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Screening *Pisum* sp. accessions for resistance to *Pseudomonas syringae* pv. *pisi*

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**Abstract.** Pea (*Pisum sativum* L.) is a main grain leguminous crop in Bulgaria. Breeding resistant pea varieties is the most economically important method for disease control especially in pea bacterial blight, caused by *Pseudomonas syringae* pv. *pisi*. The investigation was carried out during 2013 – 2014 at Dobrudzha Agricultural Institute, General Toshevo. Forty-eight *Pisum* sp. accessions were used. Two strains of *P. syringae* pv. *pisi* (B24 and NCPPB 2585) were used. The plants were inoculated twice: at phenophase budding (petioles) and grain filling (pod) with bacterial suspension in concentration 1 x 10^8 CFU/ml using 1ml syringe. The reaction of the accessions was read 10 days after inoculation. A nine-degree scale was applied. According to the mean disease index (MDI) the accessions were divided into five groups: immune (I), resistant (R), middle resistant (MR), susceptible (S), very susceptible (VS). Twenty-five *Pisum* sp. accessions had resistant to moderately resistant reaction of leaves and pods to strain NCPPB and twelve accessions to strain B24, respectively. Eleven accessions (including *P. sativum* ssp. *sativum*, *P. humile*, *P. elatius*, *P. s. var. hibernicum*, *P. abyssinicum*, *P. transcaucasicum*, *P. tibetanicum*) had resistant reaction to leaves and pods to both strains which confirms the position for different genetic control of resistance of leaves and pods. The results of our investigation showed that some *Pisum* sp. accessions could be used as a donor parent in a breeding program for bacterial or complex resistance on pea.

**Keywords:** *Pisum* sp., pea, bacterial blight, *P.s.pv. pisi*

**Introduction**

Pea (*Pisum sativum* L.) is a main grain leguminous crop in Bulgaria. The size of the yields of pea is determined by a number of abiotic and biotic factors, among which diseases are significant: viral, bacterial and fungal. Among the bacterial diseases, bacterial blight is of the most economic importance. The disease is caused by Gram-negative, aerobe phytopathogen bacteria *Pseudomonas syringae* pv. *pisi*. It is reported for the first time in North America in 1915 (Sackett, 1916), in Europe in 1985 (Grondeau et al., 1996) and in Turkey in 2010 (Benlioglu, 2010). Disease development is favored by autumn or early spring sowing of pea (Mansfield et al., 1997), rainfalls (Roberts, 1997) and temperatures 27 – 28ºC (Saskett, 1916). The bacteria is seed transmitted (Skoric, 1927; Lawyer and Chun, 2001), but it can survive in the soil up to 42 days (Hollaway and Bretag, 1997) and in the plants up to 34 weeks after harvest (Grondeau et al., 1996). Up to now eight races of *P.s. pv. pisi* (Beaven et al., 1995; Elvira-Recuenco et al., 2001; Martin-Sanz et al., 2011) and six race-specific genes for resistance in pea (*P. sativum*) (Beaven et al., 1995) to the pathogen have been identified. In Bulgaria, the disease has insignificant effect on pea production and the investigations on its etiology and epidemiology are limited.

Breeding resistant pea varieties is the most economically important method for disease control especially in pea bacterial blight (Sackett, 1916; Hollaway et al., 2007). The first step in every disease resistance breeding program is screening different accessions among the same and different species of the same genus for finding sources of resistance (Martin et al., 2008). The aim of this investigation was to determine the disease response of some *Pisum* sp. accessions to the cause agent of pea bacterial blight – *P.s. pv. pisi*.

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**Material and methods**

The investigation was carried out during 2013 – 2014 at Dobrudzha Agricultural Institute, General Toshevo. Forty seven *Pisum* sp. accessions were used: 19 – *P. sativum*, 7 – *P. s. subssp. sativum var. arvense, 4 – *P. elatius*, 2 – *P. humile*, 1 – *P. jomardi*, 1 – *P. s. var. hibernicum*, 1 – *P. thebaicum*, 5 – *P. abyssinicum*, 4 – *P. transcaucasicum*, 1 – *P. asiaticum*, 2 – *P. tibetanicum*, provided by Jonh Innes Centre, Variety Kelvedon Wonder was used as a susceptible control (Elvira-Recuenco et al., 2003). The accessions were planted in 1 m rows, 10 plants per row. Two strains of *P. syringae* pv. *pisi* (NCPPB 2585 and B24) originating from Turkey (Benlioglu et al., 2010) were used for inoculation. The strains were cultivated on YDC and stored at 4ºC. The plants were inoculated twice: at phenophase budding (petioles) and grain filling (pods) with bacterial suspension in concentration 1 x 10^8 CFU/ml using 1ml syringe. The reaction of the accessions was read 10 days after inoculation. A nine-degree scale was applied (Figure 1): Leaves: 1 – symptomless; 3 – necrosis around the penetration; 5 – necrosis with water soaked in the spot; 7 – water soaked spot in the place of penetration of the leaf surface; 9 – leaf dead. Pods: 1 – symptomless; 3 – water soaked spots up to 1.0 mm; 5 – water soaked spots 1.1 – 3.0 mm; 7 – water soaked spots 3.1 – 5.0 mm; 9 – water soaked spots > 5.0 mm. The mean disease index (MDI) was calculated (MDI = ∑(n x DI)/N), where n is number of plants; DI is disease index according to the scale; N is the number of all plants. According to MDI, the accessions were divided into five groups: MDI = 1.0 – immune (I), MDI = 1.1 – 3.0 - resistant (R), MDI = 3.1 – 5.0 - middle resistant (MR), MDI = 5.1 – 7.0 - susceptible (S), MDI > 7.0 very susceptible (VS).
Variety Kelvedon Wonder had susceptible reaction of leaves and pods to strains NCPPB 2585 and Bz4 of *P. s. pv. pisi*. Thirteen *Pisum* sp. accessions had a resistant phenotype, 18 accessions had a moderately resistant phenotype and 16 accessions had susceptible phenotype of leaves to strain NCPPB 2585 (Figure 2). Eight accessions had resistant reaction, 18 accessions had moderately resistant reaction and 21 accessions had resistant reaction of pods to the same strain. No accession had an immune or very susceptible reaction of leaves and pods to strain NCPPB 2585. Twenty-five *Pisum* sp. accessions had resistant/moderately resistant reaction of leaves and pods. Among them 6 accessions *P. sativum*, 5 – *Ps. subsp. sativum var. arvense*, 2 – *P. elatius*, 2 – *P. humile*, 1 – *Ps. var. hibernicum*, 4 – *P. abyssinicum*, 3 – *P. transcaucasicum*, 2 – *P. tibetanicum*. The rest accessions had different response of leaves and pods to this strain.

Two *Pisum* sp. accessions had resistant phenotype, 15 accessions had moderately resistant phenotype and 30 accessions had susceptible phenotype of leaves after inoculation with strain Bz4 (Figure 3). One accession had a resistant reaction, 16 accessions had moderately resistant reaction and 30 accessions had susceptible reaction of pods. No accession had an immune or very susceptible reaction of leaves and pods to strain Bz4. Twelve accessions had resistant/moderately resistant reaction of leaves and pods. Among them 2 accessions of the species *P. sativum*, *P. abyssinicum*, *P. transcaucasicum*, *P. tibetanicum* and one accession of the species *P. subsp. sativum var. arvense*, *P. elatius*, *P. humile* and *P. s. var. hibernicum*.

Complex resistance of leaves and pods to both strains of *P.s. pv. pisi* had eleven *Pisum* sp. accessions (Table 1). They had resistant or moderately resistant phenotype of leaves and pods after inoculation with strains NCPPB 2585 and Bz4 of the pathogen. These accessions can be used as a donor parent in a bacterial blight resistant breeding program in pea. In the literature the information about cross ability between different species of genus *Pisum* is limited so that future investigations will give clarity about these possibilities.

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**Results and discussion**

1. Immune (I)  3. Resistant (R)  5. Moderate Resistant (MR)  7. Susceptible (S)  9. Very Susceptible (VS)

**Figure 1.** Scale for estimation of the reaction of *Pisum* sp. accessions after inoculation of leaves and pods with *Pseudomonas syringae pv. pisi*

**Figure 2.** Distribution of 47 *Pisum* sp. accessions according to the reaction of leaves and pods to strain NCPPB 2585 of *P. s. pv. pisi*

**Figure 3.** Distribution of 47 *Pisum* sp. accessions according to the reaction of leaves and pods to strain Bz4 of *P. s. pv. pisi*
Species included in this investigation is race (strain)-specific. This indicates that the resistance in the JI 1556) vary from resistant, moderately resistant to susceptible.

The reaction of leaves and pods of the other accessions (JI 225, JI 2 and Epiphytic life is the main characteristic of the life cycle of leaves and pods to both strains of the pathogen (Table 1). The P. abyssinicum of a ( ). Schmit et al. (1993) described the resistance glasshouse and field conditions. European Journal of Plant Martin-Sanz et al., 2011) and is governed by dominant genes responses to pea bacterial blight in stems, leaves and pods under specific ( ). Elvira-Recuenco et al., 2001

specific ( ). Elvira-Recuenco et al., 2003; Kiryakov and Turkey. Plant Disease, 7, 923. Conclusion

Differences in the reaction of leaves and pods of some Pisum sp. to strain NCPPB 2585 and Bz4 of Pseudomonas syringae pv. pisi have been observed. Twenty-five Pisum sp. accessions had resistant/moderately resistant reaction of leaves and pods to strain NCPPB 2585 and twelve Pisum sp. accessions had resistant/moderately resistant reaction to strain Bz4. Eleven Pisum sp. accessions had resistant or moderately resistant phenotype of leaves and pods after inoculation with both strains of P. pv. pisi. They can be used as a donor parent in a disease resistant breeding program in pea.

References


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Table 1. Reaction of 11 Pisum sp. accessions to strains NCPPB 2585 and Bz4 of P. syringae pv. pisi

<table>
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<td>NCPPB 2585</td>
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<td></td>
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<td>Leaf</td>
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<tr>
<td>P648</td>
<td>P. sativum subsp. sativum var. arvense</td>
<td>R</td>
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<td>JI 2201</td>
<td>P. elatus</td>
<td>MR</td>
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<td>JI 241</td>
<td>P. humile</td>
<td>MR</td>
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<tr>
<td>JI 181</td>
<td>P. sativum</td>
<td>R</td>
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<tr>
<td>JI 1846</td>
<td>P. sativum var. hibernicum</td>
<td>R</td>
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<tr>
<td>JI 2385</td>
<td>P. abyssinicum</td>
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<tr>
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<td>P. abyssinicum</td>
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<td>JI 1428</td>
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<td>JI 804</td>
<td>P. tibeticum</td>
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