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QTL identification for marker assisted selection of corn (*Zea mays* L.) husk traits associated with *Fusarium* spp diseases resistance

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General Introduction

Corn Importance and Production

According to the United States Department of Agriculture (USDA), global corn production reached 1.22 billion tons in 2023. The United States is the leading producer with 389.7 million tons, followed by China with 288.8 million tons. The European Union is the fourth-largest producer with 61.5 million tons, and Italy ranks seventh within the EU with 5.3 million tons in 2023, according to the European Statistical Office (EUROSTAT).

In the European Union, corn is cultivated on 8.3 million hectares with a yield of 7.4 tons per hectare for the 2023 season, as reported by EUROSTAT. In Italy, corn yields have averaged between 8.6 and 10.6 tons per hectare over the past ten years (EUROSTAT). Although yields are above the EU average, cultivated area has been steadily declining over the last 20 years, from around 1.2 million hectares to 498 thousand hectares (EUROSTAT). This significant reduction is primarily attributed to declining profitability for corn in Italy and changes in the Common Agricultural Policy (CAP) of the European Union (Kayad et al., 2021).

Data from the International Grains Council (IGC) indicate that global maize production in 2023 is primarily used for livestock feed, accounting for 59.7% of production. Industrial use represents 25.8%, food demand accounts for 11.4%, and other uses comprise the remaining 3.1%.

These data highlight the crucial role of corn at a global level. Rising demand for crop production, driven by increasing human population, growing affluence leading to higher meat and dairy consumption, and biofuel consumption, underscores the importance of this cereal. However, growers face various challenges in maintaining production and quality standards demanded by the market. Shrinking cropland, limited resources, and environmental protection issues are just a few examples of factors that can affect corn production (West et al., 2014).

The factors affecting corn production can vary depending on the region, but corn diseases are a common issue worldwide. These diseases can be caused by abiotic factors or biotic agents. Abiotic factors include climate-induced diseases such as late and early frost, root lodging, drought, hail, and others. Additionally, poor management practices like nutrient deficiencies and herbicide toxicities can negatively impact corn health. Biotic agents responsible for corn diseases include fungi, bacteria, viruses, nematodes, and even parasitic plants (Smith & White, 2015). These biotic agents reduce annual global yield by an average of 22.5% (Savary et al., 2019). Among these pathogens, fungi are the most significant, affecting both the quantity and quality of corn production.

Corn Fungal Diseases

Fungi are heterotrophs eukaryotic organisms. These organisms present the most significant ecological and economic risks to cultivated plants.

Their biological interaction exhibits a wide spectrum, ranging from symbiosis to parasitism and many plants fungal pathogens can switch from colonize the living tissue to become necrotrophs that feed on the tissue of the plant that they have killed (Koeck et al., 2011).

In corn, fungi cause the greatest number of known diseases. These pathogens can infect leaf, stalk, ear, roots and even seed. Symptoms vary widely depending on the affected plant part and the yield loss associate with each different disease can be very different (Mueller et al., 2020; Oerke, 2006).

A great number of genera can infect corn, the most common are *Aspergillu*s, *Pythium*, *Rhizoctonia*, *Bipolaris*, *Sclerophthora*, *Stenocarpella*, *Magnaporthiopsis*, *Kabatiella*, Helminthosporium, *Ustilago*, *Puccinia* and *Fusarium* (Jeffers, 2004; Smith & White, 2015).

In this context *Fusarium* spp. are one of the most important treats in corn growing system and widely recognized as one of the world's most economically destructive genera of plant pathogens (Geiser et al., 2013).

Life cycles of *Fusarium* species in temperate regions are largely similar. Spores are dispersed through the environment by wind, water, insects, and soil carried by human activities and agricultural equipment (Blandino et al., 2015; Bragard et al., 2022; Hoffmann et al., 2021). Most of these corn-infecting fungi survive from one season to the next in crop debris or stover left in the field, while some can persist in the soil year-round. Infections can occur at the seedling stage or during the flowering phase of the plant (Brauer et al., 2020; Gai et al., 2018).

These fungi produce various secondary metabolites, including mycotoxins, which pose significant risks to human and animal health (Logrieco et al., 2002). Two particularly problematic species causing substantial quality and yield losses in corn are *Fusarium verticillioides* and *Fusarium graminearum* (Figure 1).

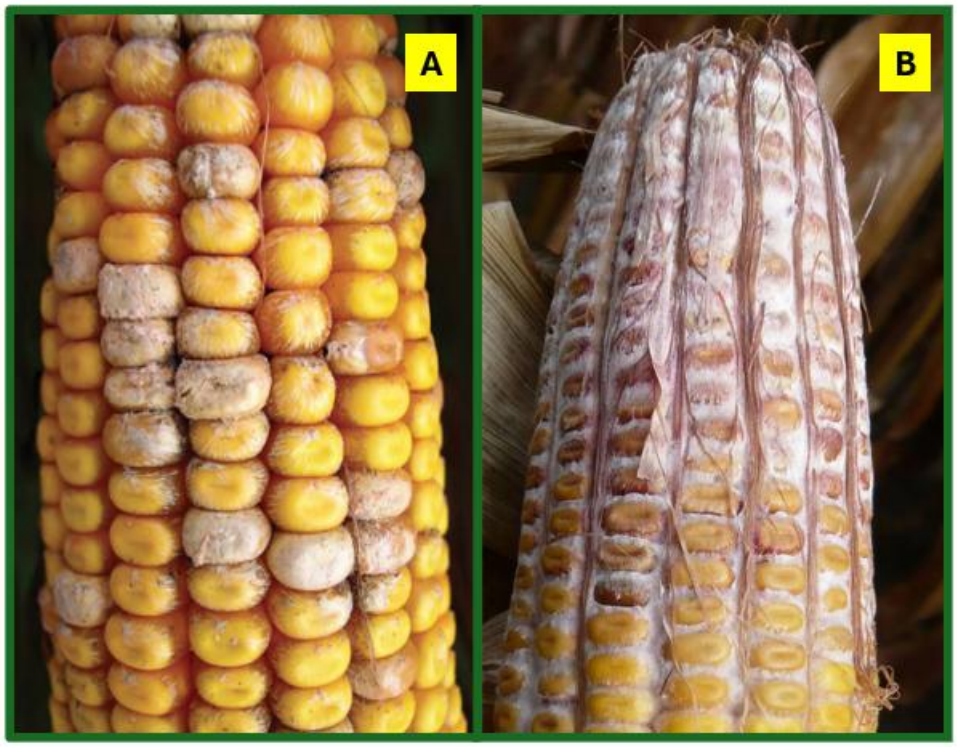

Figure 1. Fusarium ear rot caused by *Fusarium verticilloides* (A) and Gibberella ear rot caused by *Fusarium graminearum* (B).

Fusarium verticillioides

Fusarium verticillioides (Saccardo) Nirenberg (syn. *F. moniliforme* Sheldon, teleomorph *Gibberella fujikuroi* (Sawada) Wineland*)* is the causative agent of an impactful disease in corn known as Fusarium ear rot (Miedaner et al., 2020). The fungus can infect both corn seedlings and ears. It consists of a white mold scattered on the ear or kernel that may become tan or brown. In some cases, kernels can present white streaks called "starbursts". Notably, the fungus can also colonize ears without causing visible disease symptoms (Lanubile et al., 2015; Smith & White, 2015).

Infection occurs at ear through silk and wounds caused by insect or hail, mostly during the flowering period (Gai et al., 2018). Fusarium ear rot (FER) generally is favoured by warm, dry weather during the grain-filling plant stage (Mesterházy et al., 2012; Reid et al., 1999).

Insect injuries, caused by larvae feeding activity, are associated with an increasing severity of this disease (Alma et al., 2005). Larvae can acquire fungal spores from leaf surfaces and transport them into the ear (Sobek & Munkvold, 1999). In Europe and North America, the most important insect associated with FER is *Ostrinia nubilalis* (Blandino et al., 2015).

Infection of *Fusarium verticillioides* can lead to significant yield losses, reaching up to 50% of potential yield, and the production fumonisin, a class of harmful mycotoxins (LI et al., 2010; L. O. Rocha et al., 2016).

Fumonisins are a class of mycotoxins produced by various *Fusarium* species, with *Fusarium verticillioides* being the primary producer in temperate regions (Ekwomadu et al., 2020; Jestoi, 2008; Streit et al., 2013).

These mycotoxins have been characterized and classified in four different class A, B, C, and P. Among which fumonisin B and specifically FB1, FB2, and FB3 are most abundant and FB1 being the most toxic (Braun & Wink, 2018; Damiani et al., 2019).

Fumonisins has been associated with a higher incidence of cancer of the oesophagus, in addition, exposure to these fumonisins determines toxic effects on the liver and nephron (Chu & Li, 1994).

FB1 is also associated with hepatocarcinoma, stimulation and suppression of the immune system, defects in the neural-tube and nephrotoxicity (Gelderblom et al., 1988).

According to the International Agency for Research on Cancer (IARC) fumonisins FB1 and FB2 has been classified as a group 2B possible carcinogen for human (Ostry et al., 2017). In an optic of food safety European Union set a limit for cereals and all products derived for human consumption at 2 µg/kg for FB1 (European Commission, 2006).

Fusarium graminearum

Fusarium graminearum Schwabe (teleomorph *Gibberella zeae* (Schwabe) Petch) is the causative agent of Gibberella ear rot (GER) in corn but it's also known for the devastating disease caused in barley and wheat know as Fusarium head blight (Sutton, 1982; Wetter, 1999).

Ear infected by this fungus present a red or pink mold that initiates from the tip (Bottalico, 1998; Munkvold, 2017). Gibberella ear rot is favoured in environment with higher precipitation during the corn growing season (Munkvold, 2003b). High levels of moisture at silking, followed by moderate temperatures have been seen associated with a higher impact of this disease (Sutton, 1982).

Similar to *F. verticillioides*, the primary dispersal agents are rain, wind, and insects and this fungus can infect the ear through silk or wound (Desjardins et al., 2006; Parry et al., 1995). Also in this case, *Ostrinia nubilalis* is the most important insect associated with GER in temperate regions (Alma et al., 2005).

This disease has an epidemic behaviour, with years of heavy infection followed by almost complete absence (Sutton, 1982). Yield reduction can be substantial and reach even 50% kernel weigh loss (Vigier et al., 2001).

Production of mycotoxin is another characteristics of *F. graminearum*. Deoxynivalenol (DON) and zearalenone (ZEA) are the most significant mycotoxins produced (J. Wang et al., 2020).

Deoxynivalenol is a mycotoxin of the class of trichothecenes, the most common effect of is feed/food refusal but it can cause also vomit and altered immune system function.

These effects became more severe proportionally to the ingestion of this mycotoxin, but due to the reducing intake effect very severe symptoms are not common (Arunachalam & Doohan, 2013; Hou et al., 2023; O. Rocha et al., 2005). Feed refusal cause poor livestock performance and swine are the most sensitive animals (Ganesan et al., 2022).

Zearalenone is an estrogenic compound that can cause fertility disorders in both humans and animals in particular swine (Poór et al., 2015). Reduction of litter size and milk production, inflammation of the mammary glands and atrophy of the ovaries are some of the ZEA effect on swine (Nones & Scussel, 2013). The impact of ZEA in humans is not well understood, but available data suggests that sensitivity is similar to that swine (Kowalska et al., 2016)

Due to the harmful effect of these two mycotoxins different organization defined limit for feed and food contamination. For example, European commission defined for unprocessed corn a maximum of 750 µg/kg for DON and 350 µg/kg for ZEA (European Commission, 2006).

Control Methods

In this context controlling *Fusarium* diseases and their mycotoxins play a crucial role in the struggle to sustain quality and quantity of maize production. Various control methods can be implemented to mitigate the effect of these fungi (Figure 2) starting from the use of fungicide molecules. The most used class of active ingredients are triazole, acylalanine and strobilurins (Broders et al., 2007; Fernández-ortuño et al., 2010; Sukul & Spiteller, 2000). A trend of increasing of use of these type of fungicide has been seen in country where they are permitted. This increment in the usage of these molecules pose important question on the treat of the development of resistant strains (Wise & Mueller, 2011).

Another method to reduce fungal damage is to control the insect that can promote infection. The main target, in temperate regions, is *Ostrinia nubilalis*. Insecticides from various classes, such as pyrethroids, organophosphates, carbamates, and anthranilic diamides, can be used to target larvae, eggs, or both (Boiteau & Noronha, 2007; Kaçar et al., 2023). In country where fungicides are not available and genetically modified organisms (GMO) are not permitted, the use of insecticide is the most important control methods to reduce the effect of fungal diseases (Scarpino et al., 2018).

Biological control of insects is another emerging strategy. The use of natural *Bacillus thuringiensis* toxins has been proven effective against ECB (Pardo-López et al., 2013) and the use parasitoid insects like *Trichogramma* spp. are another valuable option in the control of this insect (Zang et al., 2021).

The use of insecticide and fungicide can have significant impact on the economic and environmental sustainability of maize production. Therefore, adopting sustainable agricultural practices can help mitigate these effects.

Tillage, crop rotation, plant density and sowing date are the most common agricultural practices used to control fungal diseases. Crop rotation play a crucial role in the control of different diseases not only fungal, and it was seen that rotation with non-host species favours the reduction of fungal infection (Ariño et al., 2009; Schaafsma et al., 2005). Tillage and crop residue management can also help reduce inoculum density, with conventional tillage (e.g., plowing) being more effective than reduced or no-tillage practices in controlling fungal pathogens (Pfordt et al., 2020; Tran et al., 2021). Another management methods can be the choice of the planting date. Planting date can influence the synchronization of flowering and ECB presence, with later planting dates being associated with higher fungal infections (Krnjaja et al., 2022). Plant density is another factor to consider, as high density can increase grain contamination. However, the optimal plant density can vary depending on environmental conditions and corn variety (Krnjaja et al., 2019).

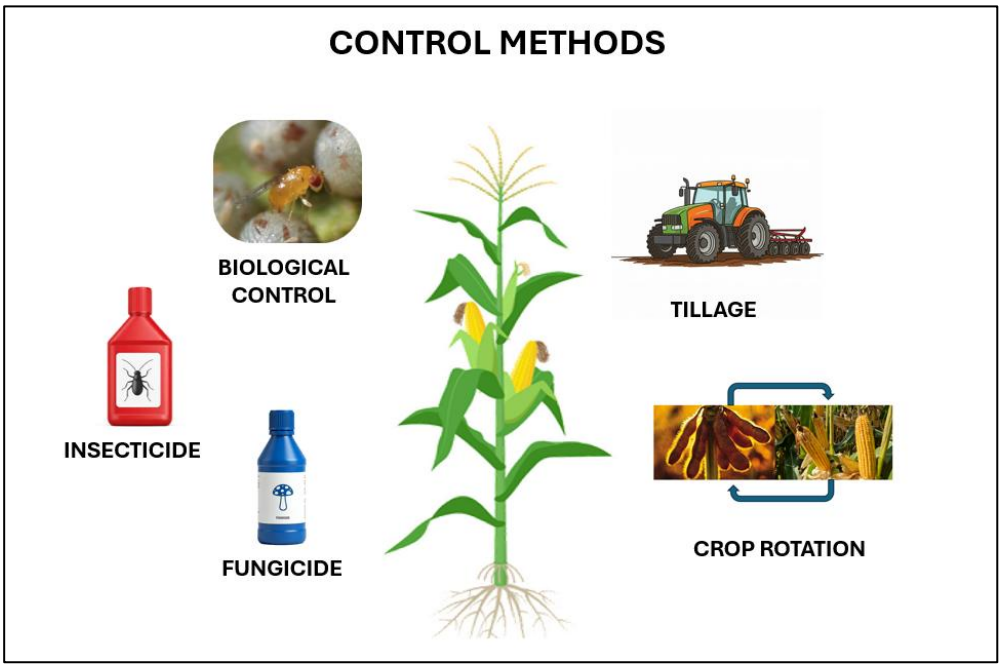

Figure 2. Control methods against fungi can be classified in two classes**:** direct controls against fungi with biological controls, fungicide and insecticides. Or with management methods like crop rotation or tillage and crop residue management.

Hybrids Selection

The combined use of agronomic practices, chemical control, and resistant hybrids is essential for effectively managing *Fusarium* diseases. While completely resistant hybrids are not yet commercially available, breeding programs have successfully excluded highly susceptible varieties (Munkvold, 2003a).

Fungal diseases resistance is a trait where the genotype × environment interaction has great influence (Bolduan et al., 2009) explaining why complete resistance has not been achieved. Various resistance mechanisms have been identified in different parts of the corn plant.

Silks, kernel and husks are the main source of resistance associated with *Fusarium* spp. infection (Headrick, 1991; Hoenisch, 1994; Landoni et al., 2020; Warfield, 1996).

Exposed silks during pollination are vulnerable to fungal infection, and varieties with excessive silk growth and unsynchronized anthesis-silking are more susceptible due to prolonged exposure (Borrás & Vitantonio-Mazzini, 2018). Silk abscission is a mechanism put in place by the plant that can reduce infection, formation of the abscission zone and senescence of the silks prevent pathogen entry to the ear (Pataky & Richter, 2007). This mechanism in normal situation prevents excess pollen tubes from accessing the ovary but it has been seen associated also with fungal disease resistance (Bassetti & Westgate, 1993). Selection of materials resistant to fungal disease is favouring hybrids that have a faster silk abscission and reduced anthesis-silking interval.

Regarding kernel resistance, selection has primarily focused on the pericarp. It has been seen that hybrids with thicker pericarps are less susceptible to fungal infection. This can be explained by the mechanical protection provided by the pericarp layer (Sampietro et al., 2009). Additionally, the accumulation of phenolic compounds, like flavonoids, can enhance kernel resistance by hardening the pericarp and acting as a physical barrier against fungal attack (Bernardi et al., 2018; Pilu et al., 2011).

Another characteristic that selection can improve in the optic of the reduction of fungal infection symptoms are husk traits. Husk leaf number, husk tightness and hask coverage are the most important. Husks play a role in the mechanical protection of the ear and hybrid with higher husk number and tighter husk that cover all the ear are the most resistant (Smith & White, 2015; Warfield, 1996; Zhang et al., 2021). Direct protection from fungal spore is not the only husk function, husk protect kernels also from insect damage and other abiotic factors (Barry et al., 1986; Y. Wang et al., 2023). All of these characteristics are important in a modern breeding program that aim to select for better resistance against *Fusarium* diseases in corn. Now days phenotypic information are not the only data used to select hybrids, genetic information is now widely used to select improved variety.

A wide range of studies have been carried out to understand the genetics behind fungal disease resistance, but genetics behind husk characteristics that are associated with *Fusarium* diseases remains relatively less investigated.

Summary of the Thesis Work

The aim of this project is to understand the phenotypic and genotypic aspects of husk traits associated with resistance to *Fusarium* spp. corn diseases in inbred and hybrids adapted to the Northen Italy environment. This work has been conducted in the frame of an industrial PhD agreement between the University of Milan and Pioneer Hi-Bred Italia Servizi Agronomici.

In this PhD thesis we initially reviewed the most important management strategies against *Fusarium* spp. fungal diseases in maize and the genetic basis which is behind hybrid resistance.

Cropping methods, insecticide against ECB, direct control with the use of fungicide are the main control methods analysed. Specific importance has been given to the selection of new variety and the source of resistance found in other studies.

In particular, ear characteristics have been taken in account as a trait that can be selected to produce better resistant hybrids. To understand which are the genetic basis of these ear traits a review of the literature has been carried out and different QTL has been found associated with husk length, husk width, husk coverage and husk number (published review; Magarini et al., 2023, first manuscript presented in this PhD thesis).

Two different approaches have been conducted to understand better which are the ear characteristics associated with resistance to *Fusarium* diseases of materials adapted to the Northern Italy environment. These experimentations have been conducted with the support of the Italian Pioneer research station.

The first approach aimed to understand the phenotypic traits of hybrids associated to Fusarium Ear Rot (FER) disease. In a single year study 38 hybrids were tested in 3 different locations: Bergamo (BG), Cremona (CR) and Vicenza (VI). Husk number, husk coverage, *F. verticillioides* severity, yield estimate, relative grain humidity and total fumonisin concentration data have been collected form each plot to understand the possible correlation between these traits.

Statistically significant correlation has been found between husk coverage and yield, husk coverage and humidity and, as confirmed in other studies, positive correlation was found between *Fusarium* severity and fumonisin content. What was interesting was that in the Vicenza location, the one that had the highest FER infection, a negative correlation between husk coverage and *Fusarium* severity was found. Confirming that higher husk coverage seems to reduce *Fusarium* infection (published paper; Magarini & Colombo, et al., 2024, second manuscript presented in this PhD thesis).

These interesting results was used as base for a second experiment carried out in a 2-year multi-locations testing network. In this second approach 74 inbred lines (42 non-stiff stalk and 32 stiff stalk) were tested to analyse the phenotypic and also the genotypes traits associated with Gibberella Ear Rot (GER), another important fungal disease in Northern Italy. These materials were planted in 2 replications for each of the three locations (Cremona, Torino and Cuneo).

Similar to the previous experiment different ear traits have been collected (husk number, husk coverage score, ear attitude score, *F. graminearum* infection score, DON and zearalenone concentration).

F. graminearum infection score has been found positive statistically correlated with husk cover and husk number. Indicating that better coverage of the husk and higher number of them are associated with less infection (infection score was collected in an inverted scale where higher score means less infection). Another significant positive correlation has been found between DON and ZEA concentration and ZEA was also found negatively correlated with ear attitude (upright ear indicate less ZEA concentration).

In addition, a high-density genotyping analysis has been conducted on these materials at the Corteva Agriscience LLC Johnston Laboratory. Phenotypic and genotypic data were used to make an association study, and several novel QTLs has been identified. Four QTLs has been found associated with husk number, two for husk cover score, eight QTLs were found for the ear attitude score and four for the *F. graminearum* infection score.

These results showed again the importance of ear traits to contrast fungal infection, and these novel identified QTLs can be useful in breeding program of materials adapted to the Italian environment (published paper; Magarini & Pirovano, et al., 2024, third manuscript presented in this PhD thesis).

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Review

Genetics and Environmental Factors Associated with Resistance to *Fusarium graminearum*, the Causal Agent of Gibberella Ear Rot in Maize

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Abstract: Maize is one of the most important food and feed sources at the worldwide level. Due to this importance, all the pathogens that can infect this crop can harm both food safety and security. Fungi are the most important pathogens in cultivated maize, and *Fusarium* spp. are one of the most important families. Reduction in yield and production of dangerous mycotoxins are the main effects of *Fusarium* spp. infection. *Fusarium graminearum* (part of the *Fusarium graminearum* species complex) is one the most important fungi that infect maize, and it is the causative agent of Gibberella ear rot (GER). The main characteristics of this species include its ability to infect various species and its varying infection pressures across different years. This fungus produces various harmful mycotoxins, such as deoxynivalenol, zearalenone, butanolide, and culmorin. Infection can start from silk channels or from ear wounds. In the first case, the environmental conditions are the most important factors, but in the second, a key role is played by the feeding action of lepidopteran larvae (in Europe, *Ostrinia nubilalis*). All these factors need to be taken into account to develop a successful management strategy, starting from cropping methods that can reduce the source of inoculum to the direct control of the fungus with fungicide, as well as insect control to reduce ear wounds. But the most important factor that can reduce the effects of this fungus is the use of resistant hybrids. Different studies have highlighted different defensive methods developed by the plant to reduce fungal infections, like fast drying of silk and kernels, chemical compounds produced by the plant after infection, and mechanical protection from insects' wounds. The aim of this paper is to review the scientific evidence of the most important management strategies against GER in maize and to highlight the genetic basis which is behind hybrid resistance to this disease, with a focus on genes and QTLs found in studies conducted across the world and with different types of maize from tropical cultivars to European flint.

Keywords: maize; *Fusarium graminearum*; GER; corn breeding; review; mycotoxin; DON; ZEA

1. Introduction

Maize (Zea mays L.) is one of the most important cultivated crops. It is the cereal that has seen the highest increase in production rate due to the high demand for maize plant products as important food resources for animals and humans, and as raw materials for use in industry and biofuels [1]. However, cultivation methods (mono-cropping) and poor gene heterogeneity in commercial hybrids have led to a serious problem of disease susceptibility [2,3]. Like all the other crops, maize has a great number of pathogens, among which fungi are some of the most critical [4]. It has been estimated that in the last decade, the average yield loss due to these pathogens ranged from 6.8% to 13.5% [5] in the USA and Canada. Fungal pathogens of maize are relevant not only for the direct damage they can cause to the plant, but also for the ability of many of these pathogens (*Aspergillus* spp. and *Fusarium* spp.) to produce mycotoxins [6] (Figure 1).

Figure 1. Fusarium ear rot caused by *Fusarium verticilloides*, Gibberella ear rot caused by *Fusarium graminearum*, Diplodia ear rot caused by *Stenocarpella maydis*, and Aspergillum ear rot caused by *As- pergillus flavus.* (**A**) Fusarium ear rot; (**B**) Gibberella ear rot; (**C**) Diplodia ear rot; and (**D**) Aspergillum ear rot.

The presence of mycotoxins in corn products (e.g., kernels and silage) can reach almost 100% of the examined samples, due to the large number of possibly toxicogenic fungi that can infect this species [7]. Several Fusarium species are known to infect maize, and among them, *F. graminearum* Schwabe is one of the most important pathogens. This fungus is sometimes still reported with its teleomorph name of *Gibberella zeae* (Schw.) Petch (Ascomycota). The complex biology of this pathogen has led researchers to define it, rather than as a single species, as the *Fusarium graminearum* species complex (FGSC) [8]. The *Fusarium graminearum* species complex is composed of 16 species: *F. acaciae-mearnsii*, *F. aethiopicum*, *F. asiaticum*, *F. austroamericanum*, *F. boothii*, *F. brasilicum*, *F. cortaderiae*, *F. gerlachii*, *F. graminearum sensu stricto*, *F. louisianense*, *F. meridionale*, *F. mesoamericanum*, *F. nepalense*, *F. ussurianum*, *F. vorosii*, and another one that is not yet formally described [9]. The most common species of the FGSC that affects cereals is *F. graminearum*, distributed at a worldwide level. Other important species in cereals are *Fusarium asiaticum*, the most common member of the complex on rice in Asia [10], which is now also present in rice in South America and the USA [11,12]; *Fusarium meridionale*, which is more prevalent in maize in South America; and *Fusarium boothii*, which is the most common species of this complex in South African maize [13]. The reasons for the dominance of one species over the others are not clear, but are correlated to differences in aggressiveness [14] and adaptation to different environments [15]. Other studies consider this fungus not only as part of the *Fusarium graminearum* species complex but also in association with *F. verticillioides* [16]. These two fungi have a complicated interaction, and the presence of one can reduce the effect of the other, but the symptoms in most cases are difficult to distinguish [17] unless the *F. verticillioides*-associated "starburst" is present, leading researchers to study these fungi together. In this review, considering the specific context of breeding resistance traits, *F. graminearum* will be considered as a single species associated with a single disease (Gibberella ear rot), and interaction with other species will be put into the background in order to focus only on the specific effects that this fungus causes in maize.

2. Fusarium graminearum (Schw.)

Fusarium graminearum (Ascomycota), also known as *Gibberella zeae* in its sexual stage, is a fungal plant pathogen diffused around the world (Figure 2).

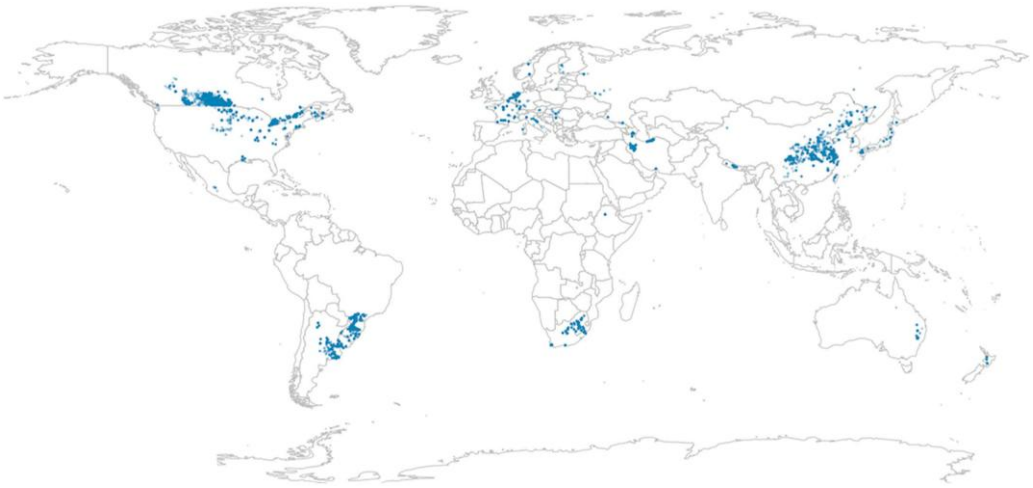

Figure 2. Countries where *Gibberella zeae* has been documented (blue dots). Modified from Del Ponte [8].

Fusarium graminearum is a homothallic and self-fertile fungus. It can have both sexual and asexual life cycles. In its diploid stage, it consists of a fruiting body (perithecium) where ascospores are formed in asci and released in spring [18]. During the haploid phase, it consists of a filamentous hypha and produces mitotic spores (macroconidia). Both ascospores and macroconidia are known as sources of infection [19,20]. It can grow at temperatures between 15 and 29 ◦C, but when the temperature is higher than 30 ◦C, its development is very limited [21,22], while on the contrary, some studies have suggested that it can even grow at temperatures below 15 ◦C [23,24] with a limit of 8 ◦C for perithecia production [25].

Fusarium verticillioides, another pathogen commonly found in cereals, has a higher temperature optimum with a peak development around 27 ◦C, and it can produce spores even at 45 ◦C [26]. *F. graminearum* can be found in a large number of cereal grains such as wheat, barley, maize, oats, rice, and rye [27,28].

This fungus can also infect other plant genera like Pisum, Trifolium, Solanum, and Coffea [29,30]. It causes a wide range of diseases in various crops, such as head blights in wheat, tuber dry rot in potatoes, and pitch canker of Pinus species [28,29,31,32].

Fusarium graminearum is the causative agent of Gibberella ear rot (GER), which is a key maize disease in temperate regions. It appears as a reddish mold and affects the ear, starting from the tip (Figure 1). Infection can occur starting from the cob or from kernel wounds. When the fungus infects the cob, it appears as a white mycelium, and this turns into a red-pink mold.

In severe cases, it can also grow on the husk leaves. In this situation, the husk, cob, and kernels become tightly bound together by the fungal mass, and they are not separable. The infection from the kernel wound has a similar development, but in this case, it seems that the fungus spreads to the top of the ear faster than to the bottom [33]. The amount of yield loss experienced in maize can significantly differ from season to season. According to Sutton's research, there have been years in Canada where serious epidemics of GER (Gibberella ear rot) occurred, while other years saw a much lower impact of this disease [34]. A similar pattern was observed in the United States, as noted by Wetter [35]. In the case of wheat and barley, the culprit behind yield loss is fusarium head blight (FHB) [32,34], a devastating disease that has resulted in losses amounting to tens of billions of dollars over the past two decades in the United States [24,36]. Ascospores are produced from the perithecia outwinter in maize, other cereals' residues, and also a wide range of mono and dicotyledonous weeds (Figure 3) [37–39] when temperatures rise to 13° C mainly at night [40].

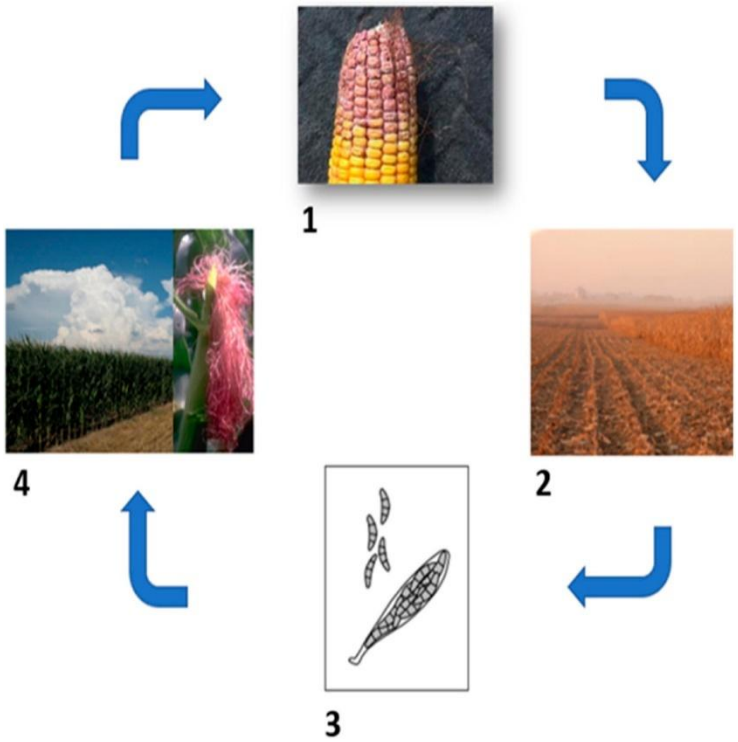

Figure 3. *Gibberella zeae* life cycle. (1) *F. graminearum* appear as a pink or reddish ear mold. (2) Inoculum outwinters in infected crop residues like corn and wheat. (3) *Fusarium graminearum* can grow at temperatures between 15 and 29 ◦C, and it produces spores starting from 13 ◦C. (4) Infection occurs at flowering via silk or insect damage.

In corn, infection occurs during the silking period. Ears are more susceptible between two and six days after the emergence of the silk, and the peak of susceptibility is during their senescence [41]. The most common propagation agents of *F. graminearum* are rain, wind, and insects [42,43]. One of the most important insects that are correlated with more severe GER infections is the larva of the European corn borer moth (*Ostrinia nubilalis* Hübner) (Lepidoptera: Crambidae). Tunneling and kernel wounds during the feeding of the larvae can favor the infection by this fungus.

The larvae can also spread the propagule with their movements inside the plant [44]. In temperate regions, *O. nubilalis* is normally bivoltine during the maize growing season, but a small number of univoltines or multivoltines can grow depending on the weather [45].

The first-generation larvae usually produce damage to leaves, while the second generation develops by feeding on the stalk or the ears. Tunnels below the ear cause breakage, while apical tunnels in the cob are linked to fungal infection due to a particular microclimatic condition that can promote fungal development [46].

3. Mycotoxins

Reduction in the yield is not the only damage caused by GER: *Fusarium gramin- earum* is also known to be a mycotoxigenic fungus. Infected maize ears can develop various types of mycotoxins, among which there are some well-established classes, such as aflatoxins, ochratoxins, trichothecenes, fumonisins, and zearalenone, and groups of minor, less-characterized, or emerging toxins for a total of over 30 different mycotoxin types [7,9,47]. The most important are deoxynivalenol and zearalenone, which cause poor livestock performance, particularly in swine. Deoxynivalenol causes feed refusal, vomit- ing, and decreased weight gain, while zearalenone causes reproductive problems [48,49]. Mycotoxins are secondary metabolites of fungi that have toxic properties to animals and humans [21,50], and they are produced by fungi when these organisms invade crops or their derived products [51,52] (Table 1).

Table 1. Comparison of mycotoxins produced by *F. graminearum* and *F. verticillioides*. Fusarium nomenclature according to Nelson [31].

Fusarium Species	Mycotoxins	Reference
F. graminearum	DON [1], ZEN [2], CUL [3], BUT [4]	[9,53]
F. verticillioides	FB1 [5], FB2 [6], FB3 [7]	[54]
F. culmorum	DON, ZEN, NIV [8]	[55]
F. oxysporum	BEA [9]	[56]
F. poae	DAS [10], NIV, FUS [11]	[54]

1 Deoxynivalenol (Vomitoxin); 2 Zearalenone; 3 Culmorin; 4 Butenolide; 5 Fumonisin B1; 6 Fumonisin B2; 7 Fumonisin B3; 8 Nivalenol; 9 Beauvericin; 10 Diacetoxyscirpenol; and 11 Fusarenone-X (4-Acetyl-NIV).

Due to the risks associated with the intake of mycotoxin-contaminated cereals, differ- ent countries and agencies, such as the FAO, FDA, and EFSA, have established regulations to limit their presence in both feed and food. For example, the European Union sets a maximum amount and guidance level for some mycotoxins in grain and derived prod- ucts [57]. While other fungi of the same genus, such as *Fusarium verticillioides* (Nirenberg), produce mostly fumonisins [54], *F. graminearum* produces various different types of toxins, of which the most important are deoxynivalenol or vomitoxin (DON), zearalenone (ZEN), butanolide (BUT), and culmorin (CUL) [9,53].

4. Deoxynivalenol

Deoxynivalenol (DON) is a mycotoxin of the trichothecene family [58]. DON and its derivates 3-acetyldeoxynivalenol (3-ADON) and 15-acetyldeoxynivalenol (15-ADON) can be produced on many cereals like corn, wheat, barley, and rice, but also on oats, rye, and sorghum [8]. DON can be produced and accumulates both in the kernels and in the stalk of maize, depending on where the fungus infects the maize plant and, unlike in wheat, it does not seem that the toxin can be transported systemically through different plant organs [59]. *Fusarium graminearum* and *Fusarium culmorum* are the two most important species that produce this toxin. Both species possess strains capable of producing deoxynivalenol (DON) as well as other toxins as their primary metabolites [60]. Both acute and chronic toxicity are associated with DON ingestion. Acute toxicity affects mostly the intestinal mucosa. Overproduction of ROS and reduced respiratory capacities in mitochondria of the host cells and intestinal microbes are the two major causes of this toxicity [61]. The chronic effect is correlated with immune system suppression caused by the inhibition of mitophagy [62]. Other effects of DON are damage to the respiratory system that can lead to asthma [63] and alteration in the expression of MAPK (mitogen-activated protein kinase) proteins that are involved in the control of cell apoptosis, differentiation, and cell growth [64]. Recent studies linked these effects to indirect damage caused by DON to mitochondria, which will ultimately lead to cell death [65].

5. Zearalenone

Zearalenone (ZEN) is a non-steroidal estrogenic mycotoxin [66]. ZEN has a crystalline structure; it is insoluble in water, is heat stable, and has a melting point of 164–165 °C [67,68]. Like DON, *Fusarium graminearum* and *Fusarium culmorum* are the two most important zearalenone fungal producer species [53]. ZEN has been found in all the most important cultivated cereals and some legumes [69–71]. Zearalenone's estrogen-like effects can cause fertility disorders both in humans and animals [72]. At high doses, ZEN induces an overproduction of ROS, thus can lead to oxidative stress. This stress can be correlated with DNA damage and mitochondrial degeneration that can lead to cell apoptosis [73,74]. At lower doses, ZEN is known for its carcinogenic activity in the liver and reproductive system [75].

6. Butenolide

Butenolide (4-acetamido-4-hydroxy-2-butenoic acid lactone or BUT) is a secondary metabolite usually co-produced with other mycotoxins (mostly deoxynivalenol) by different *Fusarium* species, mostly *F. sporotrichioides* and *F. graminearum* [53,76].

BUT is considered an emerging mycotoxin: this classification is used to define all the mycotoxins that are not legislatively regulated but have an important and increasing presence in feed and food [77]. This mycotoxin is associated with the cattle disease known as fescue foot [78,79]: it has been demonstrated to cause damage at the digestive system level due to significant cytotoxic effects caused by oxidative stress and oxidative damage [80,81]. In contrast, its toxicity in the long-term and at lower dosages has not yet been thoroughly studied, and more data are needed [82].

7. Culmorin

Culmorin (CUL) is a tricyclic sesquiterpene diol. Like butanolide and other com- pounds, it is considered an emerging mycotoxin since it is frequently observed, even in high concentrations, in grain and cereal-based products [82–84]. *F. culmorum* and *F. graminearum* are the two most important CUL producers [53]. A high concentration of this mycotoxin in contaminated grain correlates positively with the DON amount [85]. Taken singly, it seems that this compound does not affect animals or insects [86], but studies demonstrate that it can increase the toxicity of deoxynivalenol. CUL inhibits the glycosylation of DON, which produces less toxic compounds [86,87].

8. Management Strategies to Reduce Infection

Control methods to reduce or mitigate production and quality loss in maize caused by *F. graminearum* can be divided into two broad categories: direct methods that prevent the spread of fungus and infection via the use of synthetic or biological fungicides and/or insecticides, or indirect methods that include the reduction in plant stress or increasing the production of secondary metabolites to prevent the fungal infections via techniques like cropping practices and hybrid selection (Figure 4).

These control methods may not always be allowed or viable in different areas, as climatic conditions may pose limitations, and different policies in different countries may prohibit or encourage the use of some methods. Fungicide and insecticide applications are not always available, mostly due to country regulations. Cropping systems that reduce the fungus inoculum, like a crop rotation [43,89], are often not employed, despite the evident advantages that this cropping method can bring [90].

9. Control with Synthetic Fungicides

Worldwide regulations on the use of fungicides on corn can differ a lot, and they can change over the years. While certain countries, such as those in the EU, do not have any registered products specifically

designed to control Gibberella ear rot, other regions in the world, particularly in South and North America, have witnessed a growing trend in the use of foliar fungicides over the past two decades. Different commercial products are available for Gibberella ear rot management in corn [91,92]. The most used active ingredients for the control of GER are prothioconazole and quinone outside inhibitors (QoI). Prothioconazole is a demethylation-inhibiting (DMI) fungicide that interferes with the biosynthesis of ergosterol, a precursor of vitamin D2 and a crucial component of fungal cell walls [93,94]. Quinone outside inhibitors (QoI) are a group of compounds, such as strobilurins, which are active against the protein complex that produces ATP in the fungal cell's membrane, leading to cell death. In particular, QoIs inhibit the transfer of electrons between cytochrome b and cytochrome c1 by the binding of the outer quinol oxidation site (Qo site) [95]. Both groups of fungicides are already used to control Fusarium head blight in wheat with different efficacies, where prothioconazole and other triazoles have a better control effect compared to strobilurins [96–99], but in corn, different studies reported that even though these compounds can control symptoms, there are contrasting results for the reduction in mycotoxin levels [98,100,101]. The biggest differences were found in the timing of application of DMI fungicides. The most efficient time of application is at flowering (VT-R2), because most of the available products for this class of fungicide are not fully systemic, and the active ingredient is not able to move from the uptake site to the newly grown tissue [101,102]. Also, DMI fungicides are more efficient in wheat compared to corn, and this could be caused by the husks covering the corn ear, preventing full penetration by the DMI, while the pathogen bypasses this protection by entering via the silks [101]. The availability of only two classes of fungicide may cause the quick development of resistance to these active ingredients in fungal strains placed under strong selective pressure in the field. Studies have already demonstrated the presence of resistance to these fungicides in species like *Cercospora beticola*, *Mycosphaerella graminicola*, *Blumeria graminis,* and others [94,95,102,103].

10. Insecticide against a Vector

The regulations and laws regarding insecticides can vary significantly between coun- tries. However, unlike fungicides used against the pathogen, a range of insecticides are employed to combat Lepidoptera, such as *Ostrinia nubilalis* (Hübner) or *O. furnacalis* (Guenée), which feed on maize. These insecticides primarily belong to the following classes: pyrethroids, organophosphates, carbamates, and anthranilic diamides. Since, as previ- ously stated, fungicide treatments can have a different degree of success regarding the accumulation of mycotoxins, it is often more effective to reduce the damage caused by these fungi by focusing on controlling the insect pests that can facilitate the infection [104]. Pyrethroids are synthetic insecticides derived from pyrethrin, a natural insecticide active against both adults and larvae [46].

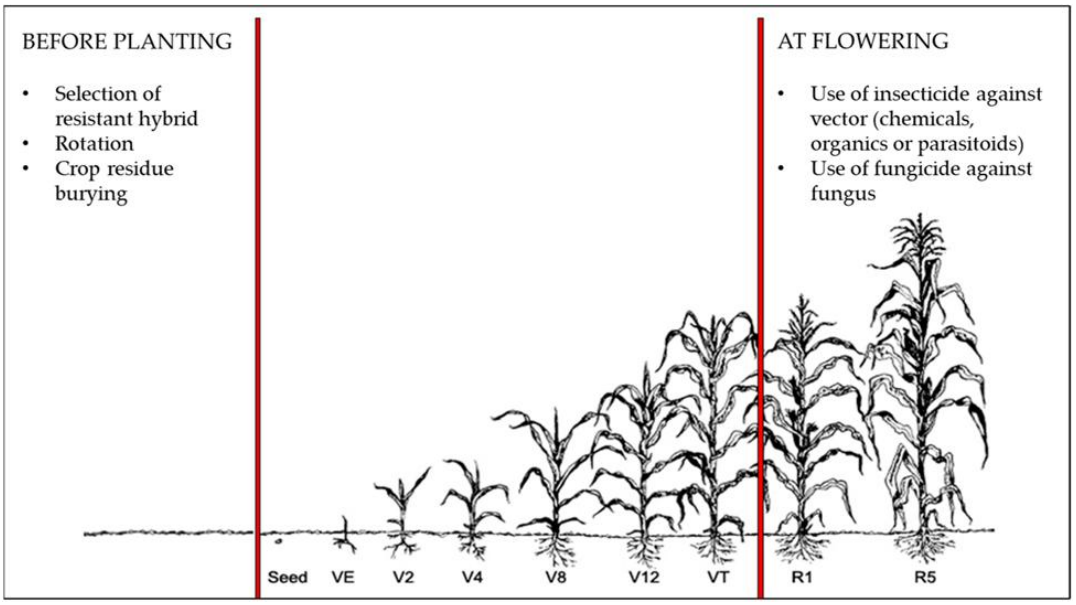

Figure 4. Controls methods in corn cultivation to reduce the impact of Gibberella ear rot (GER). Modified from Lancashire [88].

Their mode of action is described as preventing the closure of voltage-sensitive sodium channels, causing inactivation of nerves and leading to complete paralysis [105].

Organophosphates inhibit the action of acetylcholinesterase and are also effective against adults and larvae. By preventing the degradation of acetylcholine, a neurotransmitter, these compounds keep synapses in a hyperexcited state, resulting in paralysis [106,107]. Carbamates are another class of compounds that are active against the enzyme acetylcholinesterase, and therefore act in a very similar manner to organophos- phates [105]. The fourth group of insecticide compounds active against lepidopteran larvae are anthranilic diamides. These compounds cause paralysis of the insect via a different mechanism, affecting the calcium reserves in muscular cells by deregulation of the channels associated with the ryanodine receptor (RyR) [108,109]. The use of an insecticide active against lepidopteran larvae is one of the most important practices to reduce fungal infection and mycotoxin production, especially in countries where fungicides are not available or in a country where GMOs are not permitted [44,110]. For insects, like fungi, resistance to active substances is a reality and is promoted by incorrect insecticide management or uninterrupted usage of insecticides with the same mode of action [111].

11. Biological Control

The use of synthetic fungicides or insecticides is not the only method to control *F. graminearum* infection. According to reports from Reference [112], various approaches that have been successfully utilized and commercially implemented for other crops and against different fungi have been tested for controlling *F. graminearum* infection.

They include the use of plant-associated or endophytic micro-organisms, plant growth-promoting bacteria, nontoxigenic fungal strains, and plant-derived products, which have been tested in recent decades and have proven effective in controlling both symptoms and mycotoxin production. However, no resulting commercial products are yet available on the market at the current date. Another important approach toward successful biological control is targeting *O. nubilalis*. In countries where GMOs that express Cry toxin-related genes from *Bacillus thuringiensis* Berliner (Bt) are permitted, the efficacy of control against ECB and mycotoxin level has proven to be effective [113,114]. In other countries where GMOs are forbidden, there is the possibility of using isolated BT toxins as insecticides [115]. These Cry toxins form pores in the guts of the insects, at first stopping the feeding of the insect and ultimately leading to its death, usually by septicemia. There are different Cry toxins with specificity for different insect groups but, as for other types of insecticides, resistance mechanisms can be developed against this toxin. Resistance in ECB was found to be caused by a mutation in a gut protease, preventing the conversion of the toxin crystalline form into active, monomeric molecules [116]. ECB biological control can also be achieved with the use of parasitoid insects. *Trichogramma* spp. (Hymenoptera), egg parasitoids, are one of the most-used parasitoids to control ECB [117]. In recent decades, different release and distribution methods have been tested, and today, with the introduction of unmanned aerial vehicles in agriculture, the efficacy and feasibility of the use of parasitoid insects have been facilitated [118]. *Trichogramma* spp. is also considered an important factor in the management of BT toxin-resistant insects [119].

12. Cropping Methods

Agronomic practices are fundamental to achieving the highest production in a given environment. They are also one of the most important control methods to reduce the impact of different corn diseases. The most common practices used to control diseases are tillage, crop rotation, optimization of plant density and sowing date, harvest time, and all the agronomic strategies to reduce stress during the whole life cycle of the crop, like irrigation and fertilization. Crop residue management is one of the most important methods to reduce the source of inoculum, since this fungus overwinters in maize stalks and other cereal debris [37,38]. A crop rotation with non-host species is a common strategy for the management of *Fusarium graminearum* in wheat [43,89,120]. In corn, it has been demonstrated that a succession of susceptible species increases the infection rate and the symptoms of this disease [28,121,122]. The positive effects of crop rotations are also related to tillage methods. Conventional tillage associated with plowing is effective in the control of this pathogen in comparison with reduced or no tillage. The burying of crop residues accelerates their decomposition, and the subsequent underground microbial activities are effective in the reduction of inoculum density [28,122].

Of course, the mineralization of debris primarily depends on various agronomic conditions and geographical locations worldwide [123]. Planting date is another important factor in the control of different fungal diseases, including GER.

Late planting is associated with higher fungal presence; this brings a synchronization of flowering and ECB presence, resulting in greater insect damage and, consequently, higher infection rates [124,125]. Another factor that can affect *F. graminearum* infection is plant density. Higher densities are associated with higher grain contamination. Correct planting density for every cultivated area cannot be easily established because it is affected by the environment, including both persistent and seasonal factors, and different corn genetics that can be more or less suited for a high planting density [126]. In conclusion, different agronomic methods can be applied to reduce the effect of this disease, but the use of resistant hybrids is the most important method to have led to better production in terms of quantity and quality.

13. Hybrid Selection

The use of a resistant variety that does not present symptoms is considered the best practice to reduce GER infection. However, at present, the market can only offer hybrids with a varied range of tolerance, from the ones that present few symptoms to the ones that are severely infected. Even if, during the selection of new maize cultivars, the very susceptible ones are discarded, it's not uncommon to find farmers' fields with infection rates that are above the legal limit in terms of GER-correlated mycotoxins [127]. This is probably due to the fact that GER resistance is a complex quantitative trait, and the actual resistance is influenced by the genotype × environment interaction [128]. However, several studies have reported both dominant and additive genetic effects correlated with GER resistance [129–131]. Different ear defense mechanisms against fungal infection have been reported. These mechanisms are associated with silk resistance and resistance to the spread of the fungus among the kernels. It is important to note that these two mechanisms are under separate genetic control [27,132]. Kernel resistance is associated with fast drying [133], while silk resistance is associated with faster silk abscission and larger abscission zones [134]. Another type of resistance is associated with the production of defense chemical compounds like maysin and other phenolic compounds associated with antifungal activities. Maysin is a flavone glycoside active in the suppression of insects such as *Helicoverpa zea* (Boddie) and others in maize like *Sitophilus zeamais* (Motschulsky), *Euschistus servus* (Say), and *Nezara viridula* (L.) [135]. The reduction in insect damage is correlated with a reduction in fungal infection [136,137]. Phenolic compounds are produced in corn as a response to fungal infection. It seems that a more resistant variety produces more of this type of compound compared to the susceptible ones.

These compounds can also oxidate to produce quinones with an even greater antifungal effect [138,139]. Other important compounds effective against fungal infection are carotenoids. In corn, zeaxanthin has been demonstrated to be effective in the inhibition of DON production due to its effect on the DON biosynthetic pathway [140]. Physical defenses are another type of resistance, and the two major characteristics in maize correlated with the reduction in GER damage are husk tightness and ear attitude. Tight husk germplasms are correlated with a higher GER susceptibility, probably because a favorable microenvironment to fungal proliferation develops inside the ear after heavy rain [141–143]. Another ear characteristic associated with resistance to ear rot is the attitude: a pendant ear attitude is correlated to lower susceptibility to ear rot [127]. To understand the genetic aspects that are behind the phenotypical characteristics associated with GER resistance, various studies have been conducted in recent decades (Table 2).

A study conducted in Canada that evaluated 144 F2s derived from a cross between one resistant inbred line and a susceptible one found that there was no overlap in the 11 QTLs associated with silk resistance and the 18 QTLs for kernel resistance. Out of the 11 QTLs for silk resistance, 4 were located in chromosome 1, 4 QTLs were on chromosome 7, 2 QTLs were on chromosome 3, and 1 QTL was on chromosome 6. For the QTLs associated with kernel resistance, five QTLs were located on chromosome 7; three QTLs were each on chromosomes 1, 2, and 5; and one QTL was on chromosomes 3, 4, 6, and 9 [132].

Table 2. QTLs associated with GER resistance found on different corn materials.

Numbers of Materials	Materials Type	Location	QTL Found	Reference
500	European flint maize landraces	Germany	8	[144]
244	European dent lines and European flint lines	Germany	8	[145]
204	Chinese recombinant inbred line	China	23	[146]
144	Cross between resistant and susceptible Canadian lines	Canada	29	[132]
759	Cross between resistant Brazilian inbred and susceptible European flint inbred	Germany and Brazil	4	[147]
3	F2 population from resistant and susceptible Chinese inbred	China	17	[148]
298	Population from resistant and susceptible Argentinian inbred	Argentina	4	[149]

In another study of the difference between QTLs associated with GER resistance in the dent and flint materials, similarities in the Manhattan plot for GER resistance and DON accumulation were found, suggesting a possible correlation with fungal resistance and DON concentration [145]. In this study, markers associated with DON resistance for the dent and flint were found in different chromosomes.

For the dent materials, the two SNPs were found on chromosomes 2 and 5, and for the flint materials, the six SNPs were located on chromosomes 1, 3, 7, and 9 (Table 3).

Table 3. SNP markers associated with DON resistance [145].

Marker	Chr.	Bin	Position (bp)	Effect
Dent DON				
SYNGENTA1701	2	2.02	6,474,735	0.26
PZE-105154147	5	5.06	204,425,692	0.4
Flint DON				
SYN11494	1	1.01	3,708,114	0.54
PZE-101242721	1	1.11	289,238,830	0.4
PZE-103000307	3	3.00	1,233,964	0.34
PZE-107039304	7	7.02	75,985,070	0.68
PZE-109079433	9	9.05	127,490,556	0.42
SYN26913	9	9.06	147,467,181	0.45

Other studies with different types of materials like European landraces [144], Chi- nese inbreds [146,148], Argentinian genotypes [149], and crosses between European and Brazilian inbreds [147] found various QTLs associated with GER resistance in almost all the chromosomes, with considerable difference in the position and number of markers found (Table 4).

Table 4. Position and effect of different SNPs studied in four different studies. pG (%): additive effects and proportion of explained genotypic variance.

Marker/Position	Chr.	Bin	Coordinate (cM)	Range (cM)	Additive Effect	pG (%)	Reference
ZmSYNBREED_24070_673	2	-	49.00	-	5.00	15.04	[144]
ZmSYNBREED_29737_831	2	-	119.54	-	4.56	1.28	
ZmSYNBREED_30537_486	2	-	162.00	-	−3.33	2.84	
ZmSYNBREED_44869_210	4	-	162.93	-	3.27	4.35	
ZmSYNBREED_47633_944	5	-	78.30	-	3.41	3.27	
ZmSYNBREED_53695_527	6	-	31.15	-	−3.52	6.04	
ZmSYNBREED_55609_889	6	-	91.78	-	−3.14	0.46	
ZmSYNBREED_70955_321	9	-	110.30	-	−4.11	3.53	
qGER1.04	1	1.04	-	122.30–146.21	−0.26	8.85	[146]
qGER2.10	2	2.10	-	270.88–279.05	0.08	1.07	
qGER3.02	3	3.02	-	41.8–70.39	−0.31	7.75	
qGER3.06	3	3.06	-	208.96–223.41	0.13	4.92	
qGER4.05	4	4.05	-	101.37–133.51	0.11	5.24	
qGER4.09	4	4.09	-	251.87–286.56	−0.47	9.05	
qGER7.03	7	7.03	-	162.71–170.86	0.18	3.86	
qGER8.05	8	8.05	-	172.23–194.04	−0.15	6.9	
qGER9.06	9	9.06	-	124.87–146.66	−0.24	4.01	
qGER10.06	10	10.06	-	139.61–149.5	0.04	1.98	
qGER10.07	10	10.07	-	198.11–211.71	0.03	3.08	
T3 x A6_A7 q1	1	1.02	60.54	58.89–62.92	−0.96	10.17	[147]
T3 x A6_A7 q2	3	3.08	196.72	194.99–197.03	−1.33	14.86	
T3 x A6_A7 q3	5	5.06	162.53	161.56–162.71	−0.43	5.37	
T4 x A4_A5 q1	1	1.02	58.64	50.40–85.62	0.35	10.92	
T4 x A4_A5 q4	8	8.05	120.04	119.75–120.56	0.35	11.67	

T3 x A8 q1	1	1.02	60	59.93–61.04	−0.34	21.84	
qRger7.1	7	7.02	-	121.10–151.20	0.62	20.16	
qRger10.1	10	10.01–10.03	-	22.80–60.70	−0.42	10.18	
qRger2.1	2	2.01–2.02	-	6.10–33.70	0.36	7.27	
qRger2.2	2	2.02–2.03	-	33.70–75.80	−0.11	23.79	
qRger4.1	4	4.01–4.02	-	0.00–28.20	0.43	8.55	
qRger6.2	6	6.05–6.06	-	74.00–109.50	−0.65	10.47	
qRger7.2	7	7.01–7.02	-	35.60–62.00	0.43	14.09	
qRger9.1	9	9.01	-	6.00–29.30	0.38	5.97	[148]
qRger1.1	1	1.03	-	52.60–76.10	0.63	15.09	
qRger2.3	2	2.04–2.07	-	87.90–116.80	0.58	9.97	
qRger3.1	3	3.08–3.09	-	170.70–196.80	−0.41	7.25	
qRger4.2	4	4.04–4.05	-	64.00–88.00	0.76	13.55	
qRger4.3	4	4.05–4.07	-	83.20–108.80	0.53	12.03	
qRger5.1	5	5.04–5.05	-	93.00–123.3	0.6	10.62	
qRger6.1	6	6.00–6.01	-	2.90–26.10	−0.51	8.4	
qRger7.3	7	7.03–7.04	-	131.80–161.00	0.4	4.81	
qRger9.2	9	9.02–9.05	-	70.00–94.20	−0.51	10.37	

This can only confirm the nature of quantitative traits of GER resistance. Despite the challenges faced, a study aimed at identifying genes linked to this resistance was carried out, leading to the discovery of four genes located on chromosome 2 that showed a correlation with kernel resistance [150].

14. Conclusions

Interactions between *Fusarium graminearum* and corn are complex, and a great number of factors can contribute to the development of infection or resistance of the corn plant. Differences between cropping seasons seem to have a great impact on the damage caused by this disease [34,35]. Cropping methods are another important factor, but in this case, useful actions to control GER can be difficult to implement, due to economic sustainability, like a rotation, or on the contrary, environmentally sustainable methods like no tillage can increase the impact of this disease [28,89]. In the end, the use of insecticides to control the vector or fungicide to directly control the fungus may not be economically convenient and can cause the development of resistant populations [102,103,111]. The use of genetically modified organisms can be useful to control the vector, but they are not available every- where [113,114]. Biological methods to control this fungus are still in development, and no commercial products are available [112].

In conclusion, the selection of resistant hybrids is one of the most important and viable control methods. Hybrids with greater resistance will permit a reduction in the use of pesticides and, therefore, make the development of resistant pests less likely (both fungi and insects). The use of modern breeding technologies like genome prediction and marker-assisted selection can improve the development of more resistant materials in the framework of more sustainable agriculture.

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ORIGINAL ARTICLE

The role of husk traits in maize susceptibility to *Fusarium verticillioides*: A multi-location study in northern Italy

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Abstract

Fusarium disease and the consequent mycotoxin accumulation pose significant problem in maize cultivation, with fumonisins produced by *Fusarium verticil- lioides* posing a global health concern. To address this issue, a range of preventive measures (e.g. crop management techniques) can be implemented to minimize fungal infections. A promising strategy to counteract this issue involves the se- lection of genotypes with greater resistance to fungal pathogens. This approach has the potential to reduce the reliance on chemical inputs for controlling fun- gus growth or indirect infection vectors. Leveraging genetic approaches can help improve the economic sustainability of agriculture in the face of climate change challenges. In the present work, we assessed the importance of two husk leaf traits (coverage and number), their association with *F. verticillioides* infection, fumoni- sin content, and their potential influence on crop yield. The study was conducted in three locations in the North of Italy and 38 hybrids with varying resistance to *F. Verticillioides* were compared. The results obtained showed that husk cover- age has a pivotal role not only in protecting maize ears from *Fusarium* infection but have also a significant impact on crop yield: a significant positive correlation was found between husk coverage and yield in all three locations (r = 0.33185; r = 0.51327 and r = 0.51207, respectively). Furthermore, in the field of Vicenza, a significant negative correlation was found between husk coverage and Fusarium severity ($r = -0.41492$). Husk coverage emerges as an important trait that merits inclusion in maize breeding programs, given its protective role against fungal in- fections and its favourable influence on both yield and grain quality.

KEYWORDS

climate change, corn breeding, ear coverage, environmental sustainability, fumonisin, mycotoxin

1 INTRODUCTION

Maize (*Zea mays* L.) is one of the most important cereals worldwide due to its different usage that range from feed, food to industrial use, but also as model plant for scientific research. Fungi in the genus *Fusarium* are very common pathogens of maize and *Fusarium verticillioides* (Sacc.) Nirenberg (syn. *F. moniliforme* Sheldon, teleomorph *G. fujikuroi* (Sawada) Wr.) is one of them. It can infect both stalk and ear and, in some cases, also roots (Bennett et al., 2023). This fungus has two parasitizing phases, the first on a living host and the second on dead tissue (Ma et al., 2013). This necrotrophic phase gives this fungus the possibility to outwinter on crop residue and infect the following crop (Dorn et al., 2011). Infections localized on maize ear are known as Fusarium ear rot disease (FER) (Miedaner et al., 2020).

This disease can reduce yield from 10% to 50% and it is also associated with the production of harmful mycotoxins (Li et al., 2010; Rocha et al., 2016). In particular, ear infection can occur through silk and wounds caused by insect or hail (Duncan & Howard, 2010; Gai et al., 2018).

Ostrinia nubilalis (or European corn borer, ECB) is the most common insect associated with corn ear wounds in Europe and North America. It plays a fundamental role in promoting *F. verticillioides* infections, and in the sub- sequent fumonisin contamination of maize kernels in temperate regions (Blandino et al., 2015). Larvae feeding activities cause ear tunnelling and kernel wounds that can favour infection (Alma et al., 2005). In temperate areas, two generations of ECB occur per year: the first-generation larvae usually cause leaf damage when maize is at mid-late vegetative stage, while the second generation develops by feeding on the stalk or ears. The feeding activity of the second generation can cause plant break- down if the attack is concentrated under the ear, while ear tunneling is linked to mycotoxin development (Blandino et al., 2008).

Mycotoxins are secondary metabolites produced by fungi during the infection and their contamination of maize kernels represents a global threat to safety both for humans and animals (Balázs & Schepers, 2007).

Fumonisins are the most important toxins produced by *Fusarium verticillioides*, and also by *F. proliferatum*, *F. Dlamini*, *F. globosum*, *F. oxysporum*, *F. temperatum*, *F. nygamai*, *F. subglutinans*, *F. thapsinum* (Ekwomadu et al., 2020; Jestoi, 2008; Marín et al., 2004; Munkvold et al., 2019; Streit et al., 2013; Zhou et al., 2018).

These toxins are divided into four classes: A, B, C and P, of which B is dominant. Considering B fumonisins, FB1 is the most abundant, but in most of the cases the co-presence of FB2 and FB3 is reported (Peter Mshelia et al., 2020; Uwineza et al., 2022; Waśkiewicz et al., 2012). The exposure to fumonisins in animals can cause different diseases like pulmonary oedema in pigs, rat liver cancer, and leukoencephalomalacia in horses (Marasas, 2001); in mammals, it can affect liver and kidneys. In humans, the exposure to fumonisins determines teratogenic and immunotoxic effects, neural tube defects and oesophageal cancer (Dragan, 2001; Missmer et al., 2006; Stockmann- Juvala & Savolainen, 2008). Due to this harmful effect, FB1 and FB2 have been classified in group 2B as 'possibly carcinogenic to humans' by the IARC (Ostry et al., 2017). Furthermore, EFSA defined a 'Tolerable Daily Intake' value for the sum of FB1, FB2, FB3, FB4 at 1.0 μg/kg of body weight (Knutsen et al., 2018).

In maize, different methods can be implemented to reduce the infection and damage caused by *F. verticillioides*: effective field management and irrigation are crucial to create an environment unfavourable to the fungus (Ariño et al., 2009).

For instance, crop rotation plays a pivotal role in insect management, since monoculture practices have been found to elevate mycotoxin contamination in maize grains (Krnjaja et al., 2019). Another key factor is tillage and crop residue management, but their importance is still debated (Battisti et al., 2022). Many authors reported that tillage practices did not significantly affect the incidence of fumonisins in maize (Herrera et al., 2023; Marocco et al., 2008), while other studies showed that fields with previous crop stovers exhibited a greater presence of *F. verticillioides* in comparison to fields without stovers (Rossi et al., 2009; Tran et al., 2021). Also, water management is considered a key factor in controlling the fungal infection, and drought stress has been associated with higher mycotoxin production (Marín et al., 2010). In this context, climate change predictions suggest an elevated risk of mycotoxin contamination in European maize as a result of long period of drought and rising temperatures (Herrera et al., 2023). Irrigation method is also crucial and overhead irrigation, which keeps the silks excessively wet, is associated with higher infection compared to flood irrigation (Herrera et al., 2023).

While all these methods contribute to controlling at- tacks by *F. verticillioides*, relying solely on agronomic approachs is insufficient. The agronomic control should be complemented with the use of less susceptible hyrids. In the last decades, the selection of maize gen- otypes more resistant to fungal pathogens has been a significant challenge that could potentially reduce the reliance on the chemical inputs used in agriculture. The production of such chemical inputs, including pesticides and fungicides, requires substantial energy resources. By reducing the need for these chemicals through the cultivation of more resistant hybrids, we can lower energy consumption in the agricultural sector, contributing to overall energy efficiency and a smaller carbon footprint. Many authors reported that breeding for FER resistance is considered the environmentally safest and most economical approach (Eller et al., 2008; Lanubile et al., 2017; Munkvold, 2003).

Ear morphology seems to be one of the most important traits associated to FER resistance, and husk coverage is a key phenotypical trait (Morales et al., 2019). Husk leaves have a significant impact on the susceptibility of maize hybrids to *Fusarium*, as they act as the protective outer layer for the ear.

Husk leaves are important for different reasons: (i) they contribute to the production thanks to their photosynthetic activity (Cui et al., 2020); (ii) they prevent ear dehydration and keep the right moisture for the kernel growth (Cui et al., 2016; Sweeney et al., 1994; Wang et al., 2012); (iii) protection of the ear from pathogen infection, birds and pests attack (Barry et al., 1986; Warfield, 1996).

In this context, the aim of the present work was to assess the importance of two husk leaf traits (coverage and number), their association with *F. verticillioides* infection, fumonisin content, and their potential influence on crop yield.

The study was conducted in three locations in the North of Italy and 38 hybrids with varying resistance to *F. verticillioides* were compared.

2 | MATERIALS AND METHODS

2.1 | Genetic materials

The genetic material used in this study was obtained crossing the hybrid PR33A46 (FAO 500, Pioneer Hi- Bred) and an experimental inbred line obtained in our Department (FAO 700), and after many cycles of selection different inbred lines were obtained. This work was carried out in the experimental field of the University of Milan located in Landriano (PV) Italy (N45°18′, E9°15′). Crossing these inbred lines, several combinations of hybrids belonging to different FAO classes (hybrid 1–9, FAO 500; hybrid 10–30, FAO 600; hybrid 31–38, FAO 700) have been tested in three different provinces in the North of Italy (Table 1).

2.2 | Experimental design and agronomic analysis

The experiment was carried out in 2023 in three different provinces in the North of Italy: Bergamo (BG), Cremona (CR) and Vicenza (VI). These three locations are important spots for maize cultivation in the North of Italy and are frequently used by breeding companies to select less susceptible genotypes to *F. verticillioides* due to the different environment. In fact, these three locations were selected due to their different environmental conditions. In Bergamo location in the last 5 years the aver- age total rain in the period that goes from the planting to the harvest is higher compared to the other two locations (Cremona e Vicenza). For these other locations, the difference is mostly in the maximum temperature that in Cremona is higher compared to Vicenza if we consider the average of maximum temperature per days in the last 5 year (2018–2022). In Vicenza is also raining less com- pared to Cremona considering the data of the last 5 years (Figure S1). In the 2023 season, the local weather conditions of these locations are reported in Figure S2. The seeds of each genotype were sown the 5th of April with a plot seeder (0.70 × 0.20 m). The experiment was laid out in a single plot of 10 m^2 (5 × 2 m) and the three different locations chosen for this study were considered as three biological replicates.

TABLE 1 Identification number and FAO classes of the 38 hybrids tested in this study.

Hybrid	FAO	Hybrid	FAO
1	500	20	600
2	500	21	600
3	500	22	600
4	500	23	600
5	500	24	600
6	500	25	600
7	500	26	600
8	500	27	600
9	500	28	600
10	600	29	600
11	600	30	600
12	600	31	700
13	600	32	700
14	600	33	700
15	600	34	700
16	600	35	700
17	600	36	700
18	600	37	700
19	600	38	700

The experimental fields were in maize–maize rotation with standard soil fertilization (about 220 kg/ha of nitrogen). The maize plants were grown by conventional farming methods (pre-emergence herbicide was applied) and irrigation was applied as needed to avoid drought stress.

FIGURE 1 The trait husk coverage was measured with a semi-quantitative scale from 0 to 10. Representative images for scale points 0, 2, 4, 6, 8 and 10.

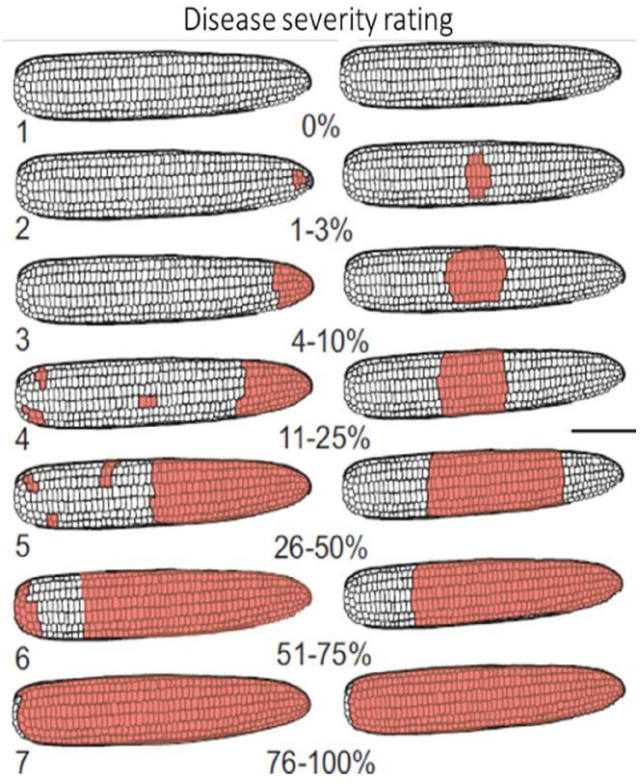

FIGURE 2 Disease severity rating of *Fusarium verticillioides*: % of surface affected by the pathogen. Assessment through a scale from 0 to 7 (modified from Reid et al., 1996).

For each plot, which corresponds to a different hybrid, the following parameters were collected on ten representative ears randomly selected:

- Husk number: counted on manually harvested ears;
- Husk coverage: semi-quantitative parameter measured using a scale from 0 to 10 (Figure 1);
- F. verticillioides severity: evaluated on ten ears by visual scoring on a scale from 1 to 7 (Disease Severity Rating, DSR), where 1 = 0%, 2 = 1–3%, 3 = 4–10%, 4 = 11–25%, 5 = 26–50%, 6 = 51–75%, 7 = 76–100% of visibly infected kernels/ear (Figure 2).

The ears were harvested by hand the second week of September, relative humidity (RU) was measured and then seeds were immediately dried to 14% of RU. The following parameters were collected:

- Relative grain humidity: measured at harvest;
- Yield estimate: the material collected per each plot was weighed and subsequently compared to the field scale, thus providing an indication of the production (t/ha).

2.3 | Measurement of total fumonisin

Ten ears of each hybrid were shelled, and the seeds obtained were mixed to create a single bulk. Bulked seeds were milled by using an electric mill (Golia 4 V, Novital Italy) to obtain a coarse grinding. Flour samples were obtained using a ball mill (Retsch MM200, Retsch GmbH Germany), grinding for 5 min at 21 oscillations s^{-1} frequency, to a final size <20 mesh.
Total fumonisin concentration in maize grains was detected according to the Celer® FUMO ELISA Test Kit HU0040032 (Eurofins Tecna, Italy).

2.4 | Statistical analysis

Statistical analysis of experimental data was performed using SPSS software (IBM SPSS Statistic 20) and PAST program (Paleontological Statistics, version 4.12). The results are presented as least square means' standard deviation. Statistically significant differences are considered for $p < 0.05$.

3 | RESULTS

3.1 | Agronomic parameters, *F. verticillioides* severity and fumonisin accumulation

In this work, 38 hybrids were cultivated in three locations to study the correlation between different parameters: number of husks, husk coverage, *F. verticillioides* severity, fumonisin concentration (ppm), grain relative humidity (%) and yield estimate (t/ha). As reported in the Materials and Methods section, we set up different plots of 10 m^2, and each plot corresponds to a different hybrid. The three different locations were considered as three biological replicates and the local weather conditions are reported in Figure S1. The parameters collected in Location 1 are reported in Table S1. In general, there was high variability in measurements among different hybrids: the number of husks ranged from an average of 6.10 (hybrid 34) to 10.50 (hybrid 4), while husk coverage ranged from 2.40 (hybrid 3) to 7.20 (hybrid 32). Fusarium severity was evaluated on a scale from 1 to 7: in Location 1 the average was always below 2.5, and fumonisin content was below the contamination threshold for human consumption (4 ppm). As in Location 1, also in Location 2 there was high variability in husk number and coverage, and *Fusarium* severity was limited. Unlike Location 1, the fumonisin content was greater than 4 ppm in 13 out 38 hybrids, while yield ranged from 8.20 t/ha (hybrid 20) to 14.12 t/ha (hybrid 17; Table S2).

In Location 3, a similar trend to previous locations was observed for no. husk, husk coverage and *Fusarium* (Table S3). The parameter that changed the most was the fumonisin content: it was over 4 ppm in 18 out 38 hybrids and was between 3 and 4 ppm in other five accessions. Relative humidity ranged from 18 to 25%, while yield exceeded 16 t/ha in hybrid no. 10, 16 and 27 (Table S3).

Table 2 represents the mean of the parameters measured in the three locations with the relative standard deviation.

Starting from husk coverage, this value ranged from a minimum of 2.10 ± 0.53 (hybrid no. 24) to 7.47 ± 0.31 in the most covered ear (hybrid no. 32).

Considering only these two accessions, husk coverage was: (i) inversely proportional to total fumonisin content (5.06 ppm in hybrid 24 and 0.66 ppm in hybrid 32); (ii) directly proportional to yield, with a value of 11.22 ± 0.69 t/ha in hybrid 24 and 14.02 ± 1.60 t/ha in hybrid 32. The other parameters will be examined in the following paragraphs.

3.2 | Correlation and multivariate analysis in three locations

A correlation analysis was performed on all the parameters collected: no. husk, husk coverage, Fusarium se- verity, total fumonisin content, relative humidity, and yield estimate. In Figure 3, negative correlations were represented in red, while positive correlations in blue. Grey boxes highlighted three statistically significant positive correlations ($p < 0.05$). The strongest correlation was found between husk coverage and yield estimate ($r = 0.5804$; $p = 0.00013$; Figure 4a), suggesting an important role of this trait on maize productivity. Additionally, husk coverage showed a positive correlation with humidity ($r = 0.3968$; $p = 0.01362$; Figure 4b) and, as expected, another positive correlation was found between *Fusarium* severity and fumonisin content ($r = 0.3407$; $p = 0.03628$; Figure 4c): a higher *Fusarium* infection corresponds to a higher quantity of fumonisins on harvested maize grains.

Furthermore, a Principal Components Analysis (PCA) was carried out on the same parameters of Figure 3: no. husk, husk coverage, Fusarium severity, total fumonisin content, relative humidity, and yield estimate. The main determinants of the clustering were highlighted in green, as shown in Figure 5. In the clustering analysis, by imposing *k* means equal to 2, the two clusters were indicated in red and yellow. It was observed that all hybrids exhibiting husk coverage below a threshold of four fell within the first cluster (marked as red circles), whereas those with a value exceeding a threshold of 6 were categorized within the second cluster (yellow circles). Focusing solely on the parameter husk coverage, the average value among hybrids in the first cluster was 4.085 ± 0.885, whereas in the second cluster was 5.462 ± 0.882.

3.3 | Correlation and multivariate analysis in Vicenza

Considering only the location most affected by *F. verticillioides* (Vicenza), two statistically significant correlations were founded. The positive correlation between husk coverage and yield was statistically significant ($r = 0.51207$; $p = 0.0010146$) and the regression plot is shown in Figure 6a. In the same location, an interesting negative correlation between husk coverage and *Fusarium* was found ($r = -0.41492$; $p = 0.0095913$): a higher husk coverage seems to reduce *Fusarium* infection (Figure 6b).

TABLE 2 Summary of agronomic parameters collected in three locations on 38 hybrids: number of husks, husk coverage, *Fusarium verticillioides* severity, fumonisin concentration (ppm), grain relative humidity (%) and yield estimate (t/ha).

Hybrid	FAO	No. husk	Husk coverage	Fusarium severity	Fumonisin (ppm)	Humidity (%)	Yield (t/ha)
1	500	9.53 ± 0.91^{a}	5.67 ± 0.86^{abcdefg}	1.22 ± 0.20^{a}	1.18 ± 0.75^{a}	21.73 ± 0.71^{abc}	14.45 ± 2.12^{a}
2	500	8.23 ± 0.31^{abcd}	5.67 ± 0.87^{abcdefg}	1.58 ± 0.10^{a}	1.41 ± 1.21^{a}	21.67 ± 1.47^{abc}	13.34 ± 2.18^{a}
3	500	7.33 ± 0.55^{bcd}	3.47 ± 1.85^{efgh}	2.37 ± 1.13^{a}	2.71 ± 2.39^{a}	19.70 ± 1.64^{c}	14.37 ± 2.12^{a}
4	500	9.27 ± 1.20^{ab}	4.30 ± 0.20^{bcdefgh}	2.12 ± 0.50^{a}	3.78 ± 3.25^{a}	21.20 ± 0.85^{bc}	13.99 ± 1.90^{a}
5	500	7.50 ± 0.61^{abcd}	4.37 ± 0.95^{bcdefgh}	2.43 ± 0.38^{a}	2.74 ± 1.91^{a}	21.70 ± 1.14^{abc}	13.46 ± 1.37^{a}
6	500	9.57 ± 0.64^{a}	4.43 ± 0.31^{bcdefgh}	1.65 ± 0.38^{a}	5.42 ± 2.92^{a}	23.57 ± 1.10^{abc}	14.38 ± 2.50^{a}
7	500	7.83 ± 0.42^{abcd}	5.40 ± 0.46^{abcdefg}	1.30 ± 0.26^{a}	4.83 ± 4.35^{a}	22.93 ± 1.42^{abc}	13.88 ± 3.09^{a}
8	500	8.97 ± 1.00^{abc}	5.27 ± 0.35^{abcdefg}	2.22 ± 0.43^{a}	3.12 ± 1.40^{a}	24.97 ± 2.41^{abc}	14.67 ± 2.97^{a}
9	500	7.37 ± 0.51^{bcd}	4.47 ± 1.06^{bcdefgh}	1.63 ± 0.49^{a}	2.30 ± 1.67^{a}	21.47 ± 3.93^{abc}	13.46 ± 1.87^{a}
10	600	7.97 ± 0.55^{abcd}	4.40 ± 0.35^{bcdefgh}	1.85 ± 0.48^{a}	2.48 ± 1.29^{a}	24.17 ± 1.63^{abc}	15.11 ± 1.71^{a}
11	600	7.97 ± 0.35^{abcd}	5.83 ± 0.45^{abcdef}	1.53 ± 0.36^{a}	3.46 ± 2.00^{a}	26.50 ± 2.76^{ab}	14.64 ± 1.35^{a}
12	600	7.30 ± 0.52^{bcd}	6.17 ± 0.31^{abc}	2.28 ± 0.39^{a}	4.13 ± 2.20^{a}	26.53 ± 1.62^{ab}	14.43 ± 1.51^{a}
13	600	7.67 ± 0.91^{abcd}	5.10 ± 0.66^{abcdefg}	2.25 ± 0.35^{a}	4.46 ± 3.67^{a}	26.20 ± 2.05^{abc}	13.87 ± 1.38^{a}
14	600	7.20 ± 0.75^{bcd}	6.73 ± 0.91^{ab}	2.02 ± 0.59^{a}	1.84 ± 0.91^{a}	26.57 ± 2.46^{ab}	14.82 ± 1.66^{a}
15	600	7.90 ± 0.56^{abcd}	4.47 ± 0.38^{bcdefgh}	1.88 ± 0.78^{a}	2.87 ± 2.48^{a}	24.53 ± 1.07^{abc}	14.49 ± 0.90^{a}
16	600	7.63 ± 0.35^{abcd}	6.67 ± 0.64^{ab}	1.50 ± 0.40^{a}	2.89 ± 2.99^{a}	25.67 ± 2.10^{abc}	15.22 ± 2.18^{a}
17	600	8.83 ± 0.21^{abc}	5.13 ± 0.76^{abcdefg}	1.57 ± 0.31^{a}	4.51 ± 3.97^{a}	23.77 ± 1.94^{abc}	14.70 ± 0.83^{a}

18	600	8.30 ± 0.69^{abc}	5.57 ± 0.58^{abcdefg}	1.83 ± 0.16^{a}	2.34 ± 1.55^{a}	25.20 ± 1.35^{abc}	16.00 ± 2.41^{a}
19	600	9.03 ± 0.93^{ab}	3.63 ± 0.35^{defgh}	1.85 ± 0.26^{a}	3.90 ± 3.23^{a}	21.93 ± 1.97^{abc}	11.08 ± 1.21^{a}
20	600	8.57 ± 0.32^{abc}	3.17 ± 0.91^{gh}	1.57 ± 0.33^{a}	1.12 ± 0.71^{a}	24.10 ± 2.52^{abc}	10.97 ± 2.41^{a}
21	600	7.93 ± 0.40^{abcd}	6.13 ± 0.71^{abcd}	1.75 ± 0.68^{a}	1.72 ± 1.36^{a}	26.97 ± 3.82^{ab}	14.79 ± 1.70^{a}
22	600	8.20 ± 0.36^{abcd}	3.77 ± 0.15^{cdefgh}	1.97 ± 0.47^{a}	2.33 ± 1.36^{a}	22.93 ± 1.29^{abc}	15.78 ± 2.45^{a}
23	600	6.87 ± 0.38^{cd}	3.70 ± 0.70^{cdefgh}	2.10 ± 0.05^{a}	2.18 ± 1.78^{a}	23.17 ± 1.53^{abc}	13.84 ± 1.27^{a}
24	600	8.70 ± 0.40^{abc}	2.10 ± 0.53^{h}	2.10 ± 0.22^{a}	5.06 ± 4.29^{a}	24.30 ± 1.56^{abc}	11.22 ± 0.69^{a}
25	600	8.60 ± 0.36^{abc}	5.20 ± 0.79^{abcdefg}	2.05 ± 0.36^{a}	5.28 ± 3.18^{a}	27.93 ± 2.84^{a}	14.06 ± 2.45^{a}
26	600	8.37 ± 0.32^{abc}	4.03 ± 0.55^{cdefgh}	1.78 ± 0.08^{a}	4.48 ± 2.37^{a}	25.50 ± 1.73^{abc}	12.87 ± 1.65^{a}
27	600	7.60 ± 0.10^{abcd}	5.97 ± 1.47^{abcde}	1.60 ± 0.05^{a}	4.81 ± 4.47^{a}	24.10 ± 1.35^{abc}	15.05 ± 1.89^{a}
28	600	7.53 ± 0.58^{abcd}	4.63 ± 0.32^{bcdefg}	1.52 ± 0.13^{a}	3.20 ± 1.06^{a}	24.60 ± 2.01^{abc}	13.81 ± 1.55^{a}
29	600	8.67 ± 0.57^{abc}	5.47 ± 0.25^{abcdefg}	1.58 ± 0.38^{a}	4.34 ± 4.46^{a}	25.60 ± 1.66^{abc}	14.84 ± 0.75^{a}
30	600	8.30 ± 1.06^{abc}	4.93 ± 1.67^{bcdefg}	1.50 ± 0.18^{a}	1.24 ± 0.72^{a}	22.43 ± 2.54^{abc}	12.57 ± 1.89^{a}
31	700	7.93 ± 0.59^{abcd}	3.63 ± 0.06^{defgh}	1.58 ± 0.53^{a}	1.07 ± 0.84^{a}	25.57 ± 3.52^{abc}	13.23 ± 1.99^{a}
32	700	7.43 ± 0.06^{abcd}	7.47 ± 0.31^{a}	1.43 ± 0.23^{a}	0.66 ± 0.32^{a}	24.83 ± 1.61^{abc}	14.02 ± 1.60^{a}
33	700	7.73 ± 0.42^{abcd}	4.00 ± 0.89^{cdefgh}	1.52 ± 0.39^{a}	2.31 ± 1.78^{a}	23.17 ± 1.70^{abc}	10.55 ± 1.09
34	700	6.23 ± 0.23^{d}	5.03 ± 0.15^{bcdefg}	1.23 ± 0.20^{a}	1.99 ± 2.64^{a}	22.83 ± 1.46^{abc}	14.26 ± 1.92^{a}
35	700	7.33 ± 1.12^{abcd}	3.80 ± 1.22^{cdefgh}	1.43 ± 0.32^{a}	0.87 ± 0.39^{a}	25.23 ± 1.37^{abc}	13.04 ± 2.50^{a}
36	700	9.03 ± 0.23^{abc}	5.17 ± 1.10^{abcdefg}	1.30 ± 0.23^{a}	1.74 ± 1.10^{a}	26.00 ± 1.57^{abc}	13.69 ± 1.69^{a}
37	700	7.47 ± 1.07^{abcd}	3.43 ± 0.21^{fgh}	1.38 ± 0.14^{a}	1.72 ± 2.30^{a}	23.17 ± 2.20^{abc}	12.90 ± 1.96^{a}
38	700	7.57 ± 0.60^{abcd}	4.40 ± 0.69^{bcdefgh}	1.50 ± 0.42^{a}	2.28 ± 1.56^{a}	23.67 ± 1.70^{abc}	12.60 ± 2.35^{a}

Note: For each parameter, different letters indicate statistically significant differences (Tukey's test, $p < 0.05$).

4 | DISCUSSION

Maize is one of the most vulnerable crops to fungal infections. Nowadays, *Fusarium* disease and the resulting accumulation of fumonisins pose a global safety hazard for human and animal health (Awuchi et al., 2021; De Ruyck et al., 2015; Magarini et al., 2023).

The methods used in the prevention of mycotoxin contamination include cropping techniques, crop residue management, disease control through chemicals or biocontrol, irrigation, and harvest timing (Battisti et al., 2022; Herrera et al., 2023; Krnjaja et al., 2019; Marín et al., 2010; Marocco et al., 2008; Rossi et al., 2009; Tran et al., 2021). However, the best method for controlling *F. verticillioides* is the selection of more resistant genotypes, even if completely immune genotypes are not available (Lanubile et al., 2017; Stagnati et al., 2019). The selection of tolerant genotypes could potentially reduce the reliance on chemical inputs. Also, many authors reported that the use of genotypes with pigmented pericarp appears promising for mitigating *Fusarium* infection (Landoni et al., 2020; Pilu et al., 2011; Sangiorgio et al., 2021; Venturini et al., 2016). Considering maize morphology, husk leaves have a significant impact on the susceptibility of maize to *Fusarium*. In this work we assessed the importance of husk leaves (husk coverage and number), their association with *F. verticillioides* infection, fumonisin content, and their potential influence on crop yield. Considering the average in the three locations, we highlighted a significant positive correlation between husk coverage and yield (Figure 4a). As reported by Ige and colleagues, husk coverage plays a crucial role in protecting ears from external attacks or infections (Ige et al., 2017).

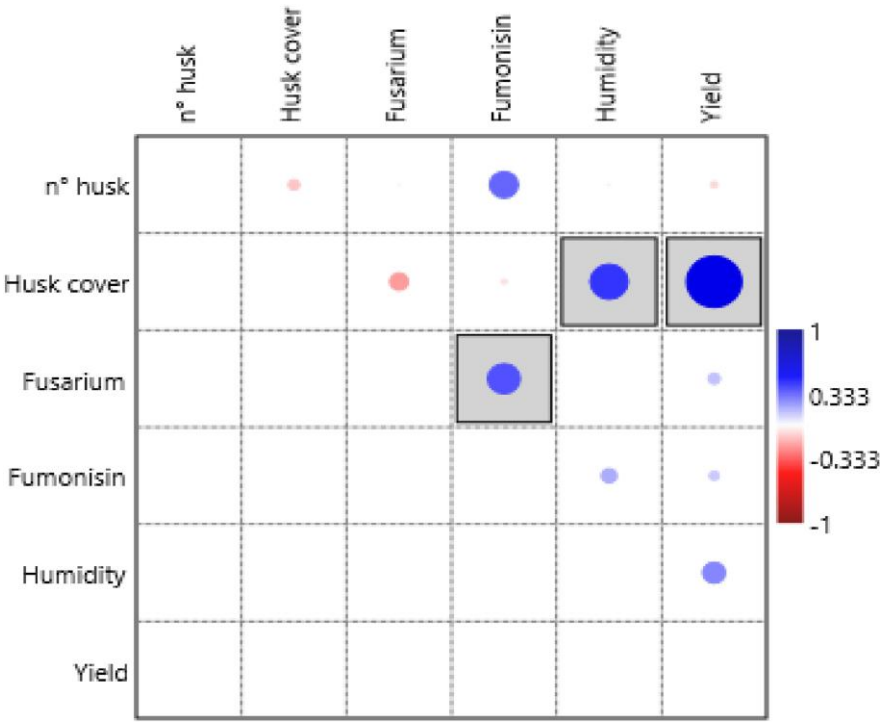

FIGURE 3 Pearson correlation plot. Negative correlations are represented in red (from 0 to −1), positive correlations in blue (from 0 to 1). The size of the circle is directly proportional to the strength of the correlation. Grey box on statistically significant correlations $p < 0.05$: husk cover/yield ($r = 0.5804$; $p = 0.00013$); husk cover/humidity ($r = 0.3968$; $p = 0.01362$); *Fusarium*/fumonisin ($r = 0.3407$; $p = 0.03628$).

In particular, many authors reported that husk tightness and husk length are important to protect silk and ear tip from fungal spore, indicating a correlation with infection resistance. Moreover, husk thickness plays an important role in reducing insect damage, as it represents a physical barrier against ear damage (Cui et al., 2020; Parsons & Munkvold, 2010). Pengelly and co-workers also re- ported that the outer husks surrounding the ear operate a C_4-like photosynthetic pathway (Pengelly et al., 2011). Hence, considering all these key functions performed by husks, we could hypothesize that this trait has a strong influence on crop yield, as confirmed by other studies that reported a positive correlation between husk leaves and grain yield (Cantrell & Geadelmann, 1981; Golam et al., 2011; Oka et al., 1997).

However, in this study it was not possible to establish which hybrid was the most productive, as the differences in yield were not statistically significant (Table 2).

Instead, significant differences were recorded in husk number, as reported in Table 2. The detected range of values, averaging from 6.23 to 9.57, underscores its nature as a quantitative trait (Zhang et al., 2022). Despite such variability, husk number did not correlate with the other parameters under evaluation (Figure 3). High variability was also observed in grain humidity at harvest, with values ranging from approximately 19 to 28% (Table 2).

In this context, a statistically significant positive correlation was found between husk coverage and grain humidity ($r = 0.3968$), highlighting the crucial role of husks in protecting ears from dehydration (Figures 3 and 4b). On the other hand, a significant negative correlation ($r = -0.41492$) was found between husk coverage and Fusarium severity in the most affected field in Vicenza (Figure 6b).

Regarding the number of husks, the other parameter related to husk leaves, it did not present a significant correlation with any of the other parameters analysed. We found this correlation considering only the Vicenza field probably because to the environmental condition in the 2023 season. In fact Vicenza location was the most affected by *F. verticillioides* infection.

Here, the GDD Accumulation is much lower (1750 GDD) than in the other two locations (both about 2250 GDD; Figure S2).

In fact, in Vicenza the temperature often exceeds 35°C, creating an environment less favourable to the development of the fungus (Figure S1). Many studies reported that the most critical stage is the silking period and sporulation is limited over 34°C, while it is absent at 45°C (Campa et al., 2005; Rossi et al., 2009).

Considering these results, it emerged that husk coverage holds greater significance compared to husk number. Indeed, ear coverage appears to play a crucial role, not only in protecting corn ears from the entry of potential pathogens but also in exerting a significant in- fluence on crop yield.

The genetic basis behind husk development is com- plex and not well understood, and only a few studies have investigated potential quantitative trait loci (QTL) and candidate genes associated with this trait. In one of the pioneering investigations by Widstrom and col- leagues (2003), four QTLs associated with husk tight- ness were identified on chromosomes 1S, 1L, 3L and 7L. The marker on chromosome 3L explained 12.7% of the variation, while the contribution of other individual markers on phenotypic variance was all below 10%. More studies were conducted on husk morphology: husk length, husk width and husk numbers were ana- lysed by many authors, and different QTLs were found in maize. The first GWAS for husk number and husk weight was performed by Zhou et al. (2016) using 3K SNP markers and identified a total of 24 and 29 SNPs, respectively associated with husk number and husk weight. In subsequent years, research studies employed higher marker density and discovered additional QTLs linked to these morphological traits (Cui et al., 2016, 2018, 2020).

However, the small effect of each QTL revealed the complex nature of these traits, suggesting that their regulation is controlled by multiple genes (Zhang et al., 2022). Recently, genotyping by sequencing (GBS) has emerged as the preferred method for constructing linkage maps in maize, facilitating the mapping of more complex traits (Wang et al., 2020). In a recent work, an ultra-high-density linkage map was constructed using GBS from a RIL population whose parents differ significantly in husk traits. The map was created to assess the genetic variance and heritability of three husk traits (husk length, width, and number) in three field environments and in the combined environment. Twenty-six QTLs and many candidate genes associated with these three husk traits were detected; only two QTLs, qHL6 and qHN4, were identified across all environments and represented major-effect QTLs for husk length and husk number, respectively (Zhang et al., 2022). These findings and the potential of new technologies are playing a pivotal role in enhancing our comprehension of the genetic basis and molecular mechanisms responsible for husk-related traits.

While there has been some progress with QTLs related to husk morphology thanks to GWAS and high-density linkage maps, the trait husk coverage remains relatively less investigated compared to husk number and other parameters related to husk morphology, as it is a semi- quantitative parameter. However, we consider husk coverage to be a crucial trait that merits more importance in maize breeding programs, given its protective role against fungal infections and its favourable influence on both yield and grain quality. Considering the results obtained in this work, future studies should further investigate this trait and attempt to identify the genes in- volved in its regulation, to select maize varieties with enhanced resistance to fungi.

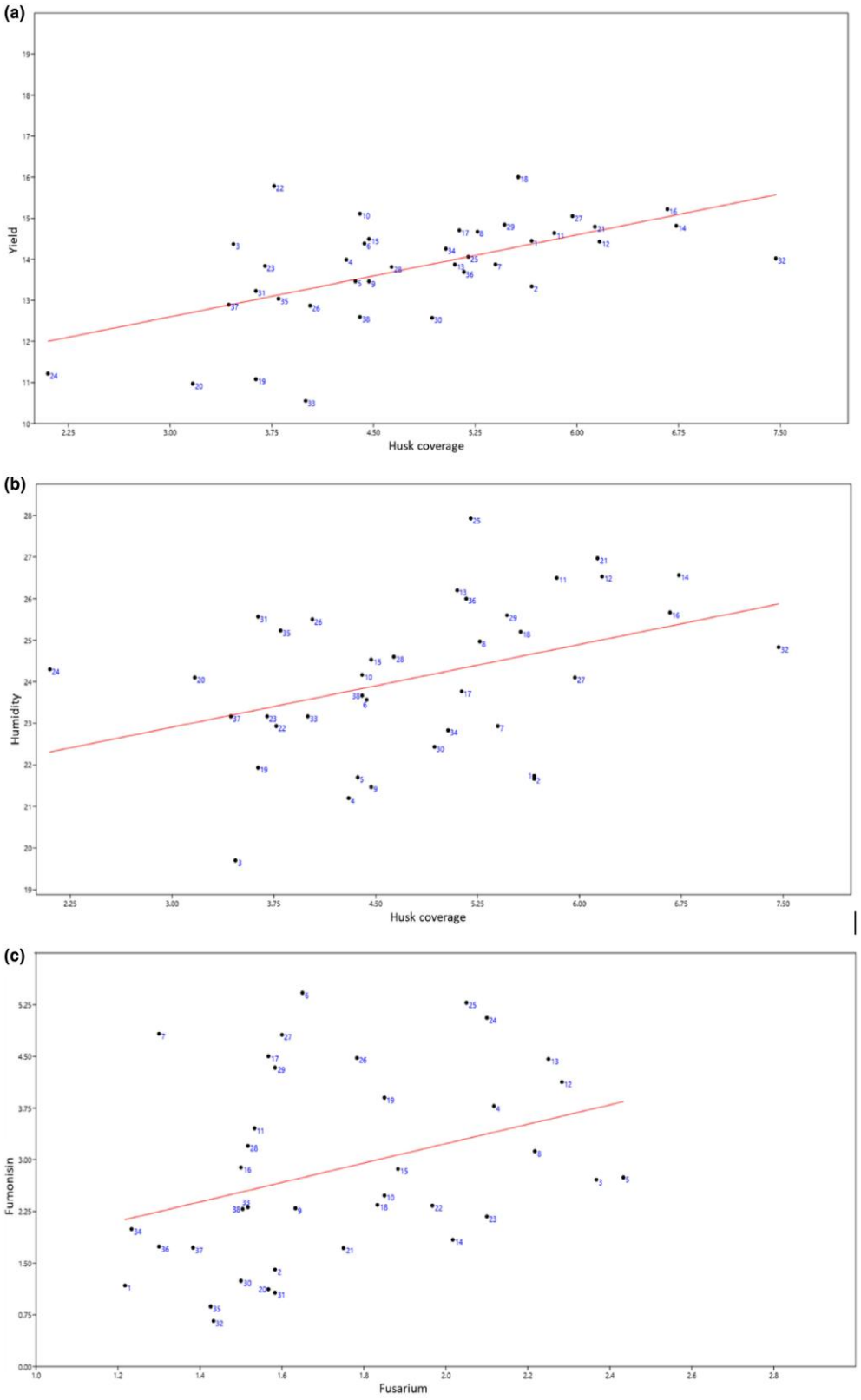

FIGURE 4 Regression plots considering the data collected in the three locations. (a) Regression plot between the husk coverage and yield ($r = 0.58041$; $p = 0.00013349$). (b) Regression plot between the husk coverage and humidity ($r = 0.39688$; $p = 0.013623$). (c) Regression plot between the *Fusarium* and fumonisin content ($r = 0.34078$; $p = 0.036289$). In red the regression line.

The potential role of husk coverage in protecting maize ears is intriguing, and further exploration of this relationship could contribute to more effective strategies for combating this detrimental disease.

In conclusion, the shift towards more sustainable farming practices not only will enhance environmental protection but will also help reduce energy consumption and carbon emissions. Maize farmers can benefit economically from cultivating more resistant hybrids, as they may experience higher yields and incur lower costs associated with chemical inputs. This can enhance the economic sustainability of agriculture in the face of climate change challenges. However, the broader context of disease management should not be overlooked, as a multifaceted approach is likely required to address this complex agricultural challenge successfully.

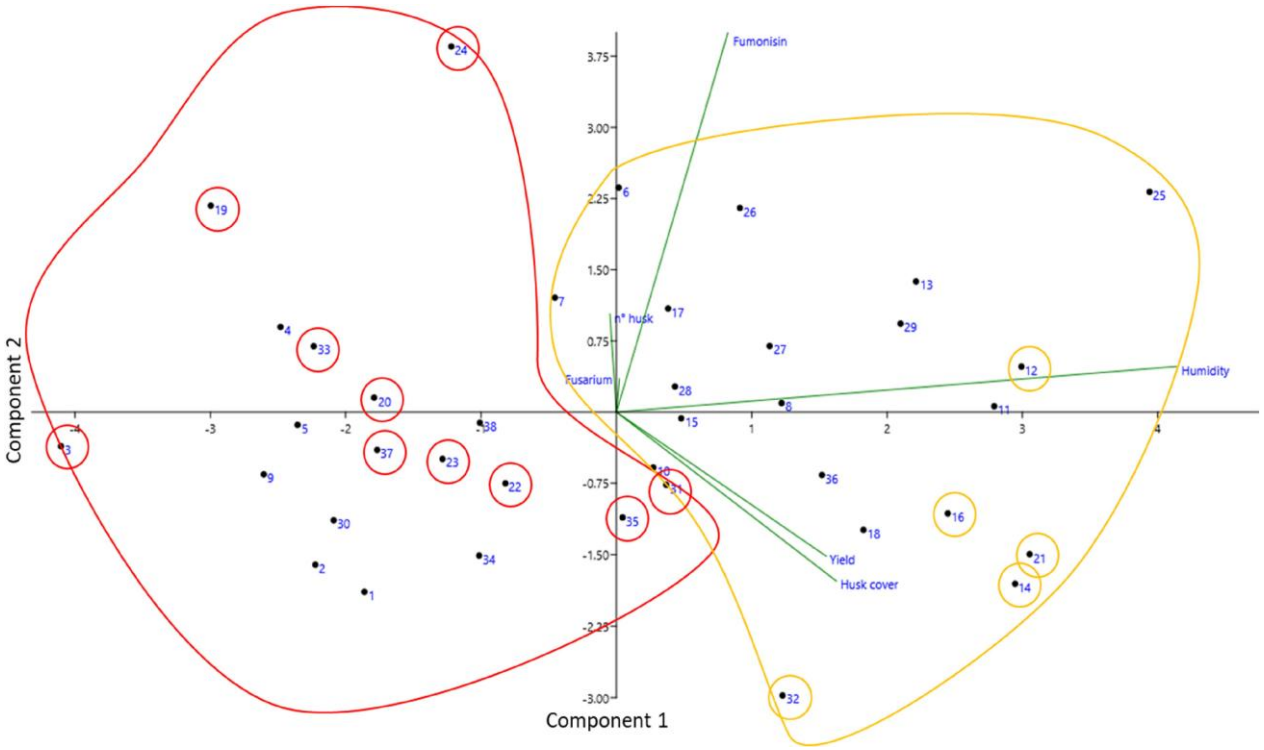

FIGURE 5 Principal components analysis (PCA) obtained using the following parameters: no. husk, husk coverage, Fusarium severity, total fumonisin content, relative humidity, and yield estimate. In green the main determinants of the clustering. In the clustering analysis by imposing *k* means equal to 2, the two clusters are indicated in red and yellow. Red circles for hybrids with husk coverage below 4; yellow circles for hybrids with husk coverage above 6.

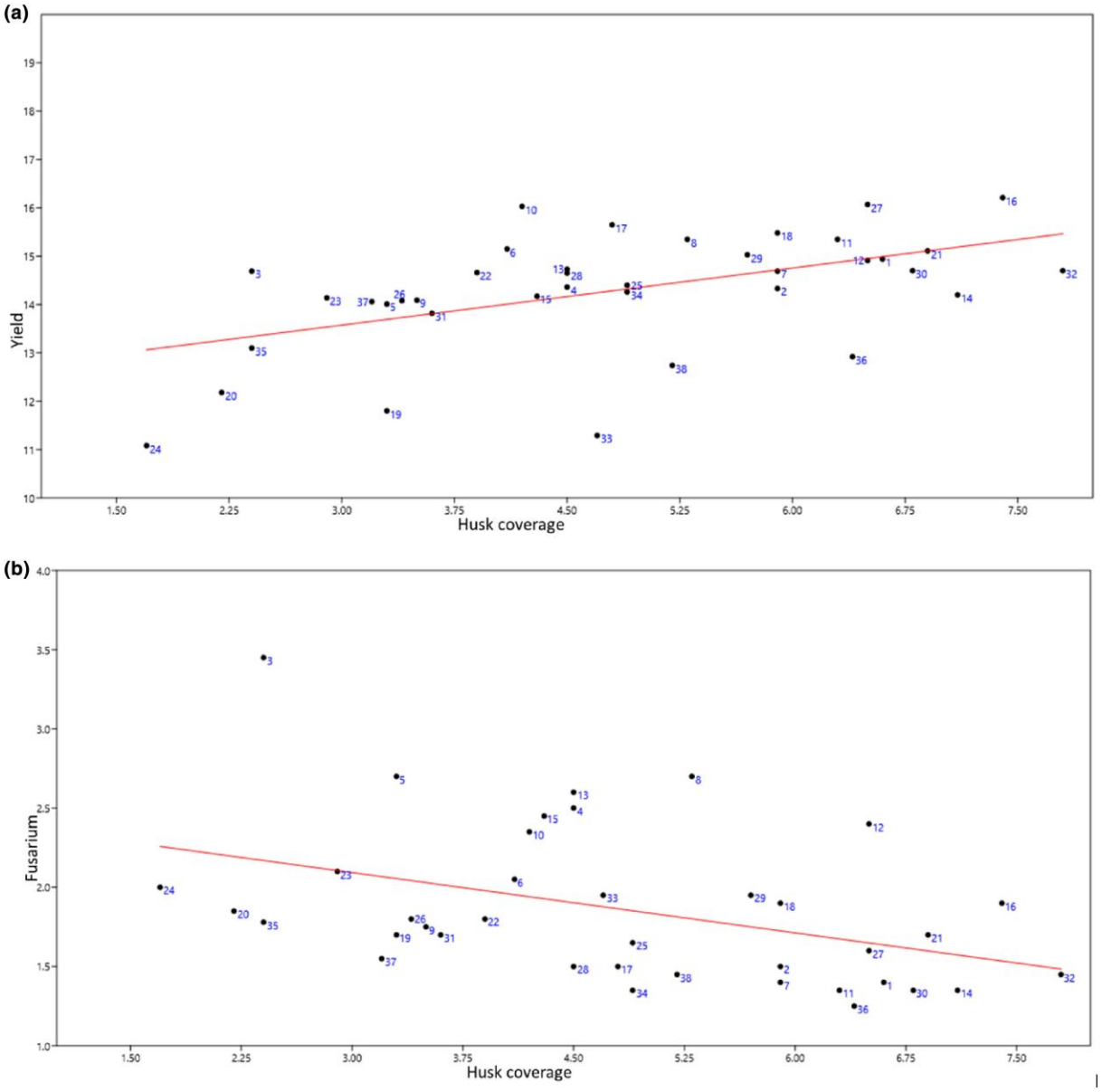

FIGURE 6 Regression plots considering the data collected in Vicenza. **(**a) Regression plot between the husk coverage and yield ($r = 0.51207$; $p = 0.0010146$). (b) Regression plot between the husk coverage and Fusarium ($r = -0.41492$; $p = 0.0095913$). In red the regression line.

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CONFLICT OF INTEREST STATEMENT

The authors have declared no conflict of interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are avail- able from the corresponding author upon reasonable request.

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Article

Quantitative Trait Loci Analysis of Maize Husk Characteristics Associated with Gibberella Ear Rot Resistance

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Abstract: Maize (*Zea mays* L.) is a vital crop susceptible to *Gibberella* ear rot (GER), a disease caused by *Fusarium graminearum*, resulting in significant yield losses and mycotoxin production. This study aimed to investigate the correlation between ear characteristics and GER resistance in 74 maize inbred lines (42 with non-stiff stalks and 32 stiff stalks) adapted to the northern Italian environment. Mycotoxin analysis was performed to assess the presence of deoxynivalenol (DON) and zearalenone (ZEA). The results showed a positive correlation between the husk traits, like the husk number and husk cover, and GER resistance in both heterotic groups. A positive correlation was also found between the DON and ZEA concentrations. In addition, we conducted a genome-wide association study (GWAS) which identified novel quantitative trait loci (QTLs) associated with the husk number, husk cover, ear attitude, and infection score. These QTLs can be utilized in marker-assisted selection for breeding new GER-resistant maize varieties. Our study provides valuable insights into the genetic basis of ear traits and their relationship with GER resistance, which can contribute to an improvement in the environmental and economical sustainability of the corn growing system.

Keywords: maize; *Fusarium graminearum*; mycotoxin; GWAS; QTL; husk; ear characteristics

1. Introduction

Maize (*Zea mays* L.) is a major cereal crop which can grow in different areas with different climates. It is an important nutritive source used as food and feed. In recent years, maize use for industry and energy production has gained importance [1,2]. In research, it is used as a model species due to its genotypic and phenotypic diversity. This shows how versatile this crop is and its importance in a wide range of markets. Like other crops, a large number of pests and diseases can affect maize production. Even though different management methods exist to reduce their effects, diseases are still a concern, causing yield losses and reducing grain quality [3,4]. Fungi are the primary pests responsible for maize diseases [5], and the genus *Fusarium* is one of the most serious types. In this genus, different species are known to infect maize, and two of the most important ones are *Fusarium verticilloides* and *Fusarium graminearum*.

Fusarium verticilloides (Sacc.) Nirenberg (syn. *F. moniliforme* Sheldon, teleomorph *G. fujikuroi* (Sawada) Wr.) can infect different parts of the plant, like the stalk, ear, and roots [6], and is the causative agent of *Fusarium* ear rot (FER), an impactful disease which can reduce yields by 10–50% and is also associated with the production of fumonisin, a harmful mycotoxin which can cause disease in both animals and humans [7–9]. The interaction between *F. verticilloides* and *F. graminearum* is complex, and the presence of one can reduce the effect of the other, sometimes leading to difficulties in distinguishing between the two species [10].

Fusarium graminearum (Schw.) (*Ascomycota*) is a fungus of the genus *Fusarium* diffused around the world which can infect different plant species like maize, barley, wheat, oats, rice, and rye [11,12]. This fungus is sometimes reported with its teleomorph name of *Gibberella zeae* (Schwabe Petch). Due to the complex biology of this pathogen, it is more common to refer to it as the *Fusarium graminearum* species complex (FGSC), the cause of different diseases in cereals [11]. It can grow in a temperature range of 15–29 ◦C. If the temperature is higher than 30 ◦C, then its development is rather limited, while on the contrary, it can grow at temperatures below 15 ◦C [13–15]. In maize, it is known as the causative agent of *Gibberella* ear rot (GER), a key disease in temperate areas. In particular, infection can occur through silk or kernel wounds caused by insect feeding, weather damage, or mechanical injury [16–18]. *Ostrinia nubilalis* (or European corn borer (ECB)) is one of the most important insects associated with GER infection in maize. Larval feeding activities create wounds and tunnels which can favor *F. graminearum* infection [19]. Yield loss associated with this disease is estimated to range from 12% to 48%, with alternating years of higher impact and years without infection [20–22].

Mycotoxin production is associated with yield loss in maize infected by *F. gramin- earum* [23,24]. Infected ears can develop various types of mycotoxins, the most important of which are deoxynivalenol (DON) and zearalenone (ZEA) [24,25]. Mycotoxins are secondary metabolites of fungi which are harmful to animals and humans [15,26]. Deoxynivalenol causes gastrointestinal irritation (feed refusal and vomiting), altered immune function, and decreased milk and meat production, while zearalenone has estrogenic-like effects which can cause fertility and reproductive problems [27,28].

Due to this risk associated with mycotoxin intake for both animals and humans, the European Union set maximum amounts and guidance levels for a wide range of mycotoxins in grain and its derived products [29].

To reduce the impact of this disease, different methods can be used. Agronomic practices like crop residue management and crop rotation are the most important techniques for reducing sources of inoculum. This fungus can overwinter in cereal debris, and a tillage system like ploughing, which permits faster residue degradation, is effective in the control of this pathogen [18,30,31]. Crop rotation with susceptible species has shown an increase in infection and symptoms of this disease, demonstrating the importance of growing a succession of non-host species as an effective control method [31,32]. Another method used to mitigate GER effects is the use of insecticide against ECB. In countries where fungicides against *F. graminearum* are registered, control with these products is widely used and is increasing. GMO Bt technology is also effective against ECB, but due to the fact that this type of genetic control Bt technology is forbidden in Italy and other European Union countries [33,34], genetic selection of resistant hybrids of maize with a classic breeding strategy plays the crucial role in the struggle against GER [35–39].

In the last few decades, the different genetics behind GER resistance have been ex- plored, and different quantitative trait loci (QTLs) were found across the 2.5 billion base pairs in all 10 maize chromosomes [40–46]. All of these studied QTLs offer an opportunity to breeders to apply genomics-assisted selection techniques. These techniques can help with the improvement in *Gibberella* ear rot resistance of new maize varieties, but due to the complex nature of this disease and the influence of the environment the application of these loci in breeding programs is still difficult. This quantitative nature of this trait and the complex interaction between plant and pathogen create obstacles to the full implementation of genomics-assisted selection techniques in breeding programs for this character [47,48].

In this context, the aim of this work was to understand the correlation between inbred corn ear characteristics and *Gibberella* ear rot resistance. In addition, through a genome-wide association study (GWAS), this study highlights the genetic basis of these characteristics with the identification of chromosomal regions associated with resistance to this disease, providing results useful for the selection of new resistant varieties, starting with inbred lines already adapted to the Italian environment.

2. Materials and Methods

2.1. Genetic Materials and Experimental Design

In the present study, 74 inbred lines (42 non-stiff stalk and 32 stiff stalk) were selected according to *Gibberella* ear rot resistance and their adaptation to the northern Italian environment (Table S1). The experiment was carried out during the 2022 and 2023 seasons in 3 different locations in northern Italy: Cremona (Location 1), Torino (Location 2), and Cuneo (Location 3). Sowing was performed with a plot planter (0.75 m × 0.14 m spacing) in a randomized complete block with two replications for each location. Each location was designed for a single-row plot experiment, and each plot was about 3 sq m (0.75 m × 4 m). The sowing, flowering, and harvesting dates of the three sites for both years are shown in Table 1.

Table 1. Planting, flowering of 50% of plots, and harvesting dates of the three locations for the 2022 and 2023 seasons.

Season	Location	Planting Date	Harvest Date	Flowering of 50% of Plots
2022	Location 1	11 May 2022	28 August 2022	18 July 2022
	Location 2	16 May 2022	19 October 2022	24 July 2022
	Location 3	26 May 2022	24 October 2022	2 August 2022
2023	Location 1	24 May 2023	2 October 2023	28 July 2023
	Location 2	21 June 2023	14 November 2023	22 August 2023
	Location 3	27 June 2023	22 November 2023	30 August 2023

The experimental fields were grown with conventional farming methods in a maize– maize succession with standard soil fertilization (about 220 kg/ha of nitrogen).

Pre- emergence herbicide was applied, and no insecticides were used. Flood irrigation was also applied every 15 days in all locations to avoid drought.

The following traits were evaluated in each plot: (1) the silk flowering date, or the date when 50% of the ears in the plot were producing silks; (2) the husk number, which was counted from manually harvested ears (average); (3) the husk coverage score, a semi- quantitative parameter measured using a scale from 0 to 9; (4) the ear attitude score, a visual evaluation of 5 ears on a scale from 1 to 9, where 1 = >90% of the ears being upright, 5 = 50% of the ears being upright, and 9 = >90% of the ears being pendant; and (5) the *Fusarium graminearum* infection score, a visual evaluation of 5 ears scored on a scale from 1 to 9 (disease severity rating (DSR)) where 1 = >75% of the ear pile being infected and 9 means no infection. All traits were collected in both replications, and data were collected from the five central ears (marked before data collection) of the plot. Data analysis was performed using SPSS software (IBM SPSS Statistic 20) and Paleon- tological Statistics (PAST, version 4.12).

2.2. *Mycotoxin Analysis*

At harvest, five ears per plot were collected with husks, and after data collection, they were dried to reach a relative humidity of 13%. After manual shelling and the creation of a bulk, the kernels were analyzed for mycotoxin contamination to assess the concentration of deoxynivalenol (DON) and zearalenone (ZEN). The bulk seeds were milled in a Pulverisette 19 electric mill (Fritsch GmbH, Idar- Oberstein, Germany) to reach a final particle size of approximately 4 mm. Five grams of flour for each sample were prepared for the mycotoxin analysis using liquid chromatography- tandem mass spectrometry. Both mycotoxins were extracted from the same sample us- ing 30 mL of a solution of acetonitrile/water/acetic acid (73:25:2 *v*/*v*). Extraction was performed by mechanical shaking for 30 min at 2300 rpm in a multitube vortex mixer (Benchmark Scientific, Sayreville NJ, USA). The extracts were filtered through a 0.2 µm polytetrafluoroethylene (PTFE) filter (Phenomenex, Torrance, CA, USA), and an aliquot of 1.8 mL was transferred to an HPLC vial and analyzed.

Analysis was carried out with a Vanquish Core HPLC (Thermo Fisher Scientific Inc., Waltham, MA, USA) in a Luna Omega Polar C18 100 × 2.1 mm column (Phenomenex, USA) coupled to an Orbitrap Explore 120 detector (Thermo Fisher Scientific Inc., USA). The total chromatographic run was 12.5 min at a flow rate of 400 µL/min. The column temperature was maintained at 45 ◦C, and the injection volume was 2.5 µL. The autosampler was operated at 10 ◦C. Data acquisition was performed using Xcalibur software (version 4.3). The system was equipped with an electrospray ionization (ESI) interface, and nitrogen was used as the drying and collision gas.

The ion source parameters were positive ion spray voltage of 3700 V, negative ion spray voltage of 2300 V, sheath gas flow rate of 55 Arb, auxiliary gas flow rate of 15 Arb, an ion transfer tube temperature of 325 ◦C, and a vaporizer temperature of 350 ◦C.

2.3. *Genotyping Data*

Genomic DNA extraction was performed on one kernel per inbred line. All 74 mate- rials were analyzed using an Infinium XT BeadChip platform (Illumina, San Diego, CA, USA). Genotyping was based on 24,000 authenticated SNPs distributed across all 10 maize chromosomes derived from the B73 reference sequence. This high-density genotyping was performed at the Corteva Agriscience LLC Johnston Laboratory using the protocol developed by the Illumina Company.

2.4. *QTL Detection*

Identification of the QTL signals was performed with a developed Corteva Agriscience model based on previously published works [49–51].

Signal detection was performed with a two-step analysis model. The first step was a genome-wide association study (GWAS). Markers were tested by fitting them one at a time while evaluating the increase in likelihood provided by each marker separately. Signals with a −Log10 value (*p* value) equal to three were marked as significant. The second step was selection of the most important signals in relation to all of the others. If a signal was selected, then no other markers were selected within 10 cM left and right of the already-selected signal. Since the inbred lines were analyzed by dividing them only by heterotic group, no clear family structure was present in the germplasm studied. The population structure term was included in the model as a random effect with marker-based relationships to complete the relationship information of these materials.

3. Results

3.1. *Phenotypic Analysis*

Seventy-four inbred lines were planted for 2 years (2022 and 2023) in three locations with two replications each to characterize the ear parameters associated with resistance to *F. graminearum*.

Table 2 shows the averages of all the parameters measured with the relative standard deviation.

High variability in measurements was found among these materials; the number of husks ranged from an average of 5.50 ± 1.57 (PHBR2) to 10.50 ± 1.73 (PHWG5), and the husk coverage score ranged from 3.5 ± 2.11 (PHT47) to 8.67 ± 0.82 (PHR32), while the ear attitude score ranged from 1 ± 0 (PHBG4, PHMK0, PHP60, PHTP9, PHV07, PHV53) to 8.56 ± 0.88 (PHR31).

Table 2. Summary of agronomic parameters, shown as average ± SD, collected in three locations over 2 years for 74 inbred lines. DON = deoxynivalenol concentration (ppm); EAS = ear attitude score; FGIS = *Fusarium graminearum* infection score; HCS = husk cover score; HN = husk number; ZEA = zearalenone concentration (ppb). For each parameter, different letters indicate statistically significant differences according to Dunn's test ($p < 0.05$).

Heterotic Group	Inbred	EAS	FGIS	HCS	HN	DON (ppm)	ZEA (ppb)
NSS	PHAP9	5.00 ± 3.62 abcde	5.92 ± 2.50 a	5 ± 1.91 acdfgh	6.50 ± 0.90 bcfghk	0.38 ± 1.07 a	30.14 ± 92.05 a
	PHG29	6.00 ± 3.46 abcde	5.67 ± 1.30 a	4.17 ± 1.59 cfh	6.92 ± 1.51 abcdefghijk	0.31 ± 0.63 a	12.36 ± 34.04 a
	PHJ90	4.33 ± 3.34 abcde	5.42 ± 2.07 a	4.17 ± 1.80 cdfh	6.33 ± 0.98 bcfgk	0.75 ± 1.24 a	76.48 ± 185.57 a
	PHK42	7.17 ± 3.13 bde	6.25 ± 2.09 a	4.17 ± 1.59 cfh	6.08 ± 1.62 bcfgk	0.04 ± 0.06 a	1.28 ± 2.25 a
	PHKW3	4.00 ± 3.36 abcde	6.00 ± 2.52 a	8.00 ± 1.35 abeg	7.25 ± 0.97 abcdefghijkl	2.32 ± 5.87 a	93.5 ± 224.41 a
	PHR31	8.56 ± 0.88 e	6.13 ± 1.55 a	4.11 ± 1.05 cdfh	6.63 ± 0.74 abcdefghijk	0.09 ± 0.23 a	2.24 ± 5.45 a
	PHT77	6.09 ± 3.02 bcde	7.91 ± 0.70 a	5.18 ± 1.08 abcdfgh	6.58 ± 1.44 abcdfghijk	0.02 ± 0.02 a	1.44 ± 4.27 a
	PH5 HK	2.67 ± 2.53 abcde	7.58 ± 1.38 a	7.17 ± 1.59 abcdefgh	6.83 ± 1.19 abcdefghijk	0.17 ± 0.36 a	6.72 ± 16.53 a
	PHK56	5.67 ± 3.55 abcde	6.17 ± 2.21 a	6.33 ± 1.56 abcdefgh	7.00 ± 0.85 abcdefghijk	0.04 ± 0.06 a	0.94 ± 2.03 a
	PHN11	7.33 ± 2.67 de	6.33 ± 2.06 a	4.00 ± 2.17 cdfh	5.50 ± 1.57 f	0.85 ± 1.49 a	41.00 ± 80.13 a
	PHN46	4.67 ± 2.53 abcde	6.67 ± 1.61 a	5.67 ± 1.3 abcdefgh	6.09 ± 0.94 bcfk	0.38 ± 1.28 a	10.44 ± 32.06 a
	PHN73	5.00 ± 2.83 abcde	5.63 ± 2.92 a	6.20 ± 1.69 abcdefgh	6.60 ± 0.84 abcfghijk	0.26 ± 0.54 a	6.24 ± 10.80 a
	PHP76	5.67 ± 3.23 bcde	6.17 ± 1.75 a	4.00 ± 1.35 ch	7.33 ± 1.07 abcdefghijkl	0.24 ± 0.51 a	5.09 ± 10.15 a
	PHPP8	4.33 ± 2.99 abcde	7.08 ± 1.51 a	6.83 ± 1.80 abcdefgh	6.83 ± 1.03 abcdefghijk	0.94 ± 2.94 a	101.87 ± 235.27 a
	PHR62	5.17 ± 3.24 abcde	7.50 ± 1.00 a	6.50 ± 1.93 abcdefgh	5.92 ± 0.90 bf	0.16 ± 0.51 a	4.60 ± 13.91 a
	PHW30	6.33 ± 2.99 bde	7.42 ± 0.90 a	8.17 ± 1.80 abe	7.25 ± 1.22 abcdefghijkl	0.28 ± 0.73 a	2.49 ± 6.94 a
	PHW53	2.00 ± 1.81 abcd	6.25 ± 1.91 a	6.33 ± 1.56 abcdefgh	8.25 ± 1.54 abcdefghijkl	0.05 ± 0.08 a	0.98 ± 2.40 a
	PHZ51	1.67 ± 1.30 abc	6.00 ± 2.41 a	6.00 ± 1.35 abcdefgh	8.25 ± 1.29 abcdefghijkl	0.08 ± 0.19 a	5.78 ± 11.62 a
	PHBE2	7.18 ± 3.28 bde	7.18 ± 0.98 a	4.82 ± 1.08 cdfgh	7.50 ± 1.31 abcdefghijkl	0.21 ± 0.54 a	3.96 ± 8.18 a
	PHJ33	2.33 ± 1.78 abcde	7.91 ± 1.04 a	5.50 ± 1.51 abcdefgh	7.17 ± 1.11 abcdefghijkl	0.10 ± 0.21 a	2.64 ± 7.22 a
	PHPM0	1.60 ± 1.35 abc	7.20 ± 1.62 a	7.40 ± 1.58 abcdefgh	8.40 ± 1.58 abcdefghijkl	0.06 ± 0.08 a	13.27 ± 28.00 a
	PHR03	4.17 ± 3.13 abcde	7.33 ± 1.67 a	5.67 ± 1.78 abcdefgh	7.50 ± 1.17 abcdefghijkl	4.85 ± 11.24 a	370.64 ± 1021.91 a
	PHR55	4.33 ± 3.34 abcde	5.09 ± 2.07 a	4.17 ± 1.03 ch	8.64 ± 1.21 adeghijkl	0.08 ± 0.17 a	1.11 ± 1.97 a
	PHVB2	5.17 ± 4.04 abcde	5.83 ± 1.95 a	5.67 ± 1.78 abcdefgh	6.58 ± 1.08 abcfghik	0.04 ± 0.07 a	19.47 ± 62.09 a
	PHBG4	1.00 ± 0.00 a	6.17 ± 2.33 a	4.17 ± 1.03 ch	7.42 ± 1.51 abcdefghijkl	0.04 ± 0.06 a	2.25 ± 5.34 a
	PHBR2	1.83 ± 1.03 abcd	7.50 ± 1.08 a	6.83 ± 1.03 abcdefgh	10.00 ± 2.52 adeijl	1.16 ± 2.62 a	7.58 ± 14.73 a
	PHBV8	4.17 ± 1.8 abcde	7.55 ± 2.34 a	6.08 ± 2.15 abcdefgh	7.82 ± 1.33 abcdefghijkl	0.03 ± 0.05 a	3.24 ± 10.72 a
	PHGV6	1.73 ± 1.85 abc	8.25 ± 1.04 a	7.73 ± 1.01 abdefg	9.45 ± 1.69 adehijl	2.11 ± 4.59 a	1.76 ± 1.05 a
	PHHH9	3.17 ± 2.33 abcde	6.89 ± 1.96 a	8.67 ± 0.78 e	9.83 ± 1.59 del	0.20 ± 0.48 a	10.46 ± 34.01 a
	PHJ31	1.33 ± 0.78 ac	6.92 ± 1.51 a	6.83 ± 2.62 abcdefgh	8.42 ± 1.44 abcdefghijkl	0.01 ± 0.01 a	0.55 ± 0.95 a
	PHJ65	1.20 ± 0.63 ac	7.17 ± 2.32 a	7.80 ± 1.40 abdefg	9.78 ± 2.28 adeghijl	7.80 ± 18.57 a	195.58 ± 476.51 a
	PHK46	2.17 ± 1.80 abcde	6.08 ± 1.98 a	8.00 ± 1.35 abeg	8.67 ± 1.50 acdeghijkl	0.99 ± 3.29 a	36.48 ± 122.12 a
	PHM57	2.00 ± 1.60 abcd	7.56 ± 0.88 a	6.83 ± 1.80 abcdefgh	7.33 ± 1.07 abcdefghijkl	0.69 ± 1.75 a	44.63 ± 134.08 a
	PHNB7	2.33 ± 1.56 abcde	6.91 ± 1.64 a	5.50 ± 1.51 abcdefgh	7.67 ± 1.15 abcdefghijkl	0.03 ± 0.07 a	0.69 ± 1.06 a
	PHP60	1.00 ± 0.00 ac	7.00 ± 2.00 a	7.20 ± 1.48 abcdefgh	9.60 ± 0.70 el	0.10 ± 0.17 a	3.04 ± 5.08 a
	PHR32	1.67 ± 1.03 abcde	7.00 ± 1.73 a	8.67 ± 0.82 abeg	7.67 ± 3.83 abcdefghijk	0.01 ± 0.01 a	0.01 ± 0.00 a
	PHR58	2.33 ± 1.30 abcde	7.27 ± 1.62 a	7.00 ± 1.48 abcdefgh	6.92 ± 1.16 abcdefghijk	0.01 ± 0.00 a	0.57 ± 0.80 a
	PHR63	3.83 ± 1.99 abcde	6.64 ± 1.63 a	8.67 ± 0.78 e	7.67 ± 1.37 abcdefghijkl	2.04 ± 6.86 a	433.41 ± 1465.63 a
	PHV53	1.00 ± 0.00 a	6.45 ± 2.07 a	6.00 ± 1.04 abcdefgh	6.50 ± 1.09 bcfghik	0.03 ± 0.05 a	33.75 ± 104.14 a
	PHW65	2.17 ± 2.33 abcd	4.44 ± 2.96 a	7.00 ± 0.00 abcdefgh	7.00 ± 1.18 abcdefghijkl	2.19 ± 6.49 a	165.75 ± 492.88 a
	PHW79	2.00 ± 1.60 abcd	6.58 ± 1.78 a	6.00 ± 2.17 abcdefgh	6.67 ± 0.78 abcfghik	0.06 ± 0.11 a	0.22 ± 0.50 a
	PHWG5	1.50 ± 0.90 abc	8.00 ± 1.41 a	8.50 ± 0.90 be	10.50 ± 1.73 l	0.20 ± 0.60 a	10.41 ± 34.16 a
	PH42B	5.67 ± 2.99 bcde	6.08 ± 2.07 a	5.00 ± 1.71 acdfgh	8.92 ± 1.98 adeghijkl	0.13 ± 0.26 a	4.90 ± 10.20 a
	PHR47	2.17 ± 1.34 abcde	7.00 ± 1.56 a	8.00 ± 1.35 abeg	7.92 ± 0.79 abcdefghijkl	0.27 ± 0.57 a	15.22 ± 33.57 a
	PHR61	2.00 ± 1.04 abcde	7.67 ± 1.61 a	7.00 ± 1.21 abcdefgh	9.83 ± 1.80 dejl	6.31 ± 20.88 a	26.50 ± 86.10 a
	PHVA9	1.17 ± 0.58 ac	7.33 ± 1.83 a	7.67 ± 1.56 abdefg	7.50 ± 0.52 abcdefghijkl	3.42 ± 11.66 a	45.91 ± 151.01 a
	PHAG6	1.50 ± 0.90 abc	6.17 ± 1.99 a	7.50 ± 1.51 abcdefg	6.67 ± 0.78 abcfghik	0.20 ± 0.42 a	2.21 ± 6.38 a
	PHBW8	4.33 ± 2.73 abcde	5.17 ± 0.98 a	4.67 ± 0.82 abcdfgh	7.33 ± 1.21 abcdefghijkl	0.01 ± 0.01 a	0.01 ± 0.00 a
	PHEW7	2.67 ± 1.87 abcde	7.80 ± 0.79 a	6.00 ± 1.81 abcdefgh	8.42 ± 1.56 abcdefghijkl	0.04 ± 0.08 a	2.35 ± 5.73 a
	PHHB4	2.83 ± 1.59 abcde	5.50 ± 2.50 a	5.17 ± 1.8 abcdfgh	6.00 ± 0.45 bf	0.09 ± 0.16 a	4.19 ± 8.31 a
	PHJR5	3.67 ± 2.31 abcde	6.92 ± 1.73 a	5.83 ± 1.8 abcdefgh	8.42 ± 1 acdeghijkl	0.23 ± 0.69 a	3.91 ± 12.86 a
	PHK29	2.00 ± 1.04 abcde	6.91 ± 1.76 a	7.33 ± 1.15 abcdefg	8.00 ± 0.85 abcdefghijkl	0.07 ± 0.19 a	0.55 ± 1.00 a
	PHK35	2.00 ± 1.35 abcd	8.10 ± 0.99 a	7.00 ± 1.48 abcdefgh	6.83 ± 0.94 abcdfghijk	0.08 ± 0.13 a	1.79 ± 4.66 a
	PHN29	5.00 ± 2.56 abcde	7.55 ± 1.13 a	6.5 ± 1.24 abcdefgh	7.18 ± 0.75 abcdefghijkl	0.09 ± 0.15 a	3.65 ± 10.29 a
	PHNJ2	1.17 ± 0.58 ac	7.78 ± 0.67 a	7.83 ± 1.34 abeg	8.08 ± 1.83 abcdefghijkl	2.59 ± 5.39 a	520.11 ± 1456.31 a

SSS	PHP38	3.83 ± 1.80 ^{abcde}	7.64 ± 1.63 ^{a}	6.83 ± 1.99 ^{abcdefgh}	6.83 ± 2.44 ^{abcdefghijk}	0.08 ± 0.17 ^{a}	1.58 ± 3.15 ^{a}
	PHT10	4.00 ± 3.02 ^{abcde}	6.50 ± 2.33 ^{a}	6.00 ± 2.34 ^{abcdefgh}	6.00 ± 0.95 ^{bcf}	2.59 ± 7.69 ^{a}	93.99 ± 243.48 ^{a}
	PHT11	1.91 ± 1.04 ^{abcde}	7.40 ± 1.07 ^{a}	6.82 ± 1.89 ^{abcdefgh}	9.55 ± 1.51 ^{adejl}	0.02 ± 0.02 ^{a}	5.42 ± 8.59 ^{a}
	PHT47	2.50 ± 2.43 ^{abcde}	6.00 ± 1.89 ^{a}	3.50 ± 2.11 ^{h}	6.83 ± 1.03 ^{abcdefghijk}	0.03 ± 0.04 ^{a}	0.56 ± 1.17 ^{a}
	PHTP9	1.00 ± 0.00 ^{a}	6.25 ± 1.42 ^{a}	5.83 ± 1.99 ^{abcdefgh}	8.08 ± 1.08 ^{abcdefghijkl}	0.40 ± 1.06 ^{a}	24.14 ± 60.63 ^{a}
	PHTV7	2.5 ± 2.11 ^{abcde}	6.73 ± 2.05 ^{a}	4.83 ± 2.17 ^{acdfgh}	9.08 ± 1.62 ^{adehijl}	0.11 ± 0.26 ^{a}	1.96 ± 4.11 ^{a}
	PHV07	1.00 ± 0.00 ^{a}	6.58 ± 1.88 ^{a}	5.50 ± 1.51 ^{abcdefgh}	7.17 ± 1.11 ^{abcdefghijkl}	0.92 ± 2.99 ^{a}	88.03 ± 298.01 ^{a}
	PHBB3	1.67 ± 1.56 ^{abc}	6.18 ± 1.94 ^{a}	6.50 ± 0.90 ^{abcdefgh}	7.92 ± 1.73 ^{abcdefghijkl}	0.15 ± 0.28 ^{a}	2.21 ± 3.59 ^{a}
	PHG86	5.00 ± 2.95 ^{abcde}	5.00 ± 2.68 ^{a}	7.33 ± 1.67 ^{abcdefgh}	8.09 ± 0.94 ^{abcdefghijkl}	0.70 ± 1.50 ^{a}	184.56 ± 422.86 ^{a}
	PHHB9	2.17 ± 1.59 ^{abcde}	6.18 ± 1.72 ^{a}	5.00 ± 1.71 ^{acdfgh}	7.33 ± 0.78 ^{abcdefghijkl}	0.07 ± 0.18 ^{a}	0.46 ± 1.56 ^{a}
	PHP85	1.73 ± 1.35 ^{abcd}	6.40 ± 2.01 ^{a}	7.73 ± 1.85 ^{abdeg}	6.91 ± 0.94 ^{abcdefghijk}	0.01 ± 0.00 ^{a}	0.68 ± 0.90 ^{a}
	PHPR5	4.67 ± 3.06 ^{abcde}	7.33 ± 1.30 ^{a}	6.17 ± 1.59 ^{abcdefgh}	7.17 ± 1.53 ^{abcdefghijkl}	0.14 ± 0.36 ^{a}	1.88 ± 3.25 ^{a}
	PHW51	2.50 ± 1.51 ^{abcde}	6.50 ± 1.73 ^{a}	5.00 ± 1.21 ^{acdfgh}	8.75 ± 0.75 ^{adehijl}	0.12 ± 0.26 ^{a}	2.54 ± 5.35 ^{a}
	PHW52	2.50 ± 1.51 ^{abcde}	5.83 ± 1.85 ^{a}	6.33 ± 1.56 ^{abcdefgh}	7.08 ± 1.00 ^{abcdefghijkl}	1.14 ± 3.61 ^{a}	51.89 ± 162.52 ^{a}
	PHEG9	1.17 ± 0.58 ^{ac}	4.91 ± 1.87 ^{a}	4.50 ± 0.90 ^{cdfh}	7.73 ± 0.9 ^{abcdefghijkl}	4.97 ± 15.60 ^{a}	446.73 ± 1468.60 ^{a}
	PHGF5	2.82 ± 3.16 ^{abcde}	6.38 ± 1.85 ^{a}	5.36 ± 1.75 ^{abcdefgh}	8.45 ± 1.04 ^{abcdeghijkl}	0.03 ± 0.04 ^{a}	2.29 ± 5.25 ^{a}
	PHJ70	3.00 ± 2.31 ^{abcde}	7.13 ± 0.99 ^{a}	6.00 ± 1.05 ^{abcdefgh}	9.63 ± 1.77 ^{adehijl}	0.11 ± 0.27 ^{a}	488.22 ± 1379.85 ^{a}
	PHMK0	1.00 ± 0.00 ^{a}	7.40 ± 1.34 ^{a}	8.50 ± 0.90 ^{be}	8.64 ± 1.57 ^{abcdefghijkl}	0.06 ± 0.13 ^{a}	2.62 ± 3.48 ^{a}
	PHT55	2.50 ± 2.28 ^{abcde}	7.90 ± 1.45 ^{a}	7.67 ± 1.30 ^{abdefg}	7.83 ± 1.19 ^{abcdefghijkl}	0.06 ± 0.10 ^{a}	3.10 ± 6.81 ^{a}

As reported in the Section 2, the harvested ears were visually scored to evaluate *F. graminearum* infection. This score, on average, ranged between 4.44 ± 2.96 (PHW65) and 8.25 ± 1.04 (PHGV6), with the higher score associated with less fungal infection on the ear. The average of the infection score parameter by location was lower in the 2022 season compared with the 2023 season (Table 3), indicating higher infection rates in the first testing season.

Table 3. *F. graminearum* infection score average (x−) and standard deviation (σ) for the three locations in two different years. Different letters indicate statistically significant differences according to Dunn's test ($p < 0.01$).

Location	Year	$\bar{x}$	σ
Location 1	2022	5.16 ^{a}	1.90
	2023	6.94 ^{b}	1.97
Location 2	2022	6.51 ^{c}	1.71
	2023	7.25 ^{d}	1.89
Location 3	2022	6.49 ^{e}	1.40
	2023	7.36 ^{f}	1.76

Mycotoxin analysis showed an almost complete absence of DON and ZEA for the first testing season, except for rather few samples, namely 16 for ZEA and 3 for DON (Figure 1). In 2023, the mycotoxin concentration was higher, even if only 19 samples for the ZEA analysis and 35 for the DON analysis (1.75 ppm) were above the threshold set by the European Union for human consumption (350 ppb) (Table S2).

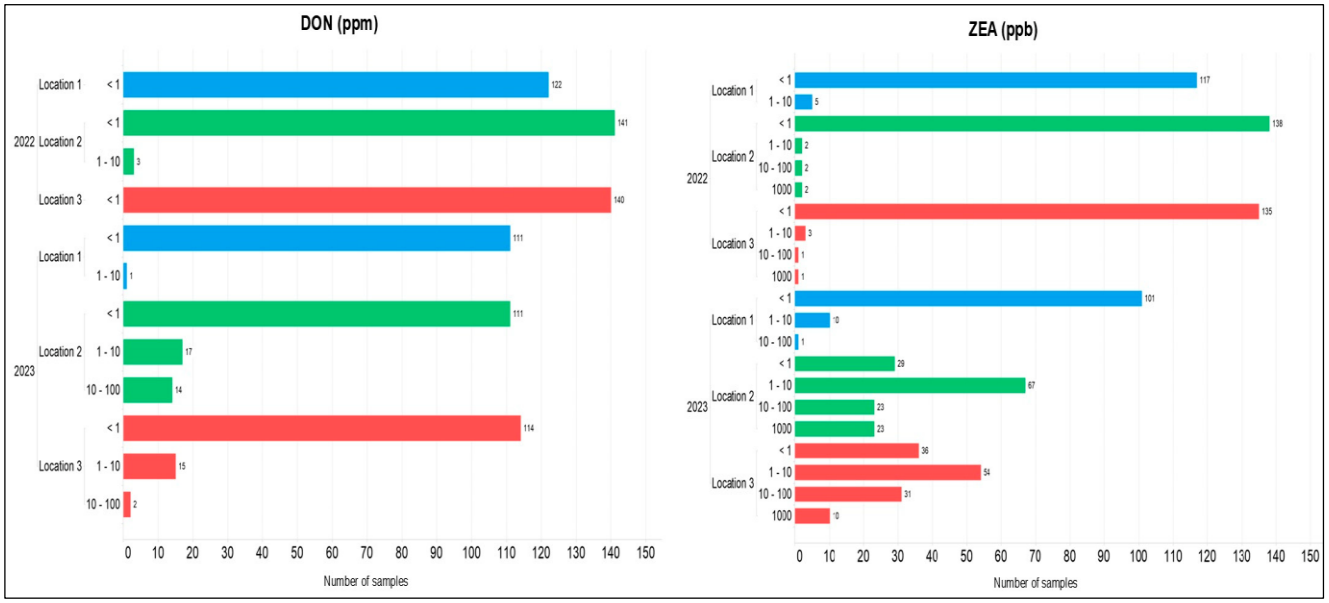

Figure 1. Number of samples collected in three locations for two seasons and their concentration of zearalenone (ZEA) in ppb and deoxynivalenol (DON) in ppm.

3.2. *Multi-Year Multi-Site Data Correlation*

The collected data were not normally distributed according to a Shapiro–Wilk test (Table S3). Therefore, a Spearman's rank correlation test was used to find possible relations between the ear parameters (Table 4). Correlation analysis was performed while considering the materials' genetic back- grounds, which in this case were their heterotic group (NSS and SSS) and also taking into account the testing year.

Positive and significant correlation between the DON and ZEA concentrations was found in both heterotic groups and for both testing years (NSS 2022: $p < 0.05$, $r_s = 0.32$; NSS 2023: $p < 0.05$, $r_s = 0.70$; SSS 2022: $p < 0.05$, $r_s = 0.21$; SSS 2023: $p < 0.05$, $r_s = 0.67$). A significant negative correlation was found between the ear attitude and ZEA concentration in both heterotic groups for the 2023 season (NSS 2023: $p < 0.05$, $r_s = -0.18$; SSS 2023: $p < 0.05$, $r_s = -0.19$).

The ear attitude was also found to significantly correlate negatively with the husk cover score (NSS 2022: $p < 0.05$, $r_s = -0.22$; NSS 2023: $p < 0.05$, $r_s = -0.19$) and husk number (NSS 2022: $p < 0.05$, $r_s = -0.31$; NSS 2023: $p < 0.05$, $r_s = -0.17$) for the NSS materials in both testing years.

In the 2022 season, for both heterotic groups, a significant positive correlation was found between the *F. graminearum* infection score and husk number (NSS 2022: $p < 0.05$, $r_s = 0.20$; SSS 2022: $p < 0.05$, $r_s = 0.21$). The *F. graminearum* infection score was also found to have a significant positive correlation with the husk cover score in the NSS heterotic group in 2023 and SSS in 2022 (NSS 2023: $p < 0.05$, $r_s = 0.19$; SSS 2022: $p < 0.05$, $r_s = 0.19$).

Table 4. Spearman's rank correlation between ear parameters and mycotoxin concentration. DON = deoxynivalenol concentration (ppm); EAS = ear attitude score; FGIS = *Fusarium graminearum* infection score; HCS = husk cover score; HN = husk number; ZEA = zearalenone concentration (ppb).
* Correlation significant at $p < 0.05$.

Material and Year	Variable	DON (ppm)	EAS	FGIS	HCS	HN	ZEA (ppb)
NSS 2022	DON (ppm)	1					
	EAS	0.05	1				
	FGIS	−0.07	0.02	1			
	HCS	−0.02	−0.22 *	0.19 *	1		
	HN	−0.04	−0.31 *	0.20 *	0.34 *	1	
	ZEA (ppb)	0.32 *	0.07	0.03	−0.04	−0.01	1
NSS 2023	DON (ppm)	1					
	EAS	−0.18 *	1				
	FGIS	−0.08	0.00	1			
	HCS	0.02	−0.19 *	−0.02	1		
	HN	0.09	−0.17 *	0.07	0.20 *	1	
	ZEA (ppb)	0.70 *	−0.18 *	−0.07	0.01	0.02	1
SSS 2022	DON (ppm)	1					
	EAS	0.09	1				
	FGIS	−0.12	−0.06	1			
	HCS	0.08	−0.07	0.14	1		
	HN	−0.04	−0.03	0.21 *	0.09	1	
	ZEA (ppb)	0.21 *	0.00	0.01	0.00	0.04	1
SSS 2023	DON (ppm)	1					
	EAS	−0.15	1				
	FGIS	−0.06	0.07	1			
	HCS	0.08	−0.08	0.19 *	1		
	HN	−0.08	−0.06	0.02	0.01	1	
	ZEA (ppb)	0.68 *	−0.19 *	−0.10	0.06	0.15	1

3.3. *QTL Analysis*

QTL analysis was performed using phenotypic data recorded over 2 years (2022 and 2023) in three locations, with two replications each. Ear characteristic QTL identification was performed separately for the SSS and NSS materials (Table 5, Figure 2).

The best linear unbiased prediction (BLUP) values for the ear traits were calculated to conduct a GWAS with 24,000 SNPs. The threshold for QTL detection was determined as a −log (p value) equal to three. No peak was selected if it was closer than 10 cM left or right to an already-selected peak.

Seven QTLs for the ear traits were found for the NSS heterotic group. Four QTLs for the husk numbers were located on chromosomes 3, 4, 7, and 10, with an effect which ranged from −0.43 to −0.25 in the number of husks. For the husk cover score, two QTLs were located on chromosomes 1 and 6, with effects on the score of 0.62 and −0.56, respectively.

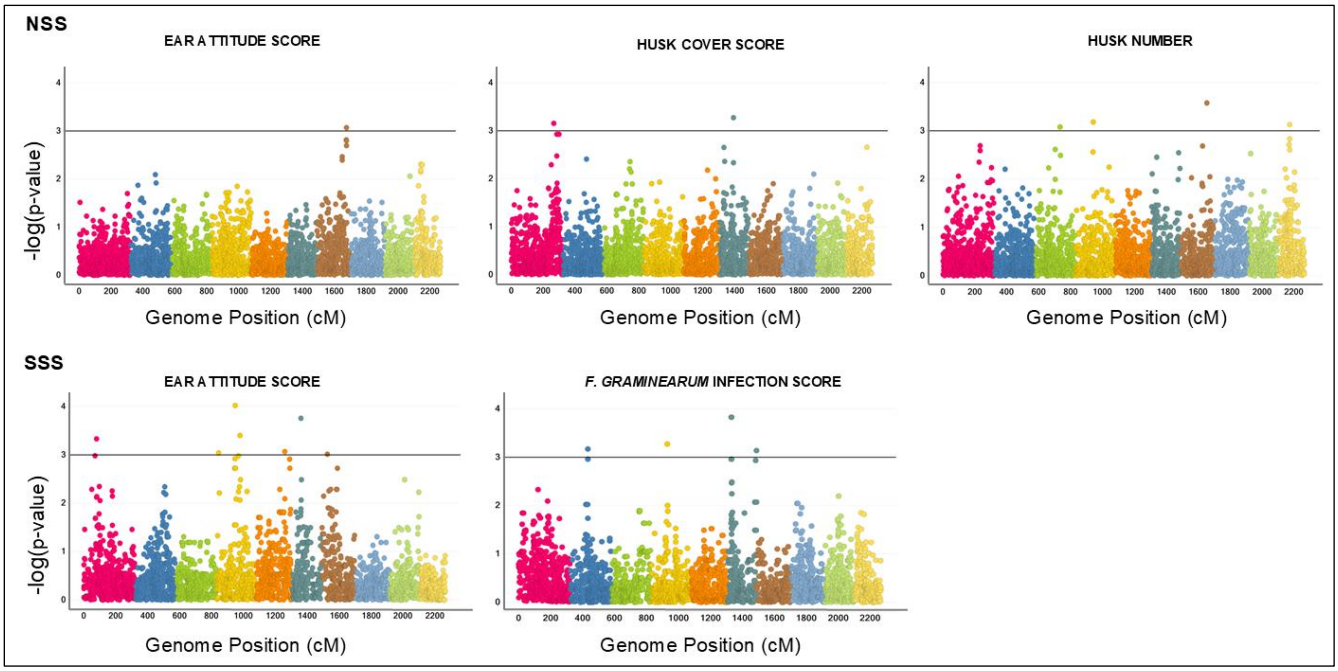

Figure 2. Manhattan plots of markers associated with traits in SSS and NSS heterotic groups. The ear attitude score, husk cover score, and husk number are the traits for the NSS group. The ear attitude score and *F. graminearum* infection score are shown for the SSS group. On the x axis, different colors represent corn chromosomes, and the genome position is expressed in centimorgans (cM). On the y axis, a −log (*p* value) threshold of 3 is shown to identify which markers were considered significant.

A QTL for the ear attitude score was located on chromosome 7, with an effect of 1.33 on the ear attitude score scale. In the SSS heterotic group, different QTLs were found for the ear attitude score and infection score. Seven QTLs were found for the ear attitude score on chromosomes 1, 4, 5, 6, and 7. The effects of these markers ranged between −1.63 and −1.25 on the scale of the score. Four QTLs were found for the infection score, with an effect on the score which ranged between 0.16 and 0.19

Table 5. Quantitative trait locus (QTL) mapping of ear traits. EAS = ear attitude score; FGIS = *Fusarium graminearum* infection score; HCS = husk cover score; HN = husk number. Chromosome positions and genome positions are expressed in centimorgans (cM). The effect and standard error of the effect (SE effect) are expressed in each trait's unit of measure. Markers listed are the ones which were above the −log (*p* value) threshold of three.

Heterotic Group	Trait	Model	Marker	Chromosome	Chromosome Position (cM)	Genome Position (cM)	Effect	SE Effect	−log (*p* Value)
NSS	EAS	GWAS	C10550D-001	7	185.98	1675.98	1.33	0.4	3.07
	HCS	GWAS	C104E04-001	1	264.81	264.81	1.01	0.3	3.16
			C1052NP-001	6	86.75	1392.75	−0.86	0.25	3.27
	HN	GWAS	C104W1Y-001	3	153.15	732.15	−1.02	0.31	3.08
			C104XB3-001	4	110.41	940.41	−1.07	0.31	3.19
			C1054NE-001	7	163.06	1653.06	−0.96	0.26	3.58
			C104PTU-001	10	71.27	2173.27	−0.88	0.26	3.13
SSS	EAS	GWAS	C104NB1-001	1	79.39	79.39	−1.48	0.42	3.33
			MZA15082-13	4	13.06	843.06	−1.57	0.47	3.04
			C104XFF-001	4	117.82	947.82	−1.25	0.32	4.02
			C104XTF-001	4	148.22	978.22	−1.31	0.37	3.4
			C105186-001	5	181.04	1259.04	−1.31	0.39	3.07

			MZA15414-29	6	52.79	1358.79	−1.63	0.43	3.76
			C104EJV-001	7	34.26	1524.26	−1.29	0.39	3.01
SSS	FGIS	GWAS	C104UT6-001	2	109.39	431.39	0.19	0.06	3.17
			C105025-001	4	97.58	927.58	0.17	0.05	3.27
			C1053JV-001	6	24.03	1330.03	0.16	0.04	3.83
			C1053E9-001	6	180.5	1486.5	0.19	0.06	3.14

4. Discussion

This work aimed to evaluate the ear's characteristics and their impact on the mitigation of GER infection in 74 maize inbreds selected for their adaptation to the northern Italian environment. A quantitative trait loci analysis was performed to understand the genetic background of these ear traits.

In this project, different parameters were collected to evaluate the ear traits, and the data of two seasons were analyzed to understand possible correlations between the characteristics. The *F. graminearum* infection score severity was higher in the 2022 season compared with 2023 in all locations, but extremely small differences were found in the my- cotoxin concentrations in both seasons, and no correlation was found between the infection score and mycotoxin concentration for either DON and ZEA. A positive and statistically significant correlation was therefore found between the DON and ZEA concentrations. This correlation was driven mostly by the few samples which presented high mycotoxin concentrations, confirming the already-known fact that *F. graminearum* can produce both of these mycotoxins [52,53].

To prevent fungal infection, different methods can be implemented, and among them, the most common are cropping techniques, crop residue management, and chemical or biological control [17,30,54,55]. However, with the optics of input reduction and environ- mental preservation, the best method for controlling *F. graminearum* is the selection of resistant genotypes, even if no completely resistant materials are available [56].

From the standpoint of the selection of resistant materials, it was observed that the ear morphology plays an important role in the susceptibility of corn to fungal diseases [38,39,57]. In this study, different ear characteristics were taken into account. Positive statistically significant correlations, even if not strong (r_s = 0.20 in NSS materials and r_s = 0.21 in SSS materials), were found between the *F. graminearum* infection score collected in terms of a reverse scale, where a higher number means less infection, and the husk number. A higher husk number can be associated with less insect ear damage due to the barrier effect which it represents [58]. Another husk trait found to be positively correlated (r_s = 0.19 in NSS in 2023; r_s = 0.19 in SSS in 2022) with the *F. graminearum* infection score was the husk cover score. Husks which can cover the ear completely play an important role in protection against both insect and fungi, as reported by several studies [59–61].

Other significant correlations found were the one between the ear attitude and husk cover score and between the ear attitude and husk number, but these were only in the SSS heterotic group.

Even if no correlation between the *F. graminearum* infection score and ear attitude was found, it has been seen that these traits are correlated with less ear rot infection due to the pendant position, which accelerates kernel dry down [62].

The genetic basis of the ear characteristics is still not well understood due to the quantitative nature of these traits. To investigate this, we performed a genome-wide association study (GWAS) to identify possible QTLs associated with ear traits which can be useful for selecting resistant against materials GER. Other studies have already explored the presence of possible QTLs associated with husk morphology, and a wide range of markers was found [63].

In this work, four QTLs were found to be associated with the husk number, and two were associated with the husk cover. Other authors have already explored these traits, but none of the markers found in our study were found before. These other studies also explored a wide range of maize agronomic characteristics like the days to silk, kernel dry down, silk resistance, kernel resistance, and specific husk traits like the husk length and width. None of these studies correlated their findings with resistance to GER, but they were concentrated on identification of the molecular pathways involved in husk development. The total phenotypic variation explained by the QTLs found in these studies which was associated with the husk number and husk cover traits ranged between 8.9% and 44.5% for the husk number and between 3.49% and 10.19% for the husk cover score [57,64–66]. Candidate genes related to metabolism, gene expression regulation, signal transduction, and flowering time regulation were also found in these studies [64,66].

For the first time, eight QTLs were found to be associated with the ear attitude score in both heterotic groups, namely one in the NSS group and seven in the SSS group. Four QTLs were also found to be associated with the *F. graminearum* infection score trait in the SSS heterotic group associated with resistance to GER. Like previous traits, our QTLs were found in different genome positions compared with other works which explored the scores associated with infection resistance. The total phenotypic variation explained ranged between 0.46% and 21.80% in these different studies [42–44]. In these other works, interesting QTLs associated with GER resistance were found. Specifically, Galiano-Carneiro reported a stable QTL across environments and populations useful for increasing GER resistance. Other studies reported finding different markers which could be used in genomic prediction-assisted breeding [42–44]. Candidate genes were also found, but in this case, they were associated with responses to broad spectrum resistance to fungi, bacteria, and oomycetes [42,67].

All of these newly found QTLs can be explained by the difference in materials, en- vironments, and growing seasons in our experiment, confirming the strong influence of genotype–environment interaction which lies behind the phenotypic expression of all these characters [47].

However additional studies are needed to better understand the importance of these markers and their effect on resistance against GER. The use of larger populations of inbred lines or studying the resistance in hybrids can greatly improve our understanding of these highly complex traits.

In conclusion, the current study reported the relationships between the ear characteris- tics and their correlation with *F. graminearum* infection and identified QTLs unique to each heterotic group associated with the ear morphology and disease resistance. Correlation was observed between the husk number, husk cover, and infection score. Another significant correlation was observed between ear attitude and both the husk cover and husk number. Our results can help with the selection of more resistant materials, and from a marker- assisted selection standpoint, these QTLs can be useful for breeders in their activity. These findings can be crucial for better control of fungal diseases without the use of additional inputs, with positive effects on the environmental and economical sustainability of corn growing systems.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agronomy14091916/s1. Table S1: Inbred line list; Table S2: Mycotoxin analysis; Table S3: Shapiro–Wilk Test.

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General Conclusion and Future Perspectives

In this PhD study, we analyzed the phenotypic and genotypic aspects of husk traits associated with resistance to *Fusarium* spp. corn diseases in materials adapted to the Northern Italian environment. We tested inbreds and hybrids to understand ear characteristics related to Fusarium ear rot (FER) and Gibberella ear rot (GER).

Given the complex genotype x environment interaction influencing resistance to these diseases, we established a comprehensive testing network across Northern Italy, utilizing field sites provided by Pioneer Hi-Bred Italia Servizi Agronomici. We collected phenotypic data for both hybrids and inbreds. Additionally, we analyzed genotypic data for inbreds to identify potential quantitative trait loci (QTLs) associated with resistance.

We found interesting correlations between ear characteristics and disease resistance in both hybrids and inbreds. In hybrids, husk coverage was correlated with higher yield, higher moisture at harvest, and lower *Fusarium* spp. infection. Based on this finding, we focused on inbreds. We found that husk cover and husk number were correlated with reduced infection in inbreds. Furthermore, we confirmed the known correlation between *Fusarium verticilloides* infection and fumonisin production, as well as the correlation between DON and ZEA concentrations in *Fusarium graminearum*-infected ears.

We used the information deduced from these correlations for an association study between phenotypic data and genomic information, identifying several novel QTLs. This study, for the first time, explored the link between ear characteristics associated with GER resistance and QTLs useful for marker-assisted selection in inbreds adapted to the Northern Italian environment.

Modern breeding programs heavily rely on marker data to develop improved materials. As completely resistant FER and GER hybrids are not yet commercially available, our data can be valuable for screening materials and selecting better varieties.

While our findings are encouraging, further research is necessary to gain a deeper understanding of traits associated with fungal disease resistance. Developing more resistant hybrids can not only improve the economic sustainability of corn production but also benefit the environment by reducing the need for inputs to achieve high yields.