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Evaluation of lentil cultivars and lines for resistance to *Fusarium oxysporum* f.sp. *lentis*

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Abstract. Lentil (*Lens culinaris Medik.*) is one of the most important food legume crops in the farming and food systems of many countries, including Bulgaria. In recent years lentil fields increased in our country. The productivity of lentil is affected by a number of biotic and abiotic stresses of which Fusarium wilt (*Fusarium oxysporum* f.sp. *lentis*-) is the most important yield-limiting factor worldwide. The major approach to control the disease is through resistance breeding. The aim of this investigation was to screen lentil cultivars and lines for resistance to *Fo.* f.sp. *lentis*. The investigations were carried out during 2013-2015. Thirty two lentil accessions were sown in 1m rows, in three replications. Barley grains (100g/m) inoculated with mix of four isolates of *F.o.* f.sp. *lentis* were used as inoculum. Disease reaction was estimated during flowering and pod filling stage according to 9 degree scale. The disease incidence (DI) of every accession was calculated according to McKinney index. Cluster analysis was used to group the accessions according to DI. ‘Genotype’ x ‘Year’ interaction was estimated by Two-way ANOVA. The results showed that middle disease incidence (MDI) was the highest in 2014 and the lowest in 2015. Our investigation confirms the results of many authors that disease incidence of *F.o.* f.sp. *lentis* on lentil was determined by the interaction of the factors ‘Genotype’ x ‘Year’. The cluster analysis grouped the accessions into two major classes. No resistant accessions were found. Six accessions showed MDI from 48.59 to 58.11 and they can be used in a genetic-improvement breeding program for fusarium wilt resistance.

Keywords: lentil, fusarium wilt, resistance, *lens culinaris*. *F. o.* f.sp. *lentis*

Introduction

Lentil (*Lens culinaris Medik.*) is one of the most important food legume crops in the farming and food systems of many countries, including Bulgaria. In recent years lentil fields increased in our country. The productivity of lentil is affected by a number of biotic and abiotic stresses of which Fusarium wilt (*Fusarium oxysporum* f.sp. *lentis*-) is the most important yield-limiting factor (Bayaa et al., 1997; Mohammadi et al., 2012; Pouralibaba et al., 2016; Al-Husien Mihov et al., 1987; Bayaa et al. 1997; El-Ashkar et al., 2002; Belabid et al., 2004). In 2016 Pouralibaba et al. (2016) developed a differential set with four lentil accessions and identified seven *Fol*-pathotypes.

Fusarium wilt in Bulgaria is caused by *Fo* and *Fusarium gibbsosum* but *Fo* has predominant distribution (Stanoeva, 2007). Cultural and pathogenetic characteristics of the pathogens, the effect of the year, genotype and inoculum dose on the disease incidence (DI), and the reaction of lentil accessions to the pathogen under field condition have been investigated but no resistant varieties and breeding lines were found (Stanoeva, 2007). Since then systematic screening of the germplasm has not been undertaken.

The aim of this investigation is to evaluate lentil cultivars and lines to *Fo* with a view to their use for breeding purposes.

Material and methods

The investigations were carried out during 2013-2015 at Dobrudzha Agricultural Institute, General Toshevo. Thirty two lentil accessions (14 cultivars and 18 lines) were sown in 1m rows in three replications. In every two rows, line ILL 4650 was used as a susceptible check (Erskine and Bayaa, 1996). Four isolates of *Fo* were used (F97.4, F99.3, F1.27 and F5.23). They were storage on filter pepper in 4-5˚C (Correl et al., 1986), then cultivated on Potato Dextrose Agar (PDA) and multiplied in PD Broth. Sterilized barley seeds were placed in plastic bags with spore suspension (1x10⁶, x25 ml/kg seeds), separately for every isolate (Stanoeva et al., 2006). The bags were kept in darkness for two weeks in 25˚C. Barley seeds (100g/m) with the different isolates were mixed and integrated in the rows before sowing (Stanoeva et al., 2006). The reaction of the plant to the disease were estimated in...
flowering and pod filling according to 9 degree scale (Bayaa et al., 1995), where:

- 1-no symptoms (Immune);
- 3-yellowing of the lower leaves (Resistant);
- 5-yellowing in 50% of leaves (Middle Resistant);
- 7-yellowing of whole plant or partial wilting (Susceptible);
- 9-the whole plant is wilting and/or died.

Disease incidence (DI) was calculated according to the McKinney index (McKinney, 1923) for every accession:

$$DI = \frac{\sum(a/b) \times 100}{NK}$$

Where:
- a - infection class frequencies;
- b - number of plants of each class;
- N - total of observed plants;
- K - highest value of the evaluation scale.

Cluster analysis was used to group the accessions according to the DI. ‘Genotype’x’Year’ interaction was estimated by Two-way ANOVA. The program used is STATISTICA 7.

**Results and discussion**

Line ILL 4605 had DI=100 during the three years of investigation (Table 1). In 2013 the line X04L24-112 had the lowest DI (48.37), followed by cultivar Stela (DI=55.34) and line X99L26-11 (DI=56.06). Line X98L27-112 showed the lowest DI (45.00) in 2014 followed by cultivar Naslada (DI=49.30). In 2015 the lowest DI had line X98L31-13 (29.25) followed by lines X00L45-111 (DI=35.42) and X04L24-112 (DI=36.67). Generally the DI of the investigated accessions were the highest in 2014 (73.06) and the lowest in 2015 (49.63). MDI is in the range of 48.59 (line X98L27-112) and 97.08 (ILL 101 x ILL 162) during the period of investigation. No immune accessions were found.

Cluster analysis grouped the accessions in two classes according to DI (Figure 1). The first class included two accessions (ILL 4605 and ILL 101 x ILL 162) which showed MDI=100 and MDI=97.08 (Table 1), respectively and could be determined as very susceptible. The second class is divided into two subclasses. The first subclass included 25 accessions with MDI from 64.52 (X98L014) to 90.07 (Palmina) and could be identified as susceptible. The other six accessions (X98L27-112, X98L31-13, Naslada, X99L26-11, Stela, X04L24-112) formed the second subclass with MDI from 48.59 to 58.11 (Table 1, Figure 1) and could be classified as Middle resistant. Screening 196 Spanish landrace for resistance to *Fol* Poularibaba et al. (2015) used similar method for disease scoring. The authors identified 12 accessions which showed more advance symptoms than the highly resistant ones, and less affected by the disease than the susceptible lines and called this resistance 'incomplete'. The authors determined this kind of resistance as polygenic where each gene added a small amount of resistance. Therefore the resistance in the six accessions in this investigation can be classified as incomplete too.

The analysis of variance of 33 accessions after inoculation with *Fol* showed significant ‘genotype’ x ‘year’ interaction, and significance of the factors ‘genotype’ and ‘year’ (Table 2). These results were expected and determined by many authors in lentil and *Fol* (Bayaa et al., 1997; Stanoeva et al., 2008, Mohammad et al., 2012). Cultivars Naslada and Tadzhikskaya 95 were of the first reported cultivars resistant to *Fol* (Mihov et al., 1987). Stanoeva et al. (2006) reported MDI=48.46 for Naslada and MDI=63.40 for Tadzhikskaya 95 using the same *Fol* isolates during 2001-2004. In this investigation cultivar Naslada showed moderately resistant phenotype (MDI=52.57) and cultivar Tadzhikskaya 95 showed susceptible phenotype (MDI=74.07). The differences in the results by Mihov et al. (1987), Stanoeva et al. (2006) and the results of our investigation are probably due to: the effect of the factor ‘year’, the virulence of the used *Fol* population and the methods for disease scoring, because the past the resistance to *Fol* used to be scored by percentage of dead plants.

**Table 1.** Disease incidence (DI) and Middle disease incidence (MDI) of 33 lentil (*Lens culinaris*) accessions after inoculation with *Fusarium oxysporum* f.sp. *lentis*

<table>
<thead>
<tr>
<th>No</th>
<th>Accessions</th>
<th>Year 2013</th>
<th>Year 2014</th>
<th>Year 2015</th>
<th>MDI</th>
<th>No</th>
<th>Accessions</th>
<th>Year 2013</th>
<th>Year 2014</th>
<th>Year 2015</th>
<th>MDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>X98L27-112</td>
<td>64.00</td>
<td>45.00</td>
<td>36.78</td>
<td>48.59</td>
<td>18</td>
<td>Nadezhda</td>
<td>74.37</td>
<td>85.20</td>
<td>56.41</td>
<td>71.66</td>
</tr>
<tr>
<td>2</td>
<td>X98L31-13</td>
<td>59.00</td>
<td>59.58</td>
<td>29.25</td>
<td>49.28</td>
<td>19</td>
<td>X04 ML057</td>
<td>76.88</td>
<td>85.00</td>
<td>55.19</td>
<td>72.36</td>
</tr>
<tr>
<td>3</td>
<td>Naslada</td>
<td>63.25</td>
<td>49.30</td>
<td>45.17</td>
<td>52.27</td>
<td>20</td>
<td>M89 LT4-1162441</td>
<td>78.37</td>
<td>73.43</td>
<td>66.17</td>
<td>72.66</td>
</tr>
<tr>
<td>4</td>
<td>X99 L26-11</td>
<td>56.06</td>
<td>69.64</td>
<td>37.83</td>
<td>54.51</td>
<td>21</td>
<td>Tadzhikskaya 95</td>
<td>64.14</td>
<td>69.68</td>
<td>88.40</td>
<td>74.07</td>
</tr>
<tr>
<td>5</td>
<td>Stela</td>
<td>55.34</td>
<td>73.81</td>
<td>41.33</td>
<td>56.83</td>
<td>22</td>
<td>X94L39-11246</td>
<td>82.64</td>
<td>86.47</td>
<td>53.67</td>
<td>74.26</td>
</tr>
<tr>
<td>6</td>
<td>X04L24-112</td>
<td>48.37</td>
<td>89.28</td>
<td>36.67</td>
<td>58.11</td>
<td>23</td>
<td>Ilina-1232</td>
<td>71.71</td>
<td>77.25</td>
<td>74.37</td>
<td>74.44</td>
</tr>
<tr>
<td>7</td>
<td>X98L014</td>
<td>68.34</td>
<td>69.44</td>
<td>55.78</td>
<td>64.52</td>
<td>24</td>
<td>X00L38-313</td>
<td>75.41</td>
<td>99.00</td>
<td>50.29</td>
<td>74.90</td>
</tr>
<tr>
<td>8</td>
<td>X00 L45-111</td>
<td>66.00</td>
<td>93.57</td>
<td>35.42</td>
<td>65.00</td>
<td>25</td>
<td>Stanka 2</td>
<td>71.71</td>
<td>87.50</td>
<td>67.64</td>
<td>75.62</td>
</tr>
<tr>
<td>9</td>
<td>XM95L33-22</td>
<td>73.79</td>
<td>80.31</td>
<td>45.00</td>
<td>66.37</td>
<td>26</td>
<td>Yanitza</td>
<td>84.60</td>
<td>81.20</td>
<td>67.14</td>
<td>77.65</td>
</tr>
<tr>
<td>10</td>
<td>Stanka 1</td>
<td>74.00</td>
<td>62.50</td>
<td>66.18</td>
<td>67.56</td>
<td>27</td>
<td>Elitza</td>
<td>82.10</td>
<td>86.37</td>
<td>68.18</td>
<td>78.88</td>
</tr>
<tr>
<td>11</td>
<td>X04 ML053</td>
<td>87.08</td>
<td>62.75</td>
<td>55.34</td>
<td>68.39</td>
<td>28</td>
<td>Bella</td>
<td>89.06</td>
<td>89.06</td>
<td>75.45</td>
<td>85.52</td>
</tr>
<tr>
<td>12</td>
<td>X04L17-22</td>
<td>66.37</td>
<td>93.26</td>
<td>45.90</td>
<td>68.51</td>
<td>29</td>
<td>X03L4-1344</td>
<td>88.46</td>
<td>86.53</td>
<td>80.00</td>
<td>85.00</td>
</tr>
<tr>
<td>13</td>
<td>X98 L33-23123</td>
<td>70.11</td>
<td>77.00</td>
<td>61.19</td>
<td>69.43</td>
<td>30</td>
<td>X94L62-421203</td>
<td>96.04</td>
<td>96.70</td>
<td>63.67</td>
<td>85.47</td>
</tr>
<tr>
<td>14</td>
<td>X99L20-11351</td>
<td>56.41</td>
<td>80.00</td>
<td>77.19</td>
<td>71.20</td>
<td>31</td>
<td>Palmira</td>
<td>95.89</td>
<td>96.13</td>
<td>78.19</td>
<td>90.07</td>
</tr>
<tr>
<td>15</td>
<td>Zornitza</td>
<td>80.91</td>
<td>79.25</td>
<td>54.13</td>
<td>71.43</td>
<td>32</td>
<td>ILL 101 x ILL 162</td>
<td>91.25</td>
<td>100.00</td>
<td>100.00</td>
<td>97.08</td>
</tr>
<tr>
<td>16</td>
<td>Ilina</td>
<td>71.71</td>
<td>77.25</td>
<td>65.37</td>
<td>71.44</td>
<td>33</td>
<td>ILL 4650</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>17</td>
<td>Mutant 17 MM</td>
<td>79.54</td>
<td>80.10</td>
<td>55.18</td>
<td>71.61</td>
<td>Average</td>
<td>67.08</td>
<td>73.06</td>
<td>49.63</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
On the basis of their vegetative compatibility, Stanoeva (2007) grouped 88 Fol isolates, derived from different regions of Bulgaria, into 76 vegetative compatibility groups (VCGs). The Fol isolates used in this investigation belong to different VCGs (Stanoeva, 2007). The vegetative compatibility was a suitable polymorphic sign for determine genetic diversity in Fol population (Correll et al., 1987) but it was difficult to use it for breeding purposes. Other investigations need to be made to identify race diversity in Bulgarian Fol population using the differential set developed by Pouralibaba et al. (2016). The results will probably be able to explain better all preliminary investigations on Fol and lentil breeding strategies for resistance to the pathogen should be planned according to the prevalent pathotypes present in the country.

**Conclusion**

Six lentil accessions (two cultivars and four lines) have had moderately resistant phenotype after inoculation with *Fusarium oxysporum f.sp. lentis*. These accessions can be used for breeding purposes. Significant 'genotype' x 'year' interaction and significance of the two factors separately have been determined after inoculation
of 33 lentil accessions with *Fusarium oxysporum* f. *lentis*.

**References**


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