Field Crops Research 205 (2017) 124–134

Contents lists available at ScienceDirect

Field Crops Research

journal homepage: www.elsevier.com/locate/fcr

Association between agronomic traits and aflatoxin accumulation in diverse maize lines grown under two soil nitrogen levels in Eastern Kenya

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ARTICLE INFO

Article history:
Received 3 April 2015
Received in revised form 6 February 2017
Accepted 9 February 2017

Keywords:
Maize
Maize maturity
Kernel traits
Soil fertility
Aflatoxin

ABSTRACT

Aflatoxin accumulation in maize is strongly influenced by the environment in which the crop is grown. To gain insights into the ways in which soil fertility influences aflatoxin, we investigated the relationships between agronomic traits and aflatoxin in diverse maize testcrosses that were grown under two nitrogen treatment levels. The experiment was conducted in Eastern Kenya, an aflatoxin-endemic area, with natural *Aspergillus flavus* inoculum. A panel of 205 maize lines was grown under low soil nitrogen (N_{low} = 26 kg/ha applied N) in the long season of 2011 and a subset of the genotypes (n = 123) was grown under high soil nitrogen (N_{high} = 114 kg/ha applied N) in the short season of 2010 and long season of 2011. Kernel traits, grain yield, days to anthesis, ear rot, and aflatoxin were analyzed for the panel. Grain yield, protein, and kernel bulk density were higher in maize grown under N_{high} compared to maize grown under N_{low}, with grain yield twice as high under N_{high}. A higher proportion of plots had grain with detectable aflatoxin under N_{low} than under the N_{high}. When the maize testcrosses were grouped into three maturity categories based on days to anthesis, aflatoxin accumulation was twice as high in the late-maturing group than in the other two categories under N_{low}. The proportion of aflatoxin contamination was higher in dent than in flint maize. However, the extent of aflatoxin accumulation did not differ significantly ($P > 0.05$) over the entire testcross panel, among maize genotypes within the maturity groups or among kernel texture groups within the maturity groups. Kernel bulk density and protein content were higher in early and intermediate groups than in the late maturity group. Grain yield did not differ among the maturity groups ($P > 0.05$), but significant positive correlations were observed between the proportion of grain yield reduction due to low soil nitrogen stress and aflatoxin in early and late maturity groups. Kernel bulk density was negatively correlated with aflatoxin in grain. No significant association was observed between aflatoxin and ear rot or kernel size. We conclude that aflatoxin mitigation strategies should include soil nitrogen amendment and breeding approaches that include selection for the correlated agronomic traits.

1. Introduction

There is growing evidence that food systems in tropical developing countries are widely contaminated by fungal toxins known as mycotoxins (Darwish et al., 2014). Mycotoxins contribute to global health problems and their management poses major challenges to food safety systems (Clarke et al., 2015; Hell and Mutegi, 2011; Williams and McDonald, 1983). Mycotoxin contamination in major foodstuffs is emerging as particularly problematic in Sub-Saharan Africa, where food production and storage conditions favor contamination and regulatory systems are not as effective as in well-resourced contexts (Hell et al., 2000). Maize, a major staple food in eastern and southern Africa, is commonly colonized by *Aspergillus flavus*, which causes Aspergillus ear rot and produces aflatoxin.

Exposure to aflatoxin is associated with a wide range of health risks (Reddy et al., 2010). Acute aflatoxin exposure can be fatal, and has claimed lives of over 470 people in the last 30 years in

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http://dx.doi.org/10.1016/j.fcr.2017.02.007
0378-4290/

Kenya (Mutiga et al., 2014). Chronic exposure causes liver cancer, and has been associated with stunting in children, nutrient absorption impairment, immunosuppression and increased morbidity (Zain, 2011). The extent to which maize is contaminated by aflatoxin depends on the interaction of maize genotypes, the environments in which they are grown and stored, as well as the timing and nature of the inoculum to which the crop is exposed. Recent surveys indicate a widespread prevalence and a high risk of exposure to the damaging toxins in eastern and southern Africa (Darwish et al., 2014; Mutiga et al., 2015; Mutiga et al., 2014; Yard et al., 2013).

Conditions conducive to aflatoxin contamination in maize are prevalent in the maize growing regions of East Africa (Hell and Mutegi, 2011), and aflatoxin contamination outbreaks in maize are common in Eastern Kenya (Daniel et al., 2011; Mutiga et al., 2014; Nyikal et al., 2004). Pre-harvest infection and contamination of maize by *A. flavus* is favored by plant stress factors such as high temperature, drought, insect damage and low soil fertility (Bruns, 2003; Kaaya et al., 2005; Payne, 1998; Wu, 2007). Most (70–80%) of the 6.5 million tons of maize produced in East Africa is cultivated by resource-poor farmers under sub-optimal agronomic conditions (Bekunda et al., 2004; Hellin and Kimenju, 2009; Seppälä, 1998). Inadequate soil nutrition can affect maize ear and kernel development, which may in turn influence susceptibility to fungal colonization (Seebauer et al., 2004). In Eastern Kenya, maize is cultivated by smallholder farmers under drought conditions in soils that are deficient in nitrogen and phosphorous (Simpson et al., 1996; Sutherland et al., 1999). A field study conducted in Italy showed that soil nitrogen level can influence aflatoxin levels; amendment with nitrogenous fertilizer reduced aflatoxin accumulation in their study (Blandino et al., 2008).

Breeding for aflatoxin resistance is one of several strategies for reducing aflatoxin exposure in maize-consuming populations (Williams, 2006). Genetic resistance to *A. flavus* infection and to aflatoxin accumulation exists in maize but are quantitatively inherited and variable in their expression (Mideros et al., 2012; Mideros et al., 2014). The two traits have low heritability and are characterized by strong genotype-by-environment (GxE) interactions (Betrán et al., 2005; Brown et al., 2008; Kebede et al., 2012; Mayfield et al., 2011; Menkir et al., 2008; Warburton and Williams, 2014; Williams, 2006). The strong GxE interactions have frustrated maize pathologists, geneticists, breeders, agronomists and farmers. Germplasm with varying degrees of *per se* resistance and with traits associated with reduced vulnerability to toxin accumulation has been identified (Betrán et al., 2005; Kebede et al., 2012; Mayfield et al., 2011; Warburton and Williams, 2014). Germplasm evaluation and studies on the genetics of resistance are underway to facilitate breeding for aflatoxin resistance in African maize (Brown et al., 2013; Warburton and Williams, 2014).

In view of the GxE interaction, it is important to understand how crop traits interact with environmental conditions to influence aflatoxin accumulation in locally-adapted maize germplasm. Traits associated with reduced aflatoxin accumulation in maize include ear morphology (e.g., husk coverage and tightness (Betrán and Isakeit, 2004; Widstrom, 1987)); kernel structure and chemical composition (e.g., hardness of the endosperm, waxy pericarp, antifungal proteins, etc. (Betrán and Isakeit, 2004; Betrán et al., 2006; Brown et al., 2006; Guo et al., 1995)); silk characteristics (Mideros et al., 2012); early maturity and adaptation (Betrán and Isakeit, 2004; Chen et al., 2004); insect resistance (Chen et al., 2004); kernel integrity (Odvody et al., 1997); and tolerance to drought and heat (Payne, 1992).

We sought insight into the relationships among soil fertility, kernel traits, and aflatoxin accumulation under natural colonization of maize by *A. flavus* in field trials in Eastern Kenya. The study utilized maize trials conducted as part of the Improved Maize for African Soils (IMAS) project, which was jointly conducted by the International Maize and Wheat Improvement Center (CIMMYT) and the Kenya Agricultural Livestock Research Organization (KALRO). As part of the IMAS project, testcrosses of a diverse maize association mapping panel were evaluated for tolerance to low soil nitrogen at two sites. We used this diverse panel to assess the relationships between various agronomic traits of maize and susceptibility to colonization by *A. flavus* (based on fungal biomass) and aflatoxin accumulation.

2. Materials and methods

2.1. Study sites

The IMAS association mapping (IMAS-AM) panel was evaluated at two KALRO experiment stations: Embu (latitude 00° 30′S and longitude 37°42′E; elevation 1510 masl) and Kiboko (latitude 02° 12′ S, longitude 37°43′ E, altitude 975 masl), in Eastern Kenya. Both sites are located in drought-prone areas, but water stress was avoided through the use of irrigation: three hours of overhead irrigation was provided in the evening every three days during the growing periods. Both sites are located within an aflatoxin-prone region (Daniel et al., 2011; Lewis et al., 2005; Mutiga et al., 2014) and plants were naturally infected by resident fungi. In this region, maize is planted in October and harvested in February for the short rains, and planted in April and harvested in September for the long rains season. The soils at the Embu station are humic nitrisol, slightly acidic (pH 5.6) and low in both nitrogen (0.2%) and phosphorus (26 ppb). The soils at Kiboko are haplic lixisol, slightly acidic (pH 5.7), and low in nitrogen (0.15%) and phosphorus (15 ppb).

2.2. Maize germplasm

The IMAS-AM panel is a diverse set of maize germplasm assembled by CIMMYT for use in breeding for low nitrogen (low N) tolerance in African maize. The panel consists of 421 inbred lines that were sourced from the CIMMYT's global tropical breeding programs, KALRO and the Agricultural Research Council (ARC) of South Africa. All inbred lines were crossed to a common tester, CML539 (CML312SR), a white sub-tropical dent maize line of intermediate maturity, good combining ability and moderate adaptation to drought and low N. The pedigrees of the maize genotypes studied and their maturity groups are shown in Supplementary Table S1.

2.3. Experimental design

The IMAS-AM panel was grown under two nitrogen regimes. In the low-nitrogen treatment (hereafter N_{low}), triple super phosphate (TSP – 0:46:0) fertilizer was applied at 100 kg/ha during planting to promote root development and early stand establishment. In addition, calcium ammonium nitrate (CAN; 26-0-0 +8 Ca) was applied as top dressing (42 days after planting) at 100 kg/ha. The total amount of 26 kg/ha N applied in this treatment was considered to be reflective of the average nitrogen application rate among smallholder farmers in sub-Saharan Africa (Mutegi et al., 2012). The N_{low} trials were conducted at Kiboko and Embu in 2012. In the second treatment (hereafter high nitrogen rate, N_{high}), 200 kg of di-ammonium phosphate (DAP;18-46-0) was applied per hectare at planting followed by a top dressing of 300 kg/ha of CAN per hectare 42 days after planting. The total amount of 114 kg/ha N applied in this treatment is within the range of previously reported high/optimal nitrogen application rates (96–160 kg/ha N) in the region (Adamtey et al., 2016; Masuka et al., 2016)). The N_{high} trials were grown at Kiboko in 2011 and 2012. Both trials were planted in two replicates in an alpha lattice experimental design. Trials were