t 69% and 8%, respectively. Defoliation was strongly expressed in *X. vesicatoria* R1T2t - Di 18%.

Varietal type Pumpkin – cv. Bulgarski ratund, cv. Kalabre

P pathotype – *X. euvesicatoria* P3 and *X. vesicatoria* P2

Bulgarski ratund (large pumpkin) was sensitive to *X. euvesicatoria* P3 (ms 0.89) and medium sensitive to *X. vesicatoria* P2 (ms 0.56). Total of the percentages with diagnostic scale 1 (43%) and 2 (24%) leaves was greater than that of the healthy leaves (34%). Defoliation index was 9%. The number of healthy leaves (50) was larger than those of the diagnostic scale 1,2,3, respectively, (33,6,2) in Bulgarski ratund infected with *X. vesicatoria* P2, which determined the lower value of ms, and was statistically proven.

Calabre (small hot pumpkin) was resistant to *X. euvesicatoria* P3 (ms 0.27), healthy leaves predominated (79%), Di was 18.4%. Diversity in the mean score of infestation (diagnostic scales 0,1,2,3) was observed when the variety was infected with *X. vesicatoria* P2 in a ratio (45:41:8:6) with Di13%. The cultivar was medium sensitive with ms 0.75.

PT pathotype - *X. euvesicatoria* P4T2p and *X. vesicatoria* P3T2p

Medium sensitive to artificial infection with *X. euvesicatoria* P4T2p (pepper isolate) were Bulgarski ratund (ms 0.71) and Calabre (ms 0.58). The correlations of Bulgarski ratund between healthy leaves and those with diagnostic symptoms for bacterial spot were 49:39:6:5:1%. The leaves with symptoms rated with diagnostic scale of 1 to 4 prevailed and strong defoliation was reported - 25%. Healthy leaves dominated, single ones were rated with diagnostic scales 2 and 3 in Calabre with ratio 56:34:6: 4% were observed. The defoliation index of hot small pumpkin was almost twice lower than large sweet pumpkin. Cultivars belong to the group of medium sensitive with ms 0.67 and 0.6 infected with *X. vesicatoria* P3T2p (pepper isolate). Healthy leaves, those evaluated with a diagnostic scale 1 and 2 were registered in Bulgarski ratund in proportion 44:44:11 and 42:54:4 in Calabre. The index of defoliation in hot small pumpkins was 13% regardless of the species.

PT pathotype - *X. euvesicatoria* P4T2t and *X. vesicatoria* P1T2t

Bulgarski ratund (ms 0.29) and Calabre (ms 0.24) were resistant to *X. euvesicatoria* P4T2t (tomato isolate). Essentially healthy leaves and single ones with diagnostic scale 1 in a ratio 71:29 and 76:24 predominated. Defoliation was weakly expressed.

The varieties were medium sensitive infected with the tomato isolate of *X. vesicatoria* P1T2t – pumpkin pepper (ms 0.56) and pungent small pumpkin (ms 0.5). Calabre was characterized by a higher percentage of healthy leaves compared to diseased ones (diagnostic scales 1 and 2) in a ratio of 53:41:6%. Ring shape necrosis covered 17% of the leaves.

Although *X. euvesicatoria* P4T2t is specialized for pepper species, the host from which it was isolated was a tomato, causing a decrease in virulence and aggressiveness to the main host.

Varietal type Kapia – cv. Ivaylovska kapia

P pathotype – *X. euvesicatoria* P3 and *X. vesicatoria* P2

Ivaylovska kapia was medium sensitive to *X. euvesicatoria* P3 (ms 0.5) and *X. vesicatoria* P2 (ms 0.52) with weakly expressed defoliation. Infected leaves had different degrees of damage in both species in the ratio 65:24:7:4 (XeuvP3) and 63:25:9:3 (XvP2).

PT pathotype - *X. euvesicatoria* P4T2p and *X. vesicatoria* P3T2p

Ivaylovska kapia was medium sensitive to artificial inoculation with the specific for the pepper species *X. euvesicatoria* P4T2p (ms 0.51) and typical of the tomato genotype *X. vesicatoria* P3T2p (ms 0.5). Healthy leaves prevailed to *X. euvesicatoria* P4T2p, the correlation between healthy and diseased leaves was 63:24:13. The percentage of symptomless leaves and those with single spots to *X. vesicatoria* P3T2p was 51:48, respectively.

PT pathotype - *X. euvesicatoria* P4T2t and *X. vesicatoria* P1T2t

Ivaylovska kapia was resistant (ms 0.25) to *X. euvesicatoria* P4T2t. Healthy and with single spots leaves prevailed in a ratio of 76:25% with Di 2.97%. The variety was medium sensitive to *X. vesicatoria* P1T2t (ms 0.38), the correlation between healthy leaves and those with diagnostic scale 1 was 62:38 %. Leaves with ring shape necrosis were 8.3%.

***X. euvesicatoria* (*Xev* P3, P4T2 p,t,)**

Shipka was medium sensitive to artificial infection with *X. euvesicatoria* P3 (ms 0.65), P4T2p (ms 0.45) and resistant to the tomato isolate P4T2t (ms 0.08). The ratio between healthy leaves was 39:57:92 %. The number of leaves rated diagnostic scales 1 and 2 was reduced, by 31:21:4 and 2:1:0, respectively (Table 2, Figure 2).

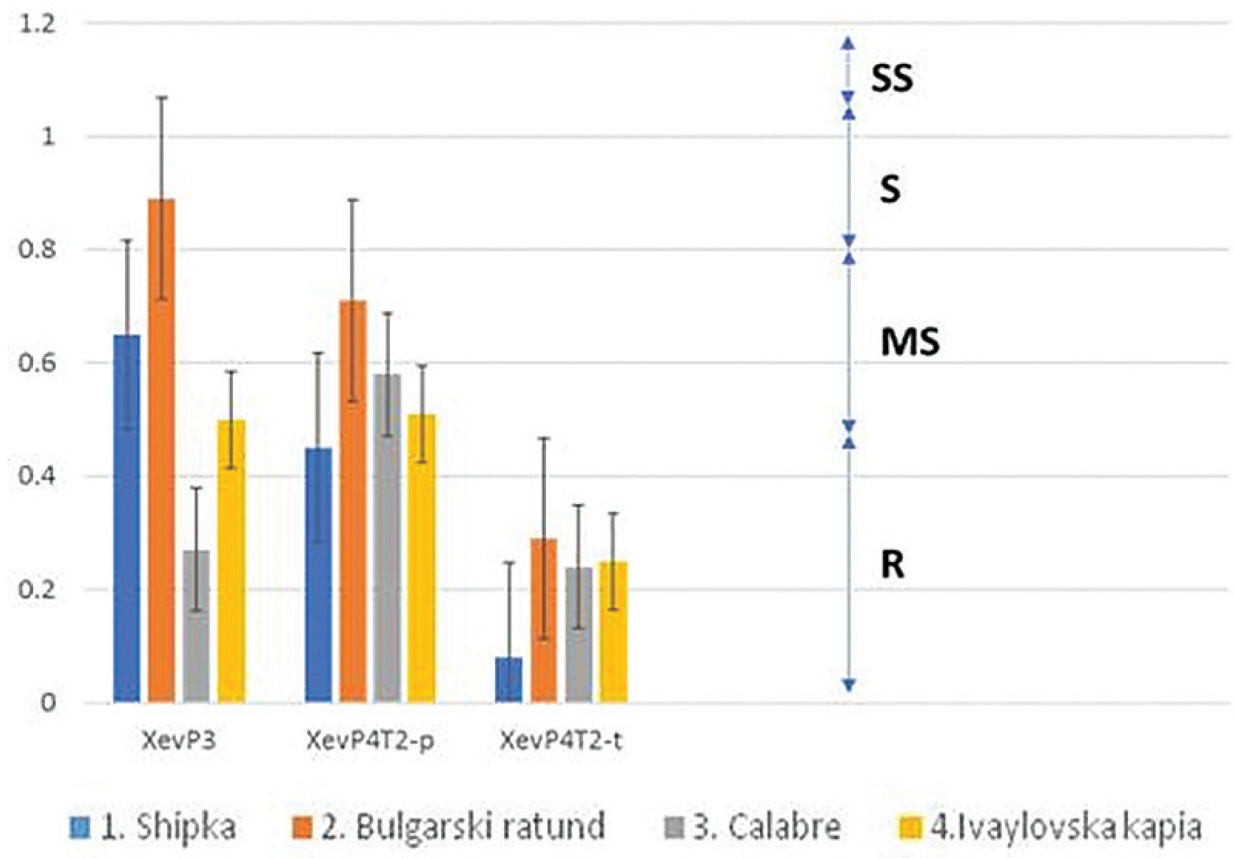

Figure 2. Grouping of pepper varietal types by mean score of infestation to *X. euvesicatoria*
immune – 0 (I); resistant 0.01-0.44 (R); medium sensitive 0.45-0.79 (MS); sensitive 0.80-1.12 (S); strongly sensitive: more than 1.13 (SS)

Varietal type – *Pumpkin* was represented by two varieties, which differ in type and content of alkaloids and capsaicin in the leaves and fruits. The Bulgarski ratund belong to the group of wide, sweet pumpkin, while pungent small fruits Calabre are characterized with a high content of capsaicin and alkaloids in the leaf tissues. These characteristics of the varieties determine their relation to the species *X. euvesicatoria*, pathotype, races and the host from which they were isolated. The Bulgarski ratund was sensitive to infection with P3 *X. euvesicatoria* (ms 0.89), medium sensitive (ms 0.71) to P4T2p and resistant (ms 0.29) to P4T2t (Table 2, Figure 2).

Pungent small pumpkin was resistant. Infected with race P3 (ms 0.27) and P4T2t (ms 0.24) of *X. euvesicatoria*, healthy leaves prevailed at a ratio of 79:76%. Calabre was characterized by medium sensitivity to P4T2p.

Among the natural populations of *X. euvesicatoria* distributed in the pepper fields in Bulgaria prevail PT, dominant race P4 in combination with a virulent race of tomato T2. Strains *X. euvesicatoria* that reproduced symptom or symptomless in pepper were specialized to the genome of genus *Capsicum* and were less pathogenic to the genus *Solanum* (Bogatzevska and Pandeva, 2009; Ignjatov et al., 2010; Vancheva et al., 2015). This characteristic determined the sensitivity of pepper varieties of different types to P4T2p of *X. euvesicatoria*, although prevailing were the healthy leaves (57:49:56:63%) and those with single spots (41:39:34:24%).

Ivaylovska kapia infected with *X. euvesicatoria* (Xev P3, P4T2 p) was medium sensitive and resistant to P4T2t isolated from tomato, healthy leaves (76%) and with single spots (25%) predominated (Table 2, Figure 2).

***X. vesicatoria* (P2, P3T2p, P1T2t)**

Shipka was medim sensitive to *X. vesicatoria* (P2, P3T2p) and resistant to P1T2t. The percentage of healthy leaves was 60:60:69, respectively, slightly reduced in diagnostic scale 1 (36:35:31), and diagnostic scale 2 by 4:5:0, respectively (Table 2, Figure 3).

The cultivars of different varietal types were medium sensitive to *X. vesicatoria*, irrespective of the race P (P2), the pathotype P3T2 and the host from which the strains were isolated (Tables 2, 3) (Figure 3).

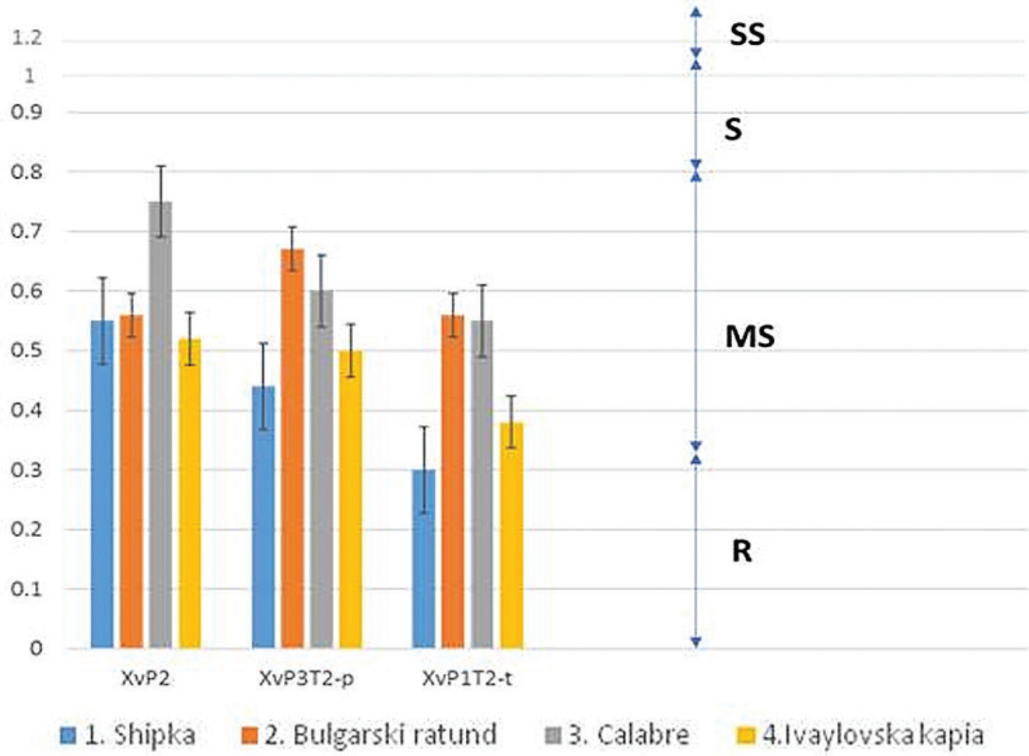

Figure 3. Grouping of pepper varietal types by mean score of infestation to *X. vesicatoria*
immune – 0 (I); resistant 0.01-0.36 (R); medium sensitive 0.37-0.79 (MS); sensitive 0.80-1.18 (S); strongly sensitive: more than 1.19 (SS).

Table 3. Impact of the species, pathotype and races to resistance of BS pepper (*X. euvesicatoria* and *X. vesicatoria*)

	ms	Di%
	Xanthomonas euvesicatoria	
XevP3	0.58 a	8.12 ab
Xev P4T2-p	0.56 a	11 a
Xev P4T2-t	0.2 c	3.78 c
	Xanthomonas vesicatoria	
XvP2	0.59 a	8.09 b
Xv P3T2-p	0.55 a	7.67 b
XvP1T2-t	0.44 ab	10.83 a

ms – mean score of infestation; Di% - index of defoliation;
*a,b… Different letters in the same column indicate significant difference ($p<0.05$).

The impact of the species, pathotype and races of the causative agents of BS and the role of the host from which they were isolated had been studied in relation to the resistance of the traditional for Bulgarian grower's pepper varietal types (*Shipka, Pumpkin, Kapia*).

X. euvesicatoria and *X. vesicatoria* are heterogeneous species (Vancheva et al., 2021), which necessitated the use of specific groups with mean score of infection with a proven statistical difference to determine the resistance of pepper accessions (Vasileva and Bogatzevska, 2021).

The strains of *X. euvesicatoria* belong to the P3 and P4T2, races P3, P4 were more aggressive in field conditions than other pepper races, and T2 was the most virulent race in the T pathotype. The population of *X. vesicatoria* PT races P1 and P3 prevailed in combination with tomato race T2, in P pathotype – P2 dominated (Vasileva and Bogatzevska, 2019; Bogatzevska et al., 2021). Races P3, P4 (P4T2p, t) of species *X. euvesicatoria* were more virulent and aggressive when inoculating pepper varietal types (*Shipka, Pumpkin, Kapia*), than races P1 (P1T2t), P2, P3 (P3T2p) of *X. vesicatoria. X. vesicatoria* was a dominant pathogen on tomato in

Bulgaria, whereas specialized for the climatic conditions of the region on pepper was *X. euvesicatoria* (Kizeva et al., 2018; Bogatzevska et al., 2021; Vancheva et al., 2021). The Bulgarian population of *X. euvesicatoria* on host pepper showed greater and strong genetic diversity between other populations in geographical regions of the world (Vancheva et al., 2021).

Shipka, Bulgarski ratund, Calabre and Ivaylovska кapia were in different groups with mean score of artificial inoculation with *X. euvesicatoria* (P3, P4T2 p, t) and *X. vesicatoria* (P2, P3T2p, P1T2t) (Tables 2 and 3, Figures 2 and 3). The pepper types were resistant to *X. euvesicatoria* P4T2t and medium sensitive to *X. euvesicatoria* P4T2p and *X. vesicatoria* P2, P3T2p, P1T2t. Shipka was resistant to *X. vesicatoria* P1T2t, and Calabre to *X. euvesicatoria* P3. Shipka and Ivaylovska kapia were medium sensitive to *X. euvesicatoria* P3, and the Bulgarski ratund was sensitive to P3 race.

Pepper varietal type *var. Pumpkin:* - large-fruited bell pepper Bulgarski ratund and pungent small fruit bell – Calabre were different in reaction to the pathogens. Bulgarski ratund was characterized with heavy defoliation. The alkaloids and capsaicin substances in the tissues of Calabre reduced the penetration and multiplication of *X. euvesicatoria* (P3, P4T2 p, t).

X. euvesicatoria P4T2t (isolate from tomato) was weakly virulent when infecting pepper accessions compared to *X. euvesicatoria* P4T2p (isolate from pepper), which specialized in the genome of pepper. Race P4 (P4T2p, t) carried *AvrBs3, AvrBs4* and was more virulent than P1 (*AvrBs*2,3), P2 (*AvrBs* 1,2), P3 (*AvrBs2, AvrBs4*) of *X. vesicatoria* in pepper plants. Race P4 in PT was in combination with virulent tomato race T2 (Vancheva, 2015; Vasileva and Bogatzevska, 2019; Bogatzevska et al., 2021). The host had a role in the pathological process, increased virulence and aggressiveness depending on the specialization of the pathogen, pathotype and race. *X. euvesicatoria* (P, PT) was a host specific pathogen and it caused disease in species from *Capsicum* genus. Apart from specific races, host–pathogen combination was important for pathogen aggressiveness, e.g *X. euvesicatoria* was found to be more aggressive on pepper than on tomato (Ignjatov et al., 2010; Kizeva et al., 2020; Bogatzevska et al., 2021).

Host resistance had been a major goal of pepper breeding programmes. Our country is characterized with large number of well-adapted local populations with specific characteristics for shape, colour, taste, and biological value (Masheva, 2014). They have been used as a starting material for the conventional breeding programs directed toward development of genotypes with improved economic and agronomic traits (Tsonev et al., 2017). Conventional resistance (R) gene-mediated bacterial spot resistance relied on known *Avr* gene–R gene interactions. Resistance of pepper varietal types and each resistance gene could be overcome by specific races of the pathogens *X. euvesicatoria, X. vesicatoria* and functional avirulence gene (*avrBS* 1,2,3,4). Pepper resistance genes differentially interacted with races of xanthomonads: Bs1 gene conferred resistance to races 0, 2, and 5; Bs2 gene conferred resistance to races 0, 1, 2, 3, 7, and 8; Bs3 gene conferred resistance to races 0, 1, 4, 7, and 9; Bs4 gene confers resistance to races 0, 1, 3, 4, and 6 (Stall et al., 2009; Barka and Lee, 2020; McAvoy et al., 2021). Pepper contains Bs5 and Bs6, which conferred resistance to *X. euvesicatoria* (Potnis et al., 2015; Timilsina et al., 2020). Therefore, a deeper understanding of the responses of plant hosts to bacterial infection in pepper will contribute to accelerating the molecular breeding process and tackling the issue of the possible evolution of BS pathogens. Genetic resistance may be ineffective because of race shifts in the bacterial populations that emerge even before resistant cultivars are deployed. Composition of races may impact the durability of plant resistance. Plant disease resistance mechanisms were complex and rigorous for sensing and adapting to the environmental changes through the genetic regulatory network (Gao et al., 2020; McAvoy et al., 2021).

The use of resistant cultivars is the most appropriate form of control and for the development of these cultivars, it is necessary to know the genetic base that controls the resistance, allowing the selection of the most appropriate breeding method. Genetic control of BS had a quantitative aspect, with higher additive effect. Therefore, it was recommended to use breeding methods that allow selection of most advanced generations when the traits were already fixed, thus reducing the environmental effects.

Conclusion

The resistance of pepper varietal types (*Shipka, Pumpkin, Kapia*) was determined by the species of causative agent of BS, pepper host specialization (P, PT) and structure of races.

The Shipka, Bulgarski ratund, Calabre and Ivaylovska кapia were classified in defined groups with a proven statistical difference depending on reaction to artificial inoculation with heterogeneous species *X. euvesicatoria* and *X. vesicatoria*.

The cultivars from different types were medium sensitive to *X. vesicatoria*, independently of race P (P2), pathotype P3T2, P1T2 and the host from which the strains were isolated. The races P3, P4 (P4T2p, t) to the species *X. euvesicatoria* were more virulent when interacting with pepper varietal types (*Shipka, Pumpkin, Kapia*) than the races P1 (P1T2t), P2, P3 (P3T2p,) of *X. vesicatoria*.

The genetic diversity between *X. euvesicatoria* and *X. vesicatoria*, their phylogenetic positions and composition of races, suggest the use of separate statistical analysis groups to determine the resistance of pepper cultivars. In breeding programs, resistance of pepper varietal types is necessary to investigate the natural populations of the pathogens, structure of races, and each resistance gene could be overcome by specific races of the pathogens *X. euvesicatoria, X. vesicatoria* and functional avirulence gene.

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