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Evaluation of pepper breeding lines and accessions to *Xanthomonas euvesicatoria* and *X. vesicatoria* and fruit traits

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Abstract

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At the Maritsa Vegetable Crops Research Institute, species *X. euvesicatoria* and *X. vesicatoria* were isolated from pepper and molecularly identified. The isolates belonging to *X. euvesicatoria* refer to pathotypes P (P6 with 2 strains and P4 with 2 strains) and PT (P4T2 with 5 strains and P2T2 with 2 strains). The predominant races of *X. vesicatoria* were PT (P1T2 with 3 strains and P2 with 2 strains). From all examined isolates, it was established that *X. euvesicatoria* was more often isolated from pepper. Of the available pepper gene pool, 13 breeding lines and 3 accessions have been studied for their reaction to *X. euvesicatoria* P6, P4T2, and *X. vesicatoria* P2 and P1T2. Immune to *X. euvesicatoria* P6, P4T2, and *X. vesicatoria* P2, P1T2 were of pepper genotypes K915, K917, K925, SOL-300 and SOL-361, the rest were classified as resistant. The studied genotypes varied by fruit characteristics – size, shape, orientation, color, and taste. Breeding line K915 has been identified as immune to all studied races of bacterial spot (*X. euvesicatoria* P6 and P4T2 and *X. vesicatoria* P2 and P1T2), which combined with its fruit traits, makes it extremely valuable for breeding activity in this direction.

Keywords: Capsicum; pathotype; resistance; PCR amplification; fruit weight; length

Introduction

Bacterial spot of pepper caused by *Xanthomonas euvesicatoria* and *X. vesicatoria* is a serious disease of economic importance (Bogatzevska et al., 2007; Kurowski et al., 2019; Potnis et al., 2015; Vancheva, 2015; Wai et al., 2015).

In recent years, the climate change resulted in long periods of drought, but also with torrential rains, which favour the development and multiplication of bacterial leaves spot on pepper. Until now, no pepper cultivar resistant to these economically important pathogens has been developed in Bulgaria.

In this sense, the first step in the breeding process is the search, identification, or creation of genetic sources of resistance from different pepper genotypes, with diverse fruit characteristics. It is the main and most significant prerequisite for the creation of bacterial spot resistant pepper cultivars with valuable economic traits for different cultivations – early, mid-early or late open field production and/or in cultivated facilities, all of which suitable for various ways of consumption – fresh or processed as fried, roasted, pickled or for powder.

X. campestris pv. *vesicatoria* consists of four groups: A, B, C and D. Therefore *X. axonopodis* pv. *vesicatoria* group A the tomato and pepper pathogenic xanthomonads were reclassified within four stand-alone species, *X. euvesicatoria* (group A), *X. vesicatoria* (group B), *Xanthomonas perforans* (group C), and *Xanthomonas gardneri* (group D) (Jones et al., 2006; Jones et al., 2004). Hence, the two species *X. euvesicatoria* and *X. perforans* were reclassified as pathovars of the same species as *X. euvesicatoria* pv. *euvesicatoria* and *X. euvesicatoria* pv. *perforans*, respectively (Constantin et al., 2016). *X. gardneri* was reclassified as a later heterotypic synonym of *X. cynarae* and named *X. cynarae* pv. *gardneri*

(Kara et al., 2016; Timilsina et al., 2015) and now is reclassified as *X. hortorum* pv. *gardneri* (Morinière et al., 2020).

Greater genetic variety and private allelic richness is observed in Bulgarian structures of *X. euvesicatoria*. The absence of exclusive differentiation between the regions and the sharing of haplotypes or clonal complexes by strains (Vancheva et al., 2021) and the diversity among *X. euvesicatoria* strains is consistent with worldwide movement of clonal compositions in seeds, whereas geographic isolation appears to be shaping the population structure of *X. vesicatoria* (Dhakal et al., 2019; Timilsina et al., 2020; Vancheva et al., 2021).

X. euvesicatoria is narrowly specialized on *Capsicum*, while the species *X. vesicatoria* is a major pathogen on tomato (Bogatzevska et al., 2007; Bogatzevska & Pandeva, 2009; Ignjatov et al., 2010; Vancheva et al., 2014).

The natural population of *X. euvesicatoria* is heterogeneous in pathotype and races. The P6 race is widespread, while the dominant race in pepper-tomato pathotype (PT) is P4 in combination with T2. Within *X. vesicatoria* pepper pathotype (P), races P0, P2 and P3 are differentiated, while races P1 and P3 were differentiated in combination with tomato race T2 (Vasileva & Bogatzevska, 2019).

The aim of the study was identification of the causative agents of bacterial spot and evaluation of the resistance of pepper breeding lines and accessions to their most common races and some fruit traits.

Materials and Methods

Isolations

The bacteria were isolated from pepper field of Maritsa Vegetable Crops Research Institute by the serial dilution method (Rudolph et al., 1990) from plant material with symptoms characteristic of bacterial spot diagnostic nutrient media (Schaad, 2001). The pathogenic properties of pure cultures were tested by tobacco injection (Klement et al., 1990) and vacuum infiltration of pepper (Vasileva & Bogatzevska, 2019).

Identification

Genus's differentiation of pathogenic isolates included the main physiological and biochemical characteristics: Gram, fluorescent pigment synthesis on King's B medium, oxidase activity, catalase activity (Schaad, 2001). Oxidase activity was determined on standard test strips Bactident Oxidase (Merck #1.13300.0001), and catalase – Bactident Catalase (Merck #1.11351).

Isolation of DNA for conducting the genetic analysis was carried out after obtaining biomass from the strains using ready kits according to the procedure described by the manufacturer – Genaxon Bioscience (Cat#: S5396; Version 230418). Identification was performed by PCR reactions with specific primers. The affiliation of the strains isolated from peppers was determined by PCR amplification of genomic DNA with the following species-specific primers: Bs-XvF/Bs-XvR – specific to the species *X. vesicatoria*, Bs-XeF/Bs-XeR and Xeu2.4/Xeu2. 5 – specific to the species *X. euvesicatoria*. The type cultures *X. vesicatoria* NBIMCC 2427 and *X. euvesicatoria* NBIMCC 8731 were included as controls.

PCR master Mix with Taq DNA reaction mixture was used for PCR. Amplification was performed on a Biorad T100 Thermal Cycler under the following conditions: denaturation for 5 min at 95°C, followed by 35 cycles of 95°C for 30 s, 58°C for 45 s, and 72°C for 45 s and a final elongation step at 72°C for 7 min. PCR reaction mixture: H_2O; Red Taq polymerase master mix 0.5x; Primer – straight 10 pmol; Primer – reverse 10 pmol; DNA 100 ng; Final volume 25 µl. The resulting PCR products were separated electrophoretically in a 1% agarose gel with added green, fluorescent dye in 1 x TBE buffer – 30 min, 100 V. 5 µl sample mixed with 2 µl dye was instilled. The gel was photographed under UV light. A 100 bp DNA marker was used.

Differentiation of pathotype and races

The pathotype of the causative agents of bacterial spot pepper was differentiated on test plants: tomato cultivar Ideal and pepper cultivar California Wonder (Bogatzevska & Sotirova, 1992). Races of the pepper-tomato pathotype (PT) of species on the genus Xanthomonas were determined. The race structures in PT were determined based on the sensitive (S) and hypersensitive reaction (HR) on the leaves of the lines – differentiators L Hawaii 7981 and L Hawaii 7998 (Jones et al., 1995) and variety Ideal according to the methodology of Bogachevska & Sotirova (2001). The races of the causative agents of bacterial spot in P on pepper (*Xanthomonas euvesicatoria*, *X. vesicatoria*) were differentiated into isogenic lines obtained from Early California Wonder: ECW10R, ECW20R, ECW30R (Kurowski et al., 2019) according to the methodology of Vasileva & Bogatzevska (2019).

Study of the reaction of breeding lines and accessions to the causative agents of bacterial spot

Thirteen breeding lines and three accessions from the pepper collection of Maritsa Vegetable Crops Research Institute were tested for races P6 and P4T2 of *X. euvesicatoria* and races P2 and P1T2 of *X. vesicatoria*. (Table 1). They were a result of many years of breeding – interspecific hy-

Table 1. Description of the studied pepper breeding lines and accessions

№	Genotypes	Description
1	K910	*C. annuum* var. *annuum x C. annuum* var. *glabriusculum*
2	K913	*C. annuum* var. *annuum x C. chinense*
3	K914	*C. annuum* var. *annuum x C. chinense*
4	K915	*(C. annuum* var. *annuum x C. chinense) x C. annuum* var. *annuum*
5	K916	*(C. annuum* var. *annuum x C. chinense) x C. annuum* var. *annuum*
6	K917	*(C. annuum* var. *annuum x C. chinense) x C. annuum* var. *annuum*
7	K919	*(C. annuum* var. *annuum x C. chinense) x C. annuum* var. *annuum*
8	K920	*(C. annuum* var. *annuum x C. chinense) x C. annuum* var. *annuum*
9	K921	*(C. annuum* var. *annuum x C. chinense) x C. annuum* var. *annuum*
10	K922	*(C. annuum* var. *annuum x C. chinense) x C. annuum* var. *annuum*
11	K924	*(C. annuum* var. *annuum x C. chinense) x C. annuum* var. *annuum*
12	K925	*(C. annuum* var. *annuum x C. frutescens) x C. annuum* var. *annuum*
13	K941	C. baccatum
14	SOL-198	C. baccatum
15	SOL-300	C. baccatum
16	SOL-361	C. baccatum

bridization, backcrossing, and subsequent self-pollinations and selections. The accessions SOL-198, SOL-300 and SOL-361 belong to *Capsicum baccatum*, a species that was not popular in Bulgaria and the Balkan region. *Capsicum annuum* is widespread in them.

The sowing of the seeds was done in mid-March in an unheated glass greenhouse. A peat-perlite mixture was used, and the substrate was previously enriched with mineral fertilizers.

Pepper plants in the first true leaf phase were infected with a bacterial suspension at a concentration of 10^8 cfu/ml from a 36 h culture, by the vacuum-infiltration method (vacuum pump 55–60 kPa (1 at = 101.3 kPa)), with strains of *X. euvesicatoria* and *X. vesicatoria*, PT and P. The inoculated plants (25–30 plants) were placed in Knop's nutrient solution and grown under laboratory conditions, at a temperature of 20–25°C (Bogatzevska et al., 2007).

Leaf symptoms and the number of fallen leaves with ring-shaped necrosis of the leaf petiole were recorded 4-5 days after infiltration on a 5-point scale (Bogatzevska et al., 2007). Mean score rate (ms) and defoliation index (Di%) were calculated (Petsi et al., 1990). The classification of the samples into groups was done depending on the average mean score (Petsi et al., 1990; Vasileva & Bogatzevska, 2021).

X. euvesicatoria resistance groups: I- immune 0; R- resistant (0.01-0.44); MS – medium sensitive (0.45–0.79); S – sensitive (0.80-1.12); SS – strongly sensitive (over 1.13). The percentage distribution depending on ms value was respectively: I – immune – 0%; R – resistant – 19%; MS – medium sensitive – 48%; S – sensitive – 4%; SS – strongly sensitive – 4%.

X. vesicatoria in the following groups: I- immune 0; R – resistant (0.01–0.36); MS – medium sensitive (0.37–0.79); S – sensitive (0.80–1.18); SS – strongly sensitive (over 1.19) (Vasileva & Bogatzevska, 2021).

The artificially inoculated pepper breeding lines and accessions were determined as immunes when did not show any symptoms of the bacterial spot agents and lack of defoliation.

To evaluate the materials for significant fruit traits, after testing the resistant plants were planted in an insect proof net house. The planting of the studied genotypes was carried out in the middle of May according to the scheme 120+40/15 cm. Seedling production and care during the growing season were in accordance with the pepper requirements and the technology for mid-early field production (Todorova et al., 2014). Fruit quantitative traits of breeding lines and accessions were studied according to Descriptors for *Capsicum* (IPGR, AVRDC and CATIE, 1995) for: length of fruit (cm), width of fruit (cm), locules of fruit (number), weight of fruit (g) and fruit wall thickness (mm). Biometric measurements were performed at maturity stage on randomly selected fruits. The genotypes were also evaluated for taste, attitude, and colour of the fruits.

The software programs used in data processing are "MS Excel Analysis ToolPak Add-Ins" 2019 and "R-4.0.3" in combination with "RStudio-0.98" and installed package "agricolae 1.2–2" (De Mendiburu, 2021).

Results and Discussion

The analysis of pepper leaves with characteristic symptoms of bacterial spot resulted in 16 pure cultures. Small,

irregular, watery, necrotic spots surrounded by a chlorotic halo typical of *X. euvesicatoria* (Figure 1A) formed on the leaves. *X. vesicatoria* (Figure 1B) formed large, solitary brown, watery lesions that may cover the entire leaf surface. A necrotic ring formed at the base of the petiole and the leaves dropped off.

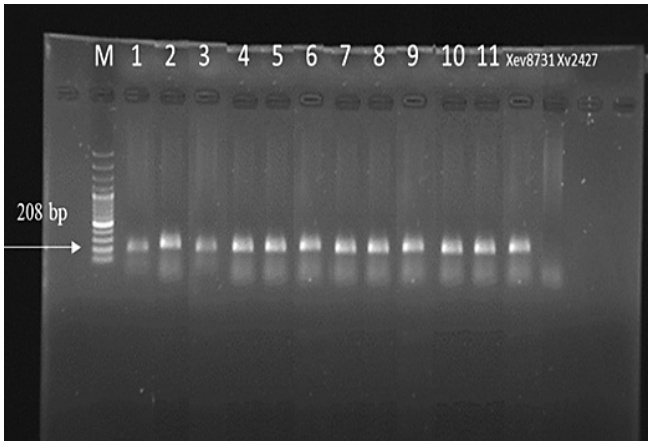
A

B

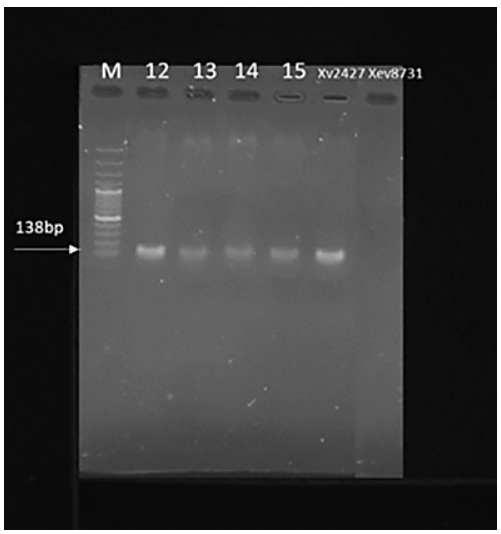
Fig. 1. Symptoms caused by:
A – *X. euvesicatoria*; B – *X. vesicatoria*

The analyses revealed that all strains with a positive signal (11 strains) formed the expected amplification products for the species *X. euvesicatoria* with the two pairs of primers used – 208bp in length and did not form the expected products with the primers for the species *X. vesicatoria*. The remaining isolates (5 strains) that showed a negative result with the primers for *X. euvesicatoria* gave a positive signal, forming an amplification product of the expected length of 138bp only in PCR-amplification with the primers specific for the *X. vesicatoria* species (Figure 2).

The isolates belonged to *X. euvesicatoria* refer to (pepper) P and (pepper-tomato) PT pathotype (11 strains.). The races determined in P were P6 (2 strains) and P4 (2 strains). Races found in PT were P4T2 (5 strains) and P2T2 (2 strains). The predominant races in PT of *X. vesicatoria* were P1T2 (3 strains) and P2 (2 strains).



A



B

Fig. 2. 16S rDNA amplification of *Xanthomonas* strains:
A – *X. euvesicatoria*; B – *X. vesicatoria*.
*** M – DNA marker**

From all examined isolates, it was established that *X. euvesicatoria* was more often isolated from pepper. Pathotype analysis revealed differences in dominant populations of pepper pathogens. Race P6 in the P pathotype of the pathogen was prevalent, followed by race P4. While in the population of *X. vesicatoria*, which was isolated in a lower frequency, P2 and P1T2 prevailed. Using these most widespread races of the pathogens, an evaluation of the reaction of pepper breeding lines and accessions was carried out.

The tested 13 breeding lines and three accessions were characterized by a different degree of attack when artificially inoculated with P and PT pathotype of *X. euvesicatoria* and *X. vesicatoria* (Table 2). A typical symptom of the causative agents on pepper was leaf drop, which was determined by

Table 2. Evaluation of resistance to races P6 and P4T2 of *X. euvesicatoria* and races P2 and P1T2 of *X. vesicatoria* in studied genotypes

№	Race[1]	NP[2]	NL[3]	HR[4]	0	1	2	3	4	ms[5]	Di%[6]
K910	1 XevP6	30	196	0	192	4	0	0	0	0.02	0.00
	1 XevP4T2	30	201	0	195	5	1	0	0	0.04	0.00
	1 XvP2	30	212	0	203	7	1	1	0	0.04	0.00
	1 XvP1T2	30	221	0	219	1	1	0	0	0.01	0.00
K913	2 XevP6	30	238	0	235	3	0	0	0	0.01	2.94
	2 XevP4T2	29	204	0	204	0	0	0	0	0.00	0.49
	2 XvP2	30	215	0	213	2	0	0	0	0.01	4.19
	2 XvP1T2	30	223	0	223	0	0	0	0	0.00	0.90
K914	3 XevP6	29	201	0	199	2	0	0	0	0.01	1.49
	3 XevP4T2	28	234	0	234	0	0	0	0	0.00	0.00
	3 XvP2	29	237	0	237	0	0	0	0	0.00	0.00
	3 XvP1T2	30	211	0	211	0	0	0	0	0.00	0.47
K915	4 XevP6	27	195	0	195	0	0	0	0	0.00	0.00
	4 XevP4T2	30	219	0	219	0	0	0	0	0.00	0.00
	4 XvP2	25	187	0	187	0	0	0	0	0.00	0.00
	4 XvP1T2	28	197	0	197	0	0	0	0	0.00	0.00
K916	5 XevP6	18	145	0	143	1	1	0	0	0.02	0.00
	5 XevP4T2	17	142	0	135	6	1	0	0	0.06	0.00
	5 XvP2	17	144	0	133	8	2	1	0	0.10	0.00
	5 XvP1T2	18	156	0	151	3	2	0	0	0.04	0.00
K917	6 XevP6	30	219	0	219	0	0	0	0	0.00	0.00
	6 XevP4T2	27	194	0	194	0	0	0	0	0.00	0.00
	6 XvP2	28	197	0	197	0	0	0	0	0.00	0.00
	6 XvP1T2	29	202	0	202	0	0	0	0	0.00	0.00
K919	7 XevP6	30	168	0	153	15	1	0	0	0.10	13.69
	7 XevP4T2	30	171	0	169	2	0	0	0	0.01	10.53
	7 XvP2	28	165	0	160	5	0	0	0	0.03	13.33
	7 XvP1T2	30	175	0	174	1	0	0	0	0.01	6.86
K920	8 XevP6	25	135	0	131	4	0	0	0	0.03	4.44
	8 XevP4T2	26	139	0	137	1	1	0	0	0.02	3.60
	8 XvP2	28	142	0	134	7	1	0	0	0.07	11.27
	8 XvP1T2	25	126	0	124	2	0	0	0	0.02	2.38
K921	9 XevP6	22	115	0	107	6	1	1	0	0.10	7.83
	9 XevP4T2	21	128	0	118	8	2	0	0	0.08	3.13
	9 XvP2	25	132	0	129	3	0	0	0	0.02	4.55
	9 XvP1T2	25	133	0	130	2	1	0	0	0.03	2.26
K922	10 XevP6	15	84	0	78	5	1	0	0	0.08	7.14
	10 XevP4T2	14	52	0	48	4	0	0	0	0.08	13.46
	10 XvP2	14	44	0	41	2	1	0	0	0.09	0.00
	10 XvP1T2	13	41	0	39	2	0	0	0	0.05	0.00
K924	11 XevP6	30	178	0	175	3	0	0	0	0.02	2.25
	11 XevP4T2	27	152	0	146	5	1	0	0	0.05	5.26
	11 XvP2	30	181	0	177	4	0	0	0	0.02	0.55
	11 XvP1T2	30	175	0	172	2	1	0	0	0.02	0.00

Table 2. Continued

K925	12 XevP6	28	155	5	150	0	0	0	0	0.00	0.00
	12 XevP4T2	27	146	0	146	0	0	0	0	0.00	0.00
	12 XvP2	27	144	2	142	0	0	0	0	0.00	0.00
	12 XvP1T2	29	171	3	168	0	0	0	0	0.00	0.00
K941	13 XevP6	30	168	0	163	3	1	1	0	0.05	7.14
	13 XevP4T2	30	171	0	169	1	1	0	0	0.02	4.68
	13 XvP2	28	159	0	154	2	3	0	0	0.05	9.43
	13 XvP1T2	27	148	0	147	1	0	0	0	0.01	2.70
SOL-198	14 XevP6	10	86	0	82	4	0	0	0	0.05	1.16
	14 XevP4T2	8	80	0	78	2	0	0	0	0.03	0.00
	14 XvP2	9	77	0	75	2	0	0	0	0.03	0.00
	14 XvP1T2	9	71	0	68	3	0	0	0	0.04	0.00
SOL-300	15 XevP6	12	79	0	79	0	0	0	0	0.00	0.00
	15 XevP4T2	11	68	0	68	0	0	0	0	0.00	0.00
	15 XvP2	11	63	0	63	0	0	0	0	0.00	0.00
	15 XvP1T2	10	55	0	55	0	0	0	0	0.00	0.00
SOL-361	16 XevP6	12	77	0	77	0	0	0	0	0.00	0.00
	16 XevP4T2	14	88	0	88	0	0	0	0	0.00	0.00
	16 XvP2	13	80	0	80	0	0	0	0	0.00	0.00
	16 XvP1T2	15	83	0	83	0	0	0	0	0.00	0.00

[1] XevP6 – *X. euvesicatoria* P6; Xev P4T2 – *X. euvesicatoria* P4T2; XvP2 – *X. vesicatoria* P2; XvP1T2 – *X. vesicatoria* P1T2; [2]NP – number of plants; [3]NL – number of leaves; [4]HR – hypersensitive reaction; [5]ms – mean score; [6]Di% – Defoliation index; 0-4 five rate scale

the defoliation index (Di) (Vasileva & Bogatzevska, 2022). Among the studied genotypes, leaf fall was most pronounced in K919 (*X. euvesicatoria* P6 – 13.69%; P4T2 – 10.53%; *X. vesicatoria* P2 – 13.33% and P1T2 6.86%); K920 (*X. vesicatoria* P2 – 11.27%); K922 (*X. euvesicatoria* P4T2 – 13.46%); K941 (*X. vesicatoria* P2 – 9.43%). Among the tested materials, those without characteristic defoliation stand out – K910, K915, K916, K917, K925, SOL-300, SOL-361.

With immune reaction to *X. euvesicatoria* P6 were pepper genotypes K915, K917, K925, SOL-300, SOL-361, and with resistance: K910, K913, K914, K916, K919, K920, K921, K922, K924, K941 and SOL-198 (Table 1, 2). Resistance to *X. euvesicatoria* P4T2 was exhibited by K910, K916, K919, K920, K921, K922, K924, K941 and SOL-198. Genotypes K913, K914, K915, K917, K925, SOL-300 and SOL-361 were immune. To *X. vesicatoria* P2 with immune reaction were K914, K915, K917, K925, SOL-300, SOL-361, and with resistant: K910, K913, K916, K919, K920, K921, K922, K924, K941 and SOL-198. The genotypes classified in the group of those immunes to P1T2 of *X. vesicatoria* were K913, K914, K915, K917, K925, SOL-300, SOL-361, and the resistant group included: K910, K916, K919, K920, K921, K922, K924, K941 and SOL-198.

Immune to *X. euvesicatoria* P6 and P4T2 and *X. vesicatoria* P2 and P1T2 were genotypes K915, K917, K925, SOL-300 and SOL-361, the rest were classified as resistant (Table 3).

It was established that resistant (11) and immune (5) genotypes predominate for *X. euvesicatoria* P6, and for P4T2 the ratio is 9:7. The reaction of the tested breeding lines and accessions to *X. vesicatoria* P2 and P1T2 was 10:6 and 9:7 resistant and immune genotypes, respectively.

Table 3. Classification of studied breeding lines and accessions according to their response towards some races of pathogens *X. euvesicatoria* and *X. vesicatoria*

Level of resistance	*X. euvesicatoria*		*X. vesicatoria*	
	P6	P4T2	P2	P1T2
Immune	K915, K917, K925, SOL-300, SOL-361	K913, K914, K915, K917, K925, SOL-300, SOL-361	K914, K915, K917, K925, SOL-300, SOL-361	K913, K914, K915, K917, K925, SOL-300, SOL-361
Resistant	K910, K913, K914, K916, K919, K920, K921, K922, K924, K941, SOL-198	K910, K916, K919, K920, K921, K922, K924, K941 SOL-198	K910, K913, K916, K919, K920, K921, K922, K924, K941, SOL-198	K910, K916, K919, K920, K921, K922, K924, K941, SOL-198

Fig. 3. Fruits of the tested breeding lines and accessions as follows (top to bottom and left to right): 1st row – K910, K913, K914, K915, K916 and K917; 2nd row – K919, K920, K921, K922 and K924; 3rd row – K925, K941, SOL – 198; SOL-300 and SOL-361

From the performed visual and morphological characterization, it was found that the tested lines and accessions varied by size, shape, orientation, colour, and taste of the fruits (Figure 3). Orange fruits were represented by two lines – K917 and K919, yellow-orange – K913, K914, K920 and K921, light red – SOL-198, SOL-300 and SOL-361. The breeding line K925 formed fruits with purple coloration determined by strong anthocyanin in intermediate stage (green stage), followed by K914. A characteristic feature of the K910 line was the very easy separation of the fruit from the calyx and pedicel.

Nine genotypes K910, K913, K914, K916, K917, K941, SOL-198, SOL – 300 and SOL – 361 had a pungent taste of the fruits. Most of them formed relatively shorter fruits as with the shortest fruits being characterized SOL-300 (2.40 cm), followed by line K914 with 2.62 cm (Table 4).

The breeding line K915 formed the longest fruits (13.33 cm), followed by K924 (12.50 cm) and K922 (11.97 cm). In terms of fruit width, the amplitude was within smaller limits – from 1.02 cm for K941 to 4.53 cm for K922. The locules of the fruits of the studied genotypes were in the range from 2 to 4. The thinnest fruit wall was established for line

Table 4. Morphological analysis of the fruit of the tested pepper genotypes

№	Genotypes	Fruit taste	Length, cm	Width, cm	Locules, number	Wall thickness, mm	Weight, g
1	K910	hot	4.42±0.32	1.25±0.18	2.17±0.41	0.84±0.34	1.99±0.24
2	K913	sweet-spicy	4.28±0.32	1.27±0.08	2.83±0.41	0.96±0.15	3.45±0.20
3	K914	hot	2.62±0.26	1.48±0.10	2.50±0.55	1.49±0.34	2.38±0.52
4	K915	sweet	13.33±1.21	4.10±0.51	2.17±0.41	3.24±0.24	62.82±4.89
5	K916	hot	11.82±1.30	1.65±0.14	2.17±0.41	2.73±0.71	13.90±2.32
6	K917	hot	3.67±0.32	1.72±0.16	3.00±0.63	1.41±0.13	3.78±0.73
7	K919	sweet	5.90±0.26	2.93±0.15	3.17±0.75	2.13±0.25	14.31±1.49
8	K920	sweet	10.67±0.40	3.23±0.30	2.00±0.00	2.91±0.62	29.66±1.88
9	K921	sweet	10.12±0.68	3.05±0.37	2.83±0.41	3.34±0.44	31.93±4.99
10	K922	sweet	11.97±0.64	4.53±0.25	2.33±0.52	3.24±0.34	54.44±2.93
11	K924	sweet	12.50±0.97	2.91±0.14	2.33±0.52	2.69±0.19	30.36±0.82
12	K925	sweet	9.50±0.91	1.82±0.16	2.50±0.55	2.74±0.13	14.76±3.69
13	K941	hot	4.67±0.46	1.02±0.15	3.00±0.00	1.30±0.21	3.19±0.39
14	SOL-198	hot	3.35±0.33	2.32±0.27	3.33±0.52	2.20±0.42	6.78±1.82
15	SOL-300	hot	2.40±0.32	2.10±0.22	4.00±0.00	1.95±0.16	4.46±1.35
16	SOL-361	hot	9.67±1.19	1.35±0.14	2.00±0.00	2.36±0.52	9.58±1.00

K910 (0.84 mm) and K913 with 0.96 mm, while the thickest – K921 with 3.34 mm. It was followed by K915 and K922 with 3.24 mm. Predominantly the sweet-tasting fruit materials had a thicker pericarp, while those with a hot taste had a thinner fruit wall. This was also the trend regarding the fruit weight. Average fruit weight varied widely from 1.99 g for K910 to 62.82 g for K915. Line K915 stands out according to the complex morphological characteristics of the fruit, which ranks first in fruit length and weight, and second in fruit width and fruit wall thickness. Line K922 was also characterized by high values for fruit length, width, and average weight, as well as for fruit wall thickness.

The obtained results revealed genes for resistance to bacterial spot being successfully transferred to *C. annuum* genotypes from germplasm of *C. annuum* var. *glabriusculum*, *C. chinense* and *C. fruitescens* through introgressive hybridization and backcrossing. The usage of *C. baccatum* accessions as parents in hybridization program with *C. annuum* would be attended with some difficulties which might be overcome (Tóth et al., 2023).

Genetic resistance may be ineffective because of race shifts in the bacterial populations that emerge even before resistant cultivars are deployed. The structure of races composition 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 data showed that the identified strains completely match the type cultures. Pepper cultivars widespread in Bulgaria are sensitive, but hot and small-fruited cultivars were less sensitive to *X. euvesicatoria* and *X. vesicatoria* (Bogatzevska & Pandeva, 2009; Vancheva et al., 2016). The cultivars from different types were medium sensitive to *X. vesicatoria*, independently of race pepper (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* (Vasileva & Bogatzevska, 2022).

Conclusions

During the study, the species *X. euvesicatoria* and *X. vesicatoria* were molecularly identified. The pathotype and races of the causative agents of bacterial spot pepper were determined.

The response of 13 breeding lines and 3 accessions from the available pepper gene pool to *X. euvesicatoria* P6 and P4T2 and *X. vesicatoria* P2 and P1T2 was studied.

The breeding lines K915, K917 and K925, and accessions SOL-300 and SOL-361 deserve special attention for future breeding purposes because degree of attack and defoliation were not recorded in them, and they were classified as immune to tested races of *X. euvesicatoria* and *X. vesicatoria*.

It can be summarized the studied genotypes are various by phenotypic characterization – size, shape, orientation, colour, and taste of the fruits and possess resistance or immunity to bacterial spot. Breeding line K915 has been identified as immune to all studied races of bacterial spot (*X. euvesicatoria* P6 and P4T2 and *X. vesicatoria* P2 and P1T2), which, combined with its fruit characteristics, makes it extremely valuable for breeding activity in this direction.

The Balkan region and Bulgaria in particular, are well-known by the presence of a rich diverse of pepper cultivars (*Capsicum annuum*) with different taste, colour and morphological characteristics of the fruit, which are sought by users for specific consumption directions. The immune and resistant breeding lines of pepper with various fruit characters established in this study are a valuable prerequisite for the successful creation of pepper cultivars resistant to bacterial spot with different ways of production and usage to meet the demands of farmers, processors, and consumers.

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