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Evaluation on reaction of late maturing maize hybrids and lines to Fusarium ear rot.

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Abstract. In order to evaluate and determine resistance rates of different corn genotypes to Fusarium ear rot, 22 inbred lines and 19 late and medium maturity hybrids in 2009 and 17 inbred lines and 14 late and medium maturity hybrids were planted in Qarakhel Agricultural Research Station in 2010. Each line and hybrid were planted separately. For each experiment a randomized complete block design with three replications was used. Plant ears were inoculated by Nail Punch method at the 10th day after anthesis. When the disease symptoms were observed, evaluation of each line and genotype was done based on percentage and severity of the disease symptom. The result in 2009 showed that 14 hybrids were tolerant. Hybrids of K3640/3 X MO17, K166B X K18, K166B X K19/1 and K3547/4 X MO17 were resistant. One hybrid was susceptible. Pure lines of K18 and K LM77007/7-2-2-1-2-1-2 were resistant. 14 tolerance lines and 6 susceptible lines were shown. In 2010 hybrids of K166B X K18 and K3653/2 X K18 were resistant. The other hybrids were tolerant. Pure lines of K3547/3 and K18 were resistant. Five tolerance lines were also shown.

Keywords: Artificial inoculation, corn, ear and Fusarium

Introduction

One of the major worldwide maize diseases, especially in tropical and subtropical regions, is Fusarium ear rot. Incidence and spread of the disease has been reported from different regions of maize fields. This disease has been reported from the cultivation regions of maize in Iran (Rahjou et al., 2009). Fusarium spp are known as common fungal pathogens of maize, causing ear, stalk, and root rots (Munkvold and Desjardins, 1997). When infection develops into Fusarium ear rot, the disease becomes of particular concern to maize producers and the processing industry, not only because it reduces grain quality (Afolabi et al., 2007) but also because F. verticillioides produces toxic secondary metabolites in maize kernels, called Fumonisins (Marasas, 2001; Marasas et al., 1981). Genetic variation for resistance to Fusarium ear rot has been investigated for a number of decades (Butron et al., 2006; Desjardins et al., 2005; Klein Schmidt et al., 2005; Presello et al., 2006; Schjoth et al., 2008). For the first time Fusarium ear rot disease was observed in Nebraska decayed corns and Sheldon (1904) reported its agent as Fusarium moniliforme. Several species of Fusarium cause seedling, root, stem and ear rot of corn all over the world (Lew et al., 2001). The most important and common pathogens related to pink ear rot in North America and many other temperate regions of the world are F. verticillioides (=moniliforme), F. proliferatum and F. subglutinans (Munkvold and Desjardins, 1997). Incidence of Fusarium ear rot depends on environmental conditions such as humidity and temperature. F. proliferatum and F. verticillioides are abundant in dry and warm regions in Europe such as Italy and Spain, and F. subglutinans is more common than F. proliferatum and F. verticillioides under cold and moist conditions (Goertz et al., 2010; Jurado et al., 2006; Logroco et al., 2002). F. verticillioides reduces seed resistance and quality, this species because of producing mycotoxins such as Moniliformine Fusain C, Fusaric acid and Fumonisins are hazardous for human and animal health and cause esophagus cancer, Epidemiological associations between maize consumption and esophagus cancer neural tube defects in newborns, and inhibition of ribosomal protein- synthesis and immunosuppression (Sun et al., 2007). Fusarium verticillioides causes Fusarium ear rot while the disease caused by F. graminearum is called Gibberella ear rot. Infections that begin in vegetative plant organs can spread through the plant and also affect the kernels (Bush et al., 2004; Koehler, 1942). Resistance to Fusarium ear rot found in maize is quantitative (Clements et al., 2004) and no complete resistance has been discovered. One way to reduce the level of mycotoxins in corn seeds to prevent infection with Fusarium spp. in the field is using low sensitive corn genotypes (Iglesias et al., 2010).

Material and methods

In order to evaluate and determine resistance rates of different corn genotypes to Fusarium ear rot, 22 inbred lines and 19 late and medium maturity hybrids in 2009 and 17 inbred lines and 14 late and medium maturity hybrids were planted in Qarakhel Agricultural Research Station in 2010. Each line and hybrid were planted separately.

The site is located in the Mazandaran province, 31°28’N latitude and 52°35’E longitude. Fields were manually planted (3 seeds per hill) in single-row plots 3 m long with 13 plants per row on 12 May. After emergence and bush establishment, plants were thinned to one plant per hill. Plots were spaced 0.75 m between rows and 0.25 m between hills. A randomized complete block design with three replications was used. In both years plants were artificially inoculated. Cultural practices were similar in both years. Fields were irrigated with a sprinkler device once per two week for 3 h. 72 treatments were planted in field condition and these treatments are mentioned at Tables 1. They were evaluated for resistance level to F. verticillioides.

To create ear rot infection spore suspension with concentration of 1×10^3 for each milliliter prepared and 7 – 10 days after pollination...
in the middle of ear (Mid ear) by injection method (Nail Punch) plants were inoculated. At harvest time, disease severity by using Jeffers et al. (1994) method in CIMMYT International Research Center with 1–6 scale for scoring calculated and cultivars responses were determined. 1. With no infection, 100% of ears are safe and infection percent is 0.2. Infection is limited to a few seeds around the inoculation site and less than or equal to 10%. 2. A quarter of grains (R) which fungal colony growth was inhibited at inoculation site and less than or equal to 10%. 3. More than half of the resistance can be related to seed physiological resistance. Among 17 hybrids, K 166B X K18 were resistant. Five lines were moderately resistant. In 2010 (Table 3) it was shown that 17 maize hybrids and 14 lines are significantly different (Ps99%). Among 17 hybrids, K 166B X K18 and K3653/2 X K18 were resistant hybrids. 15 hybrids also were moderately resistant. Among 14 lines, K3547/3 and K18 were resistant. Five lines were moderately resistant. Four lines were susceptible and three lines were highly resistant.

Results and discussion

In 2009, ANOVA (Table 1) is listed below, it is concluded that 19 maize hybrids and 22 lines are significantly different (Ps99%). Based on the resistance of cultivars, we divided them into different groups and the results are summarized in Table 2. After comparing means and ranking maize varieties, among 19 hybrids, hybrid K 3640/3 X K19 was susceptible to the disease. 14 hybrids were in moderately resistant group (MR). Four hybrids (K 3640/3 X MO17, K 3547/4 X MO17, K 166B X K18 and K166B X K191) were in resistant group (R) which fungal colony growth was inhibited at inoculation site and only a few of the seeds around the wound were infected. This resistance can be related to seed physiological resistance. Among 22 lines, K18 and KLM77007/7-2-6-3-1-2-1 were resistant. Six lines were susceptible and the highest susceptible line was K3304/1-2. 14 lines were moderately resistant. In 2010 (Table 3) it was shown that 17 maize hybrids and 14 lines are significantly different (Ps99%). Among 17 hybrids, K 166B X K18 and K3653/2 X K18 were resistant hybrids. 15 hybrids also were moderately resistant. Among 14 lines, K3547/3 and K18 were resistant. Five lines were moderately resistant. Four lines were susceptible and three lines were highly resistant.

Table 1. Variance analysis of disease severity of Fusarium ear rot on 19 hybrids and 22 lines of maize in 2009

<table>
<thead>
<tr>
<th>S.O.V Sources of variety</th>
<th>d.f.</th>
<th>Mean square (Ear rot)</th>
<th>S.O.V Sources of variety</th>
<th>d.f.</th>
<th>Mean square (Ear rot)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rep</td>
<td>2</td>
<td>17.33 ns</td>
<td>Rep</td>
<td>2</td>
<td>25.29**</td>
</tr>
<tr>
<td>Hybrid</td>
<td>18</td>
<td>128.1**</td>
<td>Line</td>
<td>21</td>
<td>182.8**</td>
</tr>
<tr>
<td>Error</td>
<td>36</td>
<td>13.24</td>
<td>Error</td>
<td>42</td>
<td>30.67</td>
</tr>
</tbody>
</table>

** and ns: Significant at 1% level and nonsignificant difference

Table 2. Mean comparison and categorizing of maize Lines and hybrids to Fusarium ear rot in 2009

<table>
<thead>
<tr>
<th>No.</th>
<th>Lines</th>
<th>Mean of disease severity, %</th>
<th>Reaction</th>
<th>Hybrids</th>
<th>Mean of disease severity, %</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>K3640/3</td>
<td>16**</td>
<td>MR*</td>
<td>K 3640/3 X MO17</td>
<td>10**</td>
<td>R*</td>
</tr>
<tr>
<td>2</td>
<td>MO17</td>
<td>27.6**</td>
<td>S</td>
<td>K 3547/4 X MO17</td>
<td>10**</td>
<td>R</td>
</tr>
<tr>
<td>3</td>
<td>K19/1</td>
<td>21.3**</td>
<td>MR</td>
<td>K SC700</td>
<td>11.3**</td>
<td>MR</td>
</tr>
<tr>
<td>4</td>
<td>A679</td>
<td>18**</td>
<td>MR</td>
<td>K SC704</td>
<td>13**</td>
<td>MR</td>
</tr>
<tr>
<td>5</td>
<td>K166B</td>
<td>13.6**</td>
<td>MR</td>
<td>K 3673/1 X K18</td>
<td>16.3**</td>
<td>MR</td>
</tr>
<tr>
<td>6</td>
<td>K3515/2</td>
<td>15.3**</td>
<td>MR</td>
<td>K3653/2 X K19</td>
<td>18.3**</td>
<td>MR</td>
</tr>
<tr>
<td>7</td>
<td>K3653/2</td>
<td>29.6**</td>
<td>S</td>
<td>K166B X MO17</td>
<td>14.3**</td>
<td>MR</td>
</tr>
<tr>
<td>8</td>
<td>K3651/1</td>
<td>28**</td>
<td>S</td>
<td>K 166B X K19</td>
<td>17**</td>
<td>MR</td>
</tr>
<tr>
<td>9</td>
<td>K3547/5</td>
<td>11**</td>
<td>MR</td>
<td>K 166B X K18</td>
<td>7**</td>
<td>R</td>
</tr>
<tr>
<td>10</td>
<td>K18</td>
<td>10**</td>
<td>R</td>
<td>K 3304/1-2 X MO17</td>
<td>23.6**</td>
<td>MR</td>
</tr>
<tr>
<td>11</td>
<td>K74/1</td>
<td>25.6**</td>
<td>S</td>
<td>K 47/2-2-1-19-1-1 X K19</td>
<td>23.7**</td>
<td>MR</td>
</tr>
<tr>
<td>12</td>
<td>K3615/1</td>
<td>25.3**</td>
<td>S</td>
<td>K166B X K19/1</td>
<td>10**</td>
<td>R</td>
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<tr>
<td>13</td>
<td>K47/2-2-1-22-1-1-1</td>
<td>22.3**</td>
<td>MR</td>
<td>K3653/2 X K19/1</td>
<td>25**</td>
<td>MR</td>
</tr>
<tr>
<td>14</td>
<td>K3304/1-2</td>
<td>41.3**</td>
<td>S</td>
<td>K3653/2 X K18</td>
<td>17.3**</td>
<td>MR</td>
</tr>
<tr>
<td>15</td>
<td>K48/3-1-2-7-1-1-1-1</td>
<td>13.3**</td>
<td>MR</td>
<td>K3653/2 X MO17</td>
<td>13.3**</td>
<td>MR</td>
</tr>
<tr>
<td>16</td>
<td>KLM77007/7-3-1-2-2-1-1</td>
<td>17.6**</td>
<td>MR</td>
<td>K 74/1 X K19</td>
<td>21**</td>
<td>MR</td>
</tr>
<tr>
<td>17</td>
<td>K LM77002/10-1-1-1-1-1-3-2</td>
<td>12.3**</td>
<td>MR</td>
<td>K 3640/3 X K18</td>
<td>18.7**</td>
<td>MR</td>
</tr>
<tr>
<td>18</td>
<td>K LM77002/10-1-1-1-1-4-2</td>
<td>24.6**</td>
<td>MR</td>
<td>K 3640/3 X K19</td>
<td>31.7**</td>
<td>S</td>
</tr>
<tr>
<td>19</td>
<td>K LM77002/10-1-1-1-1-6-1</td>
<td>19.6**</td>
<td>MR</td>
<td>K 3640/3 X K19/1</td>
<td>23.7**</td>
<td>MR</td>
</tr>
<tr>
<td>20</td>
<td>K LM77007/7-2-6-3-1-2-1</td>
<td>9.3**</td>
<td>R</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>K LM77007/7-3-1-2-1-2-1</td>
<td>17**</td>
<td>MR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>K LM77002/10-1-1-1-1-3-1</td>
<td>22.3**</td>
<td>MR</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*R = Resistant, S = Susceptible, MR = Moderately Resistant

Different letters in each column show significant difference at 5% probability (DMRT)
susceptible. The highest susceptible line was K19. For determining cultivars, through tooth-pick method, was 28% and 10%, the disease resistance and tolerance index, based on the scaling respectively. Disease severity, through silk-channel inoculation, method, among cultivars, those with less than 10% of disease 31% in dent corn and 12% in flint corn. Disease severity in natural severity were resistant and those with 11 – 25% were moderately infection is less than or equal to 10% in both dent and flint maize resistant (Tables 4). Identifying these cultivars and according to their cultivars. They inferred that flint corn, compared to dent corn, shows resistance to disease, we suggested that because of using artificial more resistance against F. verticilloides, while dent corn is more infection for evaluation, fumonisins production amount is low and susceptible. Lines were more susceptible than hybrids.

This is important in food safety. Clements et al. (2003) showed that there is high relationship between disease severity and fumonisin level and they reported that if a hybrid cultivar was identified resistant by using a reliable method such as our method in this study, its fumonisins are within its permitted level. Zamani et al. (1999) One important prevention measure to control red and pink ear ... than nail punch. To determine the resistance to the kernels. Kernel resistance blocks the spread of the fungus from ear rot by different species, a variety of things have been kernel to kernel. Resistance to Fusarium spp. is quantitatively done. However, the diversity of inoculation methods and different pathogenicity species limit the amount of useful information obtained by Gendloff et al., 1986. Czmbor and Ochodzki (2009) studied the disease severity with different infection methods by comparing the resistance of dent and flint maize cultivars against F. verticilloides. They concluded that disease severity in dent and flint maize cultivars, through tooth-pick method, was 28% and 10%, respectively. Disease severity, through silk-channel inoculation, 31% in dent corn and 12% in flint corn. Disease severity in natural infection is less than or equal to 10% in both dent and flint maize cultivars. They inferred that flint corn, compared to dent corn, shows more resistance against F. verticilloides, while dent corn is more susceptible. Lines were more susceptible than hybrids.

**Conclusion**

One important prevention measure to control red and pink ear rots is to use resistant hybrids and inbred lines. Two types of ear rot resistance have been identified in maize. Silk channel resistance prevents the fungus from invading through the silk channel down to the kernels. Kernel resistance blocks the spread of the fungus from kernel to kernel. Resistance to Fusarium spp. is quantitatively inherited, but until now no fully resistant maize genotype has been discovered. The relationships between resistance and myco toxin contamination have been documented. Hybrids that hold their ears vertically and have poor ear cover can be more susceptible to pink ear rot. Hybrids with tight husks appear to be more vulnerable to red

| Table 3. Variance analysis of disease severity of Fusarium ear rot on 17 hybrids and 14 lines of maize in 2010 |
|---------------|-----------------|---------------|---------------|-----------------|-----------------|
| S.O.V Sources of variety | df. | Mean square (Ear rot) | S.O.V Sources of variety | df. | Mean square (Ear rot) |
| Rep | 2 | 28.61** | Rep | 2 | 229.81** |
| Hybrid | 16 | 21.46** | Line | 13 | 873.37** |
| Error | 32 | 2.88 | Error | 26 | 46.17 |

** and ns: Significant at 1% level and nonsignificant difference

| Table 4. Mean comparison and categorizing of maize lines and hybrids to Fusarium ear rot in 2010 |
|---------------|-----------------|---------------|---------------|---------------|-----------------|
| No. | Lines | Mean of disease severity, % | Reaction | Hybrids | Mean of disease severity, % | Reaction |
| 1 | K3547/3 | 10' | R* | K 166B X K18 | 10' | R* |
| 2 | K3544/5 | 30.7* | S | K 74/2-1-4-2-1-1 X K18 | 12.3' | MR |
| 3 | K18 | 10' | R | K166B X Mo17 | 11' | MR |
| 4 | K3651/1 | 17.7' | MR | K 3673/1 X K18 | 11.3' | MR |
| 5 | K3545/6 | 31.7* | S | KSC700 | 10.7' | MR |
| 6 | KLM77002/10-1-1-1-1-1-2-3 | 55.3' | HS | K SC704 | 21' | MR |
| 7 | A679 | 30.3' | S | KSC706 | 11.3' | MR |
| 8 | K166A | 18.3' | MR | KSC720 | 13.3' | MR |
| 9 | K3547/5 | 12.7' | MR | KSC705 | 13' | MR |
| 10 | K74/1 | 51.3' | HS | KSC670 | 11.7' | MR |
| 11 | K19 | 60' | HS | K3653/2 XK19/1 | 12.3' | MR |
| 12 | MO17 | 35.3' | S | K3653/2 XK18 | 8.3' | R |
| 13 | K19/1 | 10.7' | MR | K3653/2 XMO17 | 10.3' | MR |
| 14 | K1264/1 | 16.7' | MR | K3640/3 X K18 | 13' | MR |
| 15 | K3640/3 X K19 | 10.7' | MR |
| 16 | K 3640/3 X K19/1 | 13.7' | MR |
| 17 | K 3547/3 X K1264/1 | 14' | MR |

*R = Resistant, S = Susceptible, MR = Moderately Resistant
Different letters in each column show significant difference at %5 probability (DMRT)
ear rot. It was cleared that disease severity can be considered a suitable and stable index for evaluating of resistance of varieties to Fusarium ear rot. In this experiment 36 corn Iranian varieties and 36 inbred lines were evaluated under artificial inoculation techniques to create a nail punch in the ear. Six cultivars (K 3640/3 X MO17, K 3547/4 X MO17, K 168B X K18 , K166B X K19/1, K 168B X K18 and K3653/2 X K18) and three inbred lines (K18, KLM7700777-2-6-3-1-2-1 and K3547/3) were R (resistant). Although many researches have been conducted to control Fusarium ear rot, using resistant or tolerant cultivars seems the most effective method for controlling the disease. It is hoped that, along using all facilities and applying the latest scientific achievements in this area together with indentifying resistant cultivars and compounds, an improved coherent and strategic program shall be conducted in the country so that a suitable solution will be obtained to control the disease. In general, disease development in this method (Nail punch) and its facility of applying in corn fields could be an accurate assessment for breeders to release the best and most resistant hybrids for introducing to farmers. Therefore, further investigations should be conducted to determine resistant cultivars to Fusarium ear rot in Iran. It is recommended that farmers and extension experts applied resistant varieties for cultivation.

Acknowledgements

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The title needs to be as concise and informative about the nature of research. It should be written with small letter /bold, 14/ without any abbreviations.

Names and affiliation of authors
The names of the authors should be presented from the initials of first names followed by the family names. The complete address and name of the institution should be stated next. The affiliation of authors are designated by different signs. For the author who is going to be corresponding by the editorial board and readers, an E-mail address and telephone number should be presented as footnote on the first page. Corresponding author is indicated with *.

Abstract should be not more than 350 words. It should be clearly stated what new findings have been made in the course of research. Abbreviations and references to authors are inadmissible in the summary. It should be understandable without having read the paper and should be in one paragraph.

Keywords: Up to maximum of 5 keywords should be selected not repeating the title but giving the essence of study.

The introduction must answer the following questions: What is known and what is new on the studied issue? What necessitates the research problem, described in the paper? What is your hypothesis and goal?

Material and methods: The objects of research, organization of experiments, chemical analyses, statistical and other methods and conditions applied for the experiments should be described in detail. A criterion of sufficient information is to be possible for others to repeat the experiment in order to verify results.

Results are presented in understandable tables and figures, accompanied by the statistical parameters needed for the evaluation. Data from tables and figures should not be repeated in the text. Tables should be as simple and as few as possible. Each table should have its own explanatory title and to be typed on a separate page. They should be outside the main body of the text and an indication should be given where it should be inserted.

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Discussion: The objective of this section is to indicate the scientific significance of the study. By comparing the results and conclusions of other scientists the contribution of the study for expanding or modifying existing knowledge is pointed out clearly and convincingly to the reader.

Conclusion: The most important consequences for the science and practice resulting from the conducted research should be summarized in a few sentences. The conclusions shouldn’t be numbered and no new paragraphs be used. Contributions are the core of conclusions.

References: In the text, references should be cited as follows: single author: Sandberg (2002); two authors: Andersson and Georges (2004); more than two authors: Andersson et al.(2003). When several references are cited simultaneously, they should be ranked by chronological order e.g.: (Sandberg, 2002; Andersson et al., 2003; Andersson and Georges, 2004).

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