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The Survey of Maize Inbred Lines for Resistance to *Fusarium verticillioides* Ear Rot

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ABSTRACT

Ear rots caused by different *Fusarium spp*. are one of the most dangerous foods and feed safety challenges in maize production, and yield losses on the average of 20% (between 1.5-40%) have been registered from artificial inoculations. Growing resistant hybrids represents one of the most efficient solutions for reducing the yield losses caused by *Fusarium spp* on the maize. So, in order to find the resistant inbred line, ten inbred lines were evaluated for their reaction to *Fusarium verticillioides* under field conditions, at the Pars Abad-e- Moghan (39° 41' N 47° 32' E, Iran) 2011, using an RBCD design with three replications. For artificial inoculation, fungal inoculum consisting of a mixture of spores was applied to ear 2 to 5 days after silk stage by Nail Punch method. At harvesting time, disease severity based on disease progress in each ear, were evaluated. The results showed Mean grain yield under natural infection condition was 106.13 grams per plant, while mean grain yield under artificial infection condition was 77.92 grams per plant which showed a reduction of 26.53% in comparison to the natural infection condition. The results also showed that MO17 and B73rfc inbred lines were resistant and susceptible Fusarium diseases. Inbred lines possessing resistance to ear rot would be valuable breeding stock for use as parents of hybrids that can be grown by farmers.

KEYWORDS: maize, *Fusarium verticillioides*, inbred lines

INTRODUCTION

Maize (*Zea mays* L.) is an important crop to which a large extent of cultivable land has been allocated. It is attacked by several fungal diseases. Fusarium is a common mould in cereal fields. The infestation (superficial contamination) and infection of Fusarium in cereals are of great concern worldwide – as plant pathogens and producers of mycotoxins (Popovski and Celar, 2013). Although cereals are important substrates, moisture level and temperature are the critical abiotic factors regulating the growth of F. *verticillioides* and the production of fumonisins. The best temperature range is 20‒28 °C for fumonisin production but low kernel moisture content (less than 22%) should reduce or prevent toxin production during storage (Fodor et al., 2006). Studies on the control of fumonisins in major food crops such as maize are still at a relatively early stage. To date, fungicides such as thiram and carbendazim are used as seed treatments to control the disease (Nayaka, et al, 2009). Because chemical control is expensive and often ineffective, moreover lead to new variants of the pathogen resistance to fungicides, improvement of host plant resistance to this fungus provides the most feasible control options (Hefny et al., 2012). Although, resistance to Fusarium ear rot is under genetic control and heritable resistance has been identified in maize, no highly resistant genotypes are known (Afolabi et al., 2007). Alessandra et al. (2010) stated that, in maize there is no evidence of complete resistance to either ear rot or fumonisin contamination and resistance to initial penetration and spreading of the pathogen in host tissue are two components responsible for resistance to *Fusarium* in maize. Therefore percentage of infected kernels is the result of resistance to both components.

Studies show that fumonisins are the most frequent mycotoxin. For example, scientists at Purdue University surveyed mycotoxin occurrence in Indiana corn harvested from 1989 to 1993. The researchers began surveying for fumonisin in 1991, and they found that fumonisins were the most frequently detected mycotoxins in Indiana corn harvested during the period 1991 to 1993. However, fumonisin contamination varied greatly from year to year. For example, in 1991, almost all (96 percent) of 328 corn samples evaluated had some Fusarium ear rot. Of the113 most severe samples, 44 percent had fumonisin levels above 5 ppm (parts per million). In contrast to 1991, all lots tested were below 5 ppm in 1992.

More recent surveys from various regions of the United States show that fumonisins are commonly present in corn, although usually at levels below those known to pose a health risk (Vincelli, 2014).

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Robertson et al. (2006) recorded moderate to high entry mean heritabilities for both fumonisin contamination (0.75) and Fusarium ear rot (0.47) suggesting that phenotypic selection against ear rot should be an effective way to improve resistance to both ear rot and fumonisin contamination. They added, resistant materials have substantially lower mycotoxin contents than susceptible ones. This indicates that the genetically controlled mechanisms of resistance to these two aspects of disease are largely the same. Therefore, selection against ear rot may be a useful strategy for selecting genotypes with lower fumonisin content.

Research and breeding efforts aimed to improve resistance of ear rot focused on accurately measuring disease severity and fumonisin concentrations to identify sources of resistance and characterizing the inheritance of ear rot and fumonisin accumulation (Robertson-Hoyt et al., 2006). Moreover information on the genetic variability exists for resistance to ear rot has been reported for the same traits(Rossouw et al., 2002).

Therefore, the present study was designed to assess (1) the resistant maize inbred lines to *Fusarium verticillioides* ear rot and (2) the relationship between ear rot severity infection and investigated traits.

MATERIALS AND METHODS

Ten maize inbred lines (named K18, K19, K19/1, MO17, K74/1, B73rfc, B73cms, K166A, A679 and K1264/1) were used for the present study. These inbred lines were evaluated for their reaction to *Fusarium verticillioides* under field conditions. The experiment was planted at the Pars Abad-e-Moghan (39° 41' N 47° 32' E, with 40-50 m above from sea level), Ardebil, Iran in 2011, using an RBCD design with three replications. The plot was made of four rows of 3 m length with the distance between rows and hills of 75 and 25 cm, respectively. One of the two middle rows in each plot were silk-channel inoculated with *Fusarium verticillioides* by Nail Punch method. Inoculation was done 6-7 days after mid silk emergence.

At harvest, ten plants per experimental unit (plot) were randomly selected from each two middle rows of plot, then their ears were hand-picked and grain yield (g plant^{-1}) and infection severity % were determined.

The severity induced Fusarium ear rot was rated as the percentage of visibly infected kernels on each ear surface as follows:

$$\textit{Infection severity}\ (\%) = \frac{No.of\ rotten\ kernels\ ear-1}{Total\ No.of\ kernels\ ear-1} \times 100$$

Then percentage of visibly infected kernels on each ear surface transformed to a 7- class rating scale in which 1 = no infection, 2 = 1 to 3%, 3 = 4 to 10%,
4 = 11 to 25%, 5 = 26 to 50%, 6 = 51 to 75%, and 7= 76 to 100% of the kernels exhibiting visible symptoms of infection, such as rot and pinkish or white mycelial growth (Afolabi et al., 2007).

Data collected was initially subjected to analyses of variance (ANOVA), using MSTAT-C computer package. Infection severity values were transformed to $\log^{10}$ (1 + incidence of discolored kernels) to normalize residuals.

RESULTS

In this study, maize inbred lines were evaluated to identify inbred lines that are resistant to fusarium ear rot. The result of ANOVA showed that studied maize inbred lines significantly affected disease severity (Table 1). Fusarium ear rot severity for all 10 inbred lines ranged from 1 (no infection) to 5 (i.e., 26 to 50% ear rot symptom), and about 90% of the inbred lines had severity levels >10% in artificial infection condition. Inbred line MO17 had lowest disease severity level with 3 (i.e., =10%) among the studied inbred lines, but inbred line B73rfc had highest disease severity with 5 (i.e., =35%) in artificial infection condition. In natural infection condition, fusarium ear rot severity for all 10 inbred lines ranged from 1 (no infection) to 2 (< 3% ear rot infection). Inbred line K19/1 had lowest disease severity level among the studied inbred lines (Table 2).

Table1: Analysis of variance for grain yield per plant and infection severity % in maize inbred lines

SOV	grain yield per plant		Infection severity %
	Natural infection	Artificial infection	
Replication	124.448	122.130	0.011
Inbred lines	2182.805**	1430.459**	0.090**
Error	109.380	59.892	0.012
C.V%	9.85	9.93	8.81

**: significant at 1% probability level

The results of analysis of variance (Table 1) showed highly significant differences for grain yield in both infection conditions, indicating the existence of genetically variability among the inbred lines. Therefore, it is possible to identify fusarium resistant and high-yielding inbred lines.

In this study, the grain yield varied from 47.57 (in inbred line MO17) to 147.42(in inbred line K166A) grams per plant in natural infection condition and from 30.33(in inbred line MO17) to 6.6 (in inbred line K1264/1) grams per plant in artificial infection condition. Mean grain yield under natural infection condition was 106.13 grams per plant, while mean grain yield under artificial infection condition was 77.92 grams per plant which showed a reduction of 26.53% in comparison to the natural infection condition. So that, at least reducing of yield per plant was observed in inbred line MO17 with 10% reduction. This line had at least disease severity. While the highest reduction in yield per plant due to by Fusarium infection occurred in inbred line B73rfc with 47.56% reduction. This line had relatively high disease severity (67.21%) (Table 2).

There were significant differences for grain yield in the responses of inbred lines to inoculation by *Fusarium verticillioides* fungi (Table 2). This result implies that maize inbred lines reacted differently when inoculated with *Fusarium verticillioides*. It also indicates presence or absence of host resistance genes in different maize genotypes against this fungus.

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Table2: Mean of grain yield per plant, disease incidence (1%) and disease severity for studied inbred lines

Lines	Grain yield per plant			Disease incidence(1%)		Disease severity	
	Natural infection	artificial infection	Reduction %	Natural infection	artificial infection	Natural infection	artificial infection
K18	98.44	81.11	17.60	1.67	11.67	2	4
K19	137.17	109.42	20.23	1.67	21.67	2	4
K19/1	98.10	72.83	25.76	0.00	11.67	1	4
MO17	47.57	30.33	36.23	1.67	10.00	2	3
K74/1	114.91	72.33	37.05	1.67	25.00	2	4
$B73_{rfc}$	114.42	60.00	47.56	1.67	35.00	2	5
$B73_{cms}$	94.29	76.89	18.46	0.67	23.33	2	4
K166A	147.42	96.00	34.88	0.00	18.33	2	4
A679	100.28	86.92	13.33	0.00	15.00	2	4
K1264/1	108.73	93.33	14.16	0.00	11.67	2	4
Mean	106.13	77.92	26.53	0.90	18.33	2	4

Average yield losses ranged between 13.33 - 47.56%. The inbred lines A679 and K1264/1 were more tolerant to Fusarium ear rot, and B73rfc was most susceptible (Table 2). The most important prerequisites for a successful breeding program for resistance to ear rot are presence of genotypic variation for host-plant response to the pathogen and availability of techniques to reliably detect these differences. In the present study a considerable amount of genetic variation was detected from the analysis of variance. Our results agreed with that obtained by Hefny et al (2012) and Naidoo et al (2002) who confirmed the presence of genetic variation for resistance to ear rot diseases.

To estimate the effect of ear rot damage on maize yields, correlation coefficients were calculated between severity infection % with grain yield per plant and its reduction (%) due to fuzarium infection (Table 3). Correlations between severity infection % and grain yield per plant (in natural and artificial infection) recorded low and non-significant values. Grain yield reduction (%) due to fuzarium infection showed positive and significant ($p < 0.10$) correlations with severity infection %. The moderate positive correlation coefficient of severity infection % with grain yield reduction % was confirmed by those results obtained by Hefny et al. (2012) who recorded negative correlation between severity infection % and grain yield.

Table 3: Correlations between infection severity % and grain yield per plant and its reduction (%) due to fuzarium infection

Traits	Disease severity (%)
Grain yield per plant (natural infection)	0.432
Grain yield per plant (artificial infection)	0.026
Grain yield reduction(%) due to fuzarium infection	$0.560^{\times}$

×: Correlation is significant at the 10% probability level

In general, MO17 Inbred line was resistant fusarium diseases. Inbred line possessing resistance to ear rot would be valuable breeding stock for use as parents of hybrids that can be grown by farmers. As Hooker and Draganic (1980) suggested that one resistant inbred parent is sufficient for obtaining a hybrid resistant to stalk rot. It can be concluded that, resistance to *F. verticillioides* should be directly evaluated by resistance itself rather than other agronomic traits in breeding programs as reported by Hefny et al. (2012).

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