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*Short Communication*

# EVALUATION OF ETHIOPIAN MAIZE CULTIVARS FOR RESISTANCE TO *FUSARIUM VERTICILLIOIDES* AND FUMONISIN ACCUMULATION

**Hadush Tsehaye[1,2,*], May Bente Brurberg[2,3], Leif Sundheim[2,3], Arne Tronsmo[4], Dereje Assefa[1], Anne Marte Tronsmo[2]**

[1]Mekelle University, Department of Dryland Crop and Horticultural Sciences, P.O. Box 231, Mekelle, Tigray, Ethiopia. *Email: had031@yahoo.com; hadush.tsehaye@gmail.com
[2]Norwegian University of Life Sciences, Faculty of Biosciences, Department of Plant Sciences, P.O. Box 5003, NO-1432 Ås, Norway
[3]Norwegian Institute for Bioeconomy Research, Biotechnology and Plant Health Division, P.O. Box 115, NO-1431 Ås, Norway
[4]Norwegian University of Life Sciences, Faculty of Chemistry, Biotechnology and Food Science, P.O. Box 5003, NO-1432 Ås, Norway

**ABSTRACT:** The objective of this study was to find sources of resistance to *Fusarium* ear rot and fumonisin accumulation in maize germplasm. Totally 15 maize cultivars were evaluated by means of silk channel inoculation using a fumonisin producing *F. verticillioides* isolate in field trials during 2013 and 2014 cropping seasons. *Fusarium* ear rot severity was determined at harvest, and fumonisin content was quantified using competitive Enzyme Linked Immunosorbent Assay (ELISA). The percentage of infected kernels per maize ear after inoculation, ranged from 5% to 60% in 2013 and from 3% to 40% in 2014. Fumonisin accumulation in maize cultivars ranged from 2700 to 76300 µg/kg in 2013 and from 1800 to 52700 µg/kg in 2014. Maize cultivars Berihu, Melkassa-2, Melkassa-7, Melkassa-4, BHQP542 and MHQ-138 showed low level of ear rot (3.9% to 22.9%) and total fumonisins (2300 to 17300 µg/kg) across the two years experiment. Cultivars that had low disease severity are useful in breeding programs aiming at developing cultivars resistance to fumonisin accumulation.

**Keywords/ phrases: *Fusarium* ear rot, *Fusarium verticillioides*, fumonisin, maize**

## INTRODUCTION

*Fusarium verticillioides* (Sacc.) Nirenberg is the most common fungal pathogen of maize in Ethiopia (Amare Ayalew, 2010; Hadush Tsehaye *et al.*, 2017). This fungal pathogen causes root, stalk and ear rot diseases in maize (Munkvold and Desjardins, 1997). The main problem associated with *F. verticillioides* infection of maize is the contamination of grains with harmful mycotoxins, known as fumonisins that poses a serious adverse effect on the health of humans and domestic animals (Voss *et al*., 2007). Taking into consideration the risk it presents to human and animal health, legislation to limit the amount of fumonsin in maize products intended for human food and animal feed have been established in some parts of the world (EU commission, 2007). The Joint FAO/WHO Expert Committee on Food Additives had set guidelines for the maximum tolerable limit of fumonisins in food intended for human consumption and recommended 4000 µg/kg in unprocessed raw maize and 2000 µg/kg in maize meal (CAC, 2014). In Ethiopia, no formal guidelines for tolerable fumonisin levels in food are available, although widespread fumonisin contamination have been reported in maize grain samples, with some samples exceeding the international standards (Amare Ayalew, 2010; Hadush Tsehaye *et al.*, 2017). Fumonisin levels in maize grains produced in Ethiopia could range 300 to 2300 µg/kg (Amare Ayalew, 2010) and from 25 to 4500 µg/kg (Hadush Tsehaye *et al.*, 2017). In

*Author to whom all correspondence should be addressed order to reduce the potential health hazard associated with consumption of fumonisin-contaminated grains, effective management of the disease at field level is required. Breeding for *Fusarium* resistant maize cultivars and cultivation of these is the most effective and environmentally safe method for controlling *Fusarium* ear rot disease and to reduce the risk of fumonisin-contaminated grains (Mesterházy *et al.*, 2012). Differences exist among maize cultivars for resistance to *F. verticillioides* infection and fumonisin accumulation (Afolabi *et al.*, 2007; Clements *et al.*, 2004; Mesterházy *et al.*, 2012).

*Fusarium* ear rot epidemics are sporadic in nature, and disease severity varies across years and are strongly influenced by the climatic conditions. Thus, selection of resistant varieties based on artificial inoculation is preferred to that based on natural infection, because artificial inoculation often enhances disease severity (Afolabi *et al.*, 2007; Clements *et al.*, 2004). Inoculation by penetrating the husk with pin bars or injecting inoculum down the silk channel are effective and reliable methods (Mesterházy *et al.*, 2012). It is clearly indicated that data from natural infection and artificial inoculation correlates well, when silk channel inoculation is used (Mesterházy *et al.*, 2012). Greatest disease severities often develop, when the ears are inoculated at the blister or $R_2$ plant growth stage (Reid and Zhu, 2005).

To incorporate resistance to *Fusarium* ear rot and fumonisin contamination into agronomical elite maize cultivars, identification of cultivars with adequate resistance level is crucial. However, in Ethiopia, very little is known regarding the resistance of maize cultivars to *Fusarium* ear rot caused by *F. verticillioides* and fumonisin accumulation in grains. Therefore, the objective of this study was to assess differences in Ethiopian maize cultivars for resistance to *F. verticillioides* infection and fumonisin accumulation after artificial inoculation, and to select maize cultivars with good level of resistance.

## MATERIALS AND METHODS

### *Experimental design and treatments*

The study was carried out at Mekelle (13$^{o}$ 29′ N latitude and 39$^{o}$ 30′ E longitude, at an altitude of 2130 m above sea level) in 2013 and 2014. Fifteen maize cultivars (see Table 1) were evaluated for resistance to *Fusarium* ear rot and fumonisin accumulation caused by *F. verticillioides*. The experiment was conducted using randomized complete block design with three replications. Each cultivar was assigned to three plots that were 3 m long and 4.5 m wide with 5 rows per plot (0.75 m between rows and 0.25 m between plants in the row). The trials were fertilized with Phosphorous ($P_2O_5$) and Nitrogen (N) fertilizers at a rate of 46 kg/ha and 64 kg/ha, respectively. Urea was applied in split form. Preparation of the fungal inoculum was carried out according to Reid and Zhu (2005). Ten plants were randomly selected in each plot, and the primary ears of the plants were inoculated with 4 mL conidial suspension ($1 \times 10^6$ conidia/mL) of a fumonisin producing *F. verticillioides* isolate (SR-6952) through the silk channel at the blister ($R_2$) growth stage according to Afolabi *et al.* (2007). This isolate was obtained from infected grains of maize in Sire area, central –western Ethiopia (Hadush Tsehaye *et al.*, 2017).

### *Data collection and analysis*

At the time of harvest, the inoculated ears were handpicked separately, husk-leaves carefully removed, and evaluated immediately for severity of ear rot symptoms. The severity of *F. verticillioides* infection was visually assessed by determining the percentage (0 - 100%) of each ear surface covered by visible symptoms of fungal infestation on kernels (Clements *et al.*, 2004). Reaction of cultivars was categorized into different susceptibility/resistance groups as described by Ashnagar *et al*. (2012) as follows: resistant (cob infection ≤ 10%), moderately resistant (infection > 10% but ≤ 25%), susceptible (infection > 25% but ≤ 50%) and highly susceptible (infection > 50%). After rating, grains were shelled from cobs and bulked by plot. Grain subsample of 100 g were taken randomly after dividing the bulk samples using riffle type grain divider (Basak brand, 13100 model, Turkey) and ground for fumonisin analysis. Total fumonisin content in the samples was determined using an Enzyme Linked Immunosorbent Assay (ELISA) kit (RIDASCREEN®Fumonisin, R-Biopharm AG, Darmstadt, Germany) according to the manufacturer's instruction. Comparisons between maize cultivars in ear rot severity and fumonisin content in grains was performed with Fisher's protected least significance (LSD) test using the Statistical Analysis System (SAS) software, version 9.4 (SAS institute, Cary, NC, USA). Pearson's correlation coefficients were determined for the relationship between ear rot severities and fumonisin concentration in grains.

## RESULTS

*Fusarium* ear rot severity was significantly influenced by year ($p < 0.012$) and maize cultivars ($p < 0.0001$). *Fusarium* ear rot severity was significantly lower in 2014 than in 2013. In 2013, ear rot severity ranged from 4.9% to 60.3%, with mean ear rot of 32.3%; while in 2014, maize ear rot severity ranged from 2.8% to 40.8% (mean of 21.8% rot on the cobs). Among the 15 maize cultivars, two (Berihu and Melkassa-7) were in the resistant group (< 10% infection), while four (Melkassa-2, Melkassa-4, MHQ138 and BHQP542) were moderately resistant (Table 1).

**Table 1. Reaction of maize cultivars to *Fusarium* ear rot disease at Mekelle, northern Ethiopia in 2013 and 2014.**

| Maize cultivars | *Fusarium* ear rot disease severity (%) | | | Reaction * |
|---|---|---|---|---|
| | 2013 | 2014 | Mean | |
| Berihu | 4.9 g | 2.8 j | 3.9 i | R |
| BH140 | 49.3 ab | 25.3 bcde | 37.3 bcd | S |
| BH540 | 40.2 bcd | 22.3 cde | 31.3 cdef | S |
| BHQP542 | 27.1 e | 18.7 efg | 22.9 gf | MR |
| Gibe1 | 53.3 ab | 31.7 abc | 42.5 ab | S |
| Gibe2 | 42.2 bcd | 20.4 def | 31.3 cde | S |
| Melkassa2 | 15.3 f | 9.0 hi | 12.1 hi | MR |
| Melkassa3 | 31.1 de | 29.6 abcd | 30.5 def | S |
| Melkassa4 | 27.0 e | 11.7 gh | 19.4 gh | MR |
| Melkassa5 | 34.3 cde | 38.9 a | 36.7 bcd | S |
| Melkassa6Q | 25.7 e | 26.6 bcde | 26.2 efg | S |
| Melkassa7 | 7.2 g | 4.9 ij | 6.1 i | R |
| MH130 | 43.4 bc | 34.0 ab | 38.7 bc | S |
| MHQ138 | 25.7 e | 12.6 fgh | 19.2 gh | MR |
| NSCM411881(32) | 60.3 a | 40.8 a | 50.6 a | HS |
| Mean | 32.3 | 21.8 | 27.1 | - |
| P-value | <.0001 | <.0001 | <.0001 | - |

Within each column, means followed by the same letter do not differ significantly according to Fisher's least significant difference test (LSD) at 0.05 probability level.
* R = Resistant, MR = Moderately Resistant, S = Susceptible, HS= highly susceptible

Fumonsin content was affected significantly by year ($p < 0.018$) and maize cultivars ($p < 0.0001$). Total fumonisins content in maize grains ranged from 2700 to 76300 µg/kg in 2013 with a grand mean of 33200 µg/kg, and from 1800 to 52700 µg/kg in 2014 with a grand mean of 23100 µg/kg. Maize cultivars with low level of total fumonisins in both years include the local landrace Berihu, Melkassa-2, Melkassa-7, Melkassa-4, BHQP542 and MHQ-138 with mean concentrations of 2300 to 17300 µg/kg (Fig. 1). The majority of cultivars that showed best resistance to *F. verticillioides* are early maturing type, except BHQP542, which is a late maturing type. Ranking order of some maize cultivars differed between years in their responses to ear rot and fumonisin contamination. In 2013, the highest amount of fumonisin accumulated in grains of cultivar Gibe-1 (76300 µg/kg), NSCM-411881(32) (67800 µg/kg) and BH-140 (58700 µg/kg). In 2014, the highest fumonisin content was recorded in cultivar Melkassa-5 (52700 µg/kg), followed by MH-130 (51400 µg/kg) and NSCM-411881(32) (48900 µg/kg) (Fig. 1). Combined analysis for 2013 and 2014 showed that fumonisin content in grains was significantly correlated ($r = 0.918$, $p < 0.001$) with *Fusarium* ear rot severity.

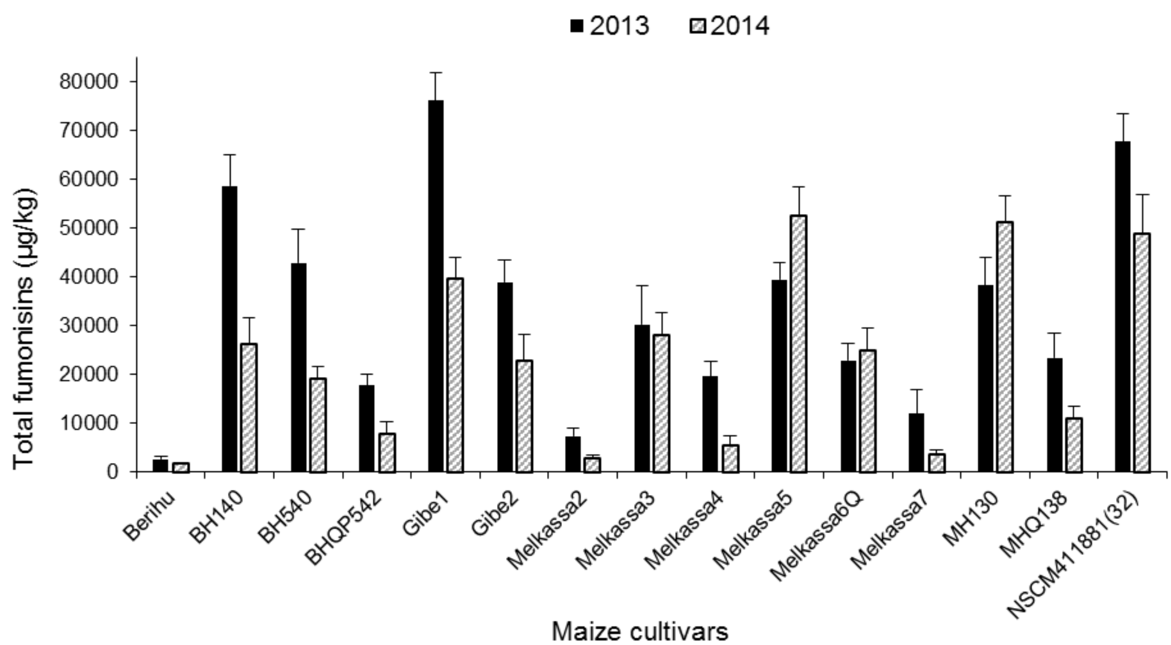


**Figure 1. Total fumonisin contamination in grains of different maize cultivars inoculated with *Fusarium verticillioides* in Mekelle, in 2013 and 2014. Vertical bars represent standard error of the mean.**

## DISCUSSION

Maize cultivars with consistently low ear rot disease severity and fumonisin accumulation in grains were identified in the two years of inoculation trials. This may indicate that potential resistance genes against *F. verticillioides* and the toxin it produces exists in Ethiopian maize germplasms. Maize cultivars with low disease severity and fumonisin level may be important as sources of resistance to *F. verticillioides* for introduction into advanced breeding programs, as this pathogen is the most common fungal pathogen associated with maize grains in Ethiopia (Amare Ayalew, 2010; Hadush Tsehaye *et al.*, 2017).

*Fusarium verticillioides* established kernel infection in all maize cultivars studied and none of the cultivars tested were free from fumonisin contamination. In the case of silk channel inoculation, ears with infected kernels indicate that silk resistance was not present or was not adequate to deter the fungus from reaching the kernels, before kernel resistance developed (Reid and Zhu, 2005). Traits such as delayed silk senescence and induction of phenolic compounds (3-deoxyanthocyanidins) may contribute to resistance in maize cultivars (Santiago *et al.*, 2015). The present study also indicates that late-maturing maize cultivars tend to exhibit significantly higher ear rot severity and fumonisin content across the two years of inoculation experiment. Similarly, Battilani *et al.* (2008) and Löffler *et al.* (2010) were observed higher susceptibility to fumonisin accumulation in late-maturing (slow drying) maize cultivars compared to early types. This may be caused by the slower reduction in grain moisture content in late-maturing cultivars as water availability plays an important role for fungal growth (Battilani *et al.*, 2008; Santiago *et al.*, 2015).

Fumonisin contamination of maize cultivars is influenced by several phenotypic traits and chemical composition of the kernels. Physical properties of the grain pericarp, such as intact kernel, pericarp thickness and wax content are associated with low fumonisin contamination (Sampietro *et al.*, 2009). Maize hybrids with flinty (hard outer layer) endosperm characteristics have been considered as more resistant to *Fusarium* ear rot and fumonisin accumulation than hybrids with dent (softer) endosperm (Desjardins *et al.*, 2005). This was attributed to higher amylase contents in kernels of flinty maize than dent type (Santiago *et al.*, 2015). However, there are contradictory reports, and Löffler *et al.*, (2010) reported that maize inbred lines with flint endosperm characteristics are more susceptible to fumonisin contamination than dent types. Chemical composition of maize kernels particularly amylopectin is a key component in inducing fumonisin production (Santiago *et al*., 2015).

In the present study, the year of inoculation had a significance influence on disease severity and fumonisin concentration. The lower level of ear rot severity and fumonisin concentration in 2014 compared with 2013 may be attributed to variation in weather factors (temperature and rainfall) affecting disease development. Temperature was consistently higher in 2013 than 2014 through the months of July to August, while precipitation was low in 2013 (Data not shown). This indicates the prevalence of stressful weather condition after inoculation in 2013 than in 2014 for the plant in the field. Stressful climatic conditions such as high temperature and low precipitation during flowering and early grain-filling period have been associated with increased *F. verticillioides* infection and fumonisin contamination of maize grains (Cao *et al*., 2014). Precipitation during harvest has also been associated with increased fumonisin contamination in maize kernels, because it delays kernel dying and favors fungal growth (Cao *et al*., 2014; Santiago *et al*., 2015).

The observed strong positive association between ear rot severity and fumonsin content suggests that selection for resistance to ear rot may eventually result in resistance to fumonisin contamination in maize grains. Similarly, Afolabi *et al*. (2007) and Löffler *et al*. (2010) have detected strong association between visible ear rot severity and fumonisin content in maize grains. Therefore, visual rating of ear rot disease severity might be used as initial selection criteria to exclude cultivars accumulating high levels of fumonisin, because visual ear rot rating is easier, less expensive and large number of maize accessions can be considered for evaluation. However, significant amount of fumonisin may also be produced in symptomless or in kernels with minimal ear rot severity (Munkvold and Desjardins, 1997). Thus, cultivars selected on the basis of ear rot rating should be further evaluated for fumonisin accumulation analysis to avoid cultivars with minimal ear rot but greater fumonisin concentration.

In conclusion, the present study has shown a significant level of variation in resistance to *Fusarium* ear rot and fumonisin accumulation among the Ethiopian maize cultivars tested. The results document the presence of certain sources of resistance to *Fusarium* ear rot and fumonisin contamination within the Ethiopian maize germplasms. The cultivars that display low ear rot disease severity and fumonisin level may be useful for the development of resistant maize cultivars in the maize breeding program. Further extensive evaluations should continue by including several maize genotypes grown in Ethiopia to increase the chances for selecting maize material with higher resistance levels.

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