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

Andrea Magarini[1] | Federico Colombo[1] | Elena Cassani[1] | Martina Ghidoli[1] | Michela Landoni[2] | Stefano Sangiorgio[1] | Roberto Pilu[1]

[1]Department of Agricultural and Environmental Sciences—Production Landscape, Agroenergy, Università degli Studi di Milano, Milan, Italy

[2]Department of Earth and Environmental Sciences, University of Pavia, Pavia, Italy

Correspondence
Roberto Pilu, Department of Agricultural and Environmental Sciences—Production Landscape, Agroenergy, Università degli Studi di Milano, Via G. Celoria 2, 20133 Milan, Italy.
Email: salvatore.pilu@unimi.it

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Abstract

Fusarium disease and the consequent mycotoxin accumulation pose significant problem in maize cultivation, with fumonisins produced by *Fusarium verticillioides* 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 selection of genotypes with greater resistance to fungal pathogens. This approach has the potential to reduce the reliance on chemical inputs for controlling fungus 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, 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. The results obtained showed that husk coverage 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 infections and its favourable influence on both yield and grain quality.

KEYWORDS

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

Andrea Magarini and Federico Colombo contributed equally to this work.

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 subsequent 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 breakdown 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 attacks by *F. verticillioides*, relying solely on agronomic approaches is insufficient. The agronomic control should be complemented with the use of less susceptible hybrids. In the last decades, the selection of maize genotypes 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.

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

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 average 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 compared 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. 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.

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

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.

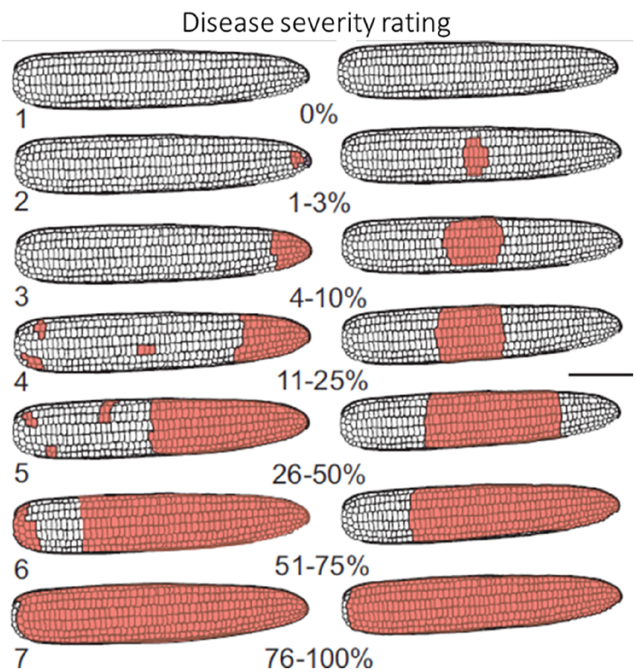

FIGURE 2 Disease severity rating of *Fusarium verticillioides*: % of surface affected by the pathogen. Assessment through a scale from 0 to 7 (modified from Reid et al., 1996).

- *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 4V, 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 severity, 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

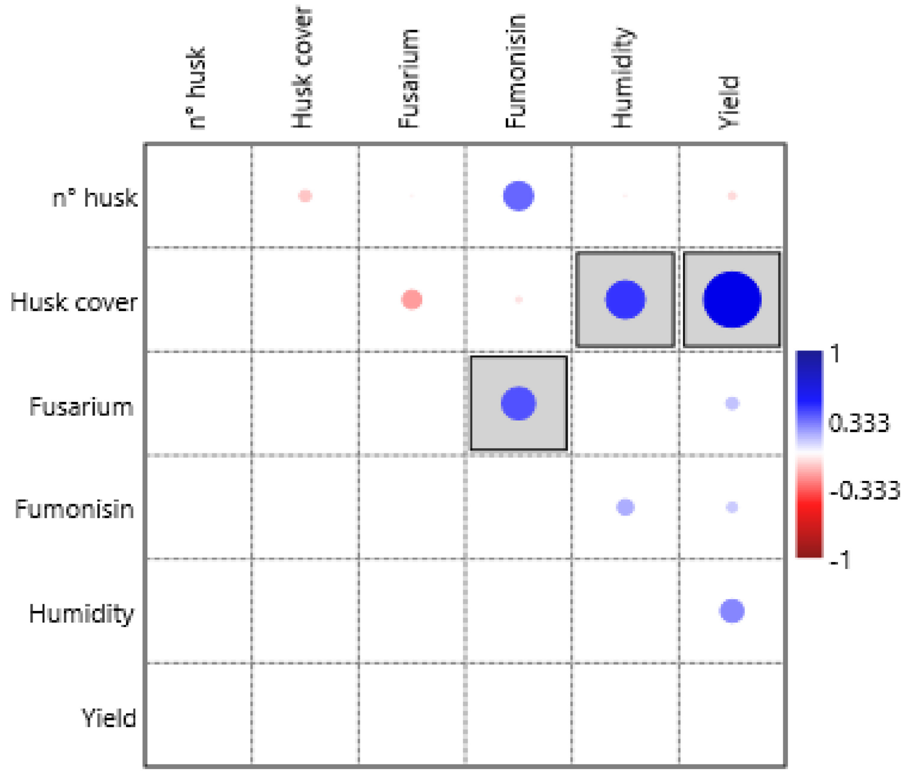

FIGURE 3 Pearson correlation plot. Negative correlations are represented in red (from 0 to −1), positive correlations in blue (from 0 to 1). The size of the circle is directly proportional to the strength of the correlation. Grey box on statistically significant correlations $p<0.05$: husk cover/yield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

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). 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 reported 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%

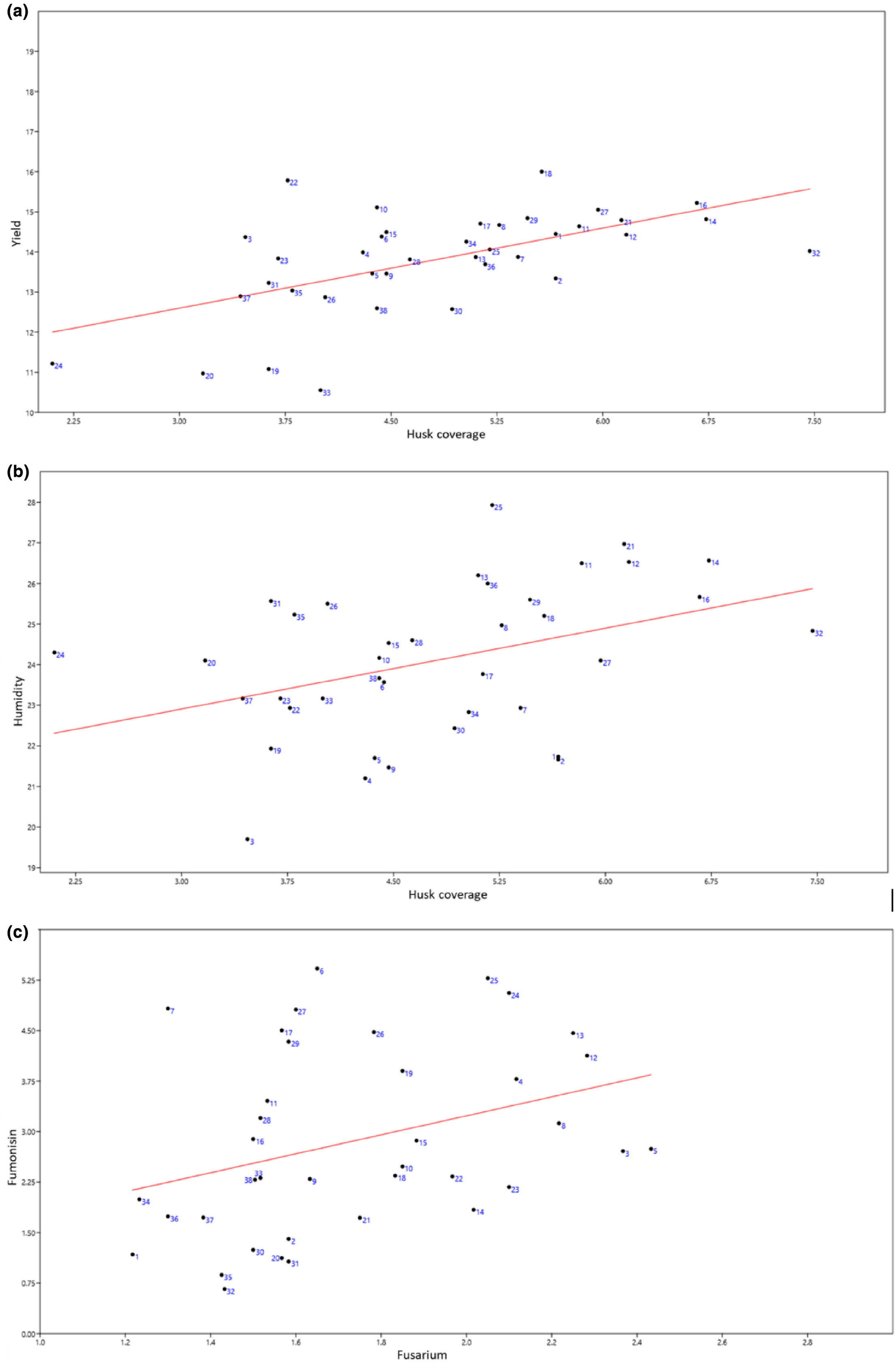

FIGURE 4 Regression plots considering the data collected in the three locations. (a) Regression plot between the husk coverage and yield ($r = 0.58041$; $p = 0.00013349$). (b) Regression plot between the husk coverage and humidity ($r = 0.39688$; $p = 0.013623$). (c) Regression plot between the *Fusarium*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

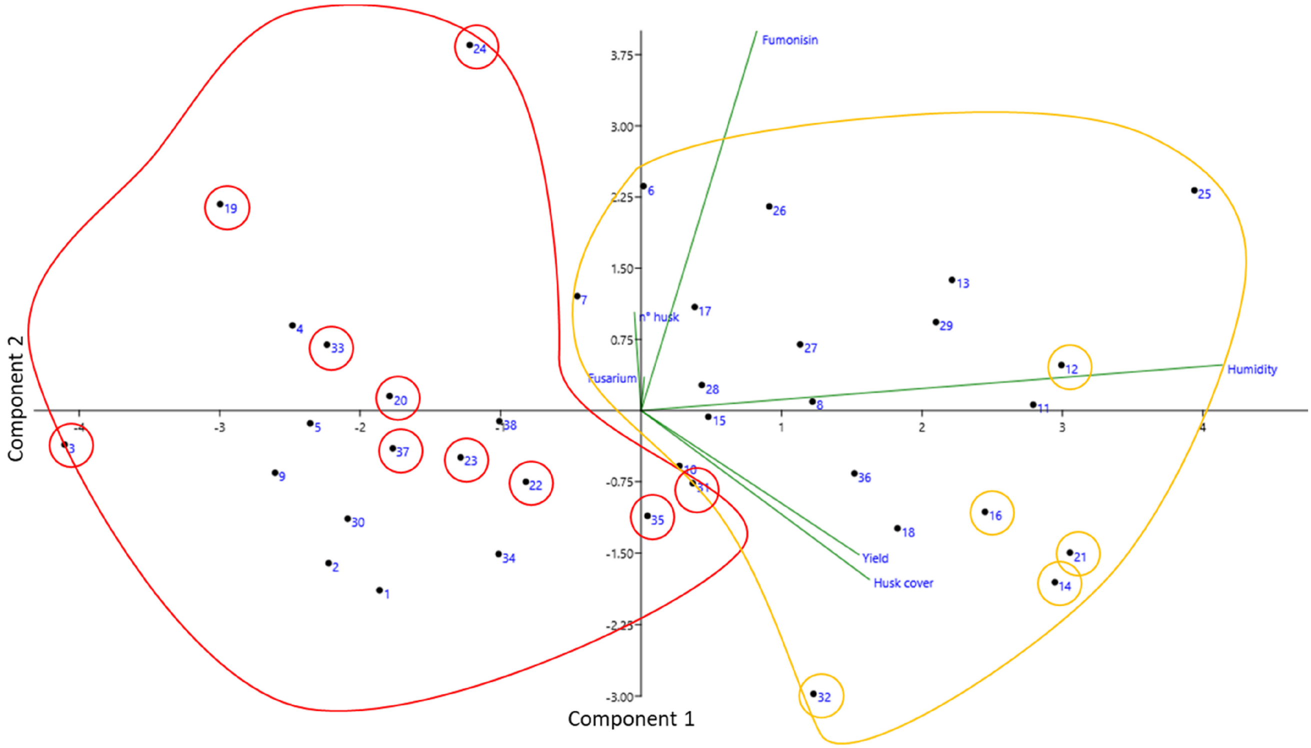

FIGURE 5 Principal components analysis (PCA) obtained using the following parameters: no. husk, husk coverage, Fusarium severity, total fumonisin content, relative humidity, and yield estimate. In green the main determinants of the clustering. In the clustering analysis by imposing *k* means equal to 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.

(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 influence on crop yield.

The genetic basis behind husk development is complex 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 colleagues (2003), four QTLs associated with husk tightness 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 analysed 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)

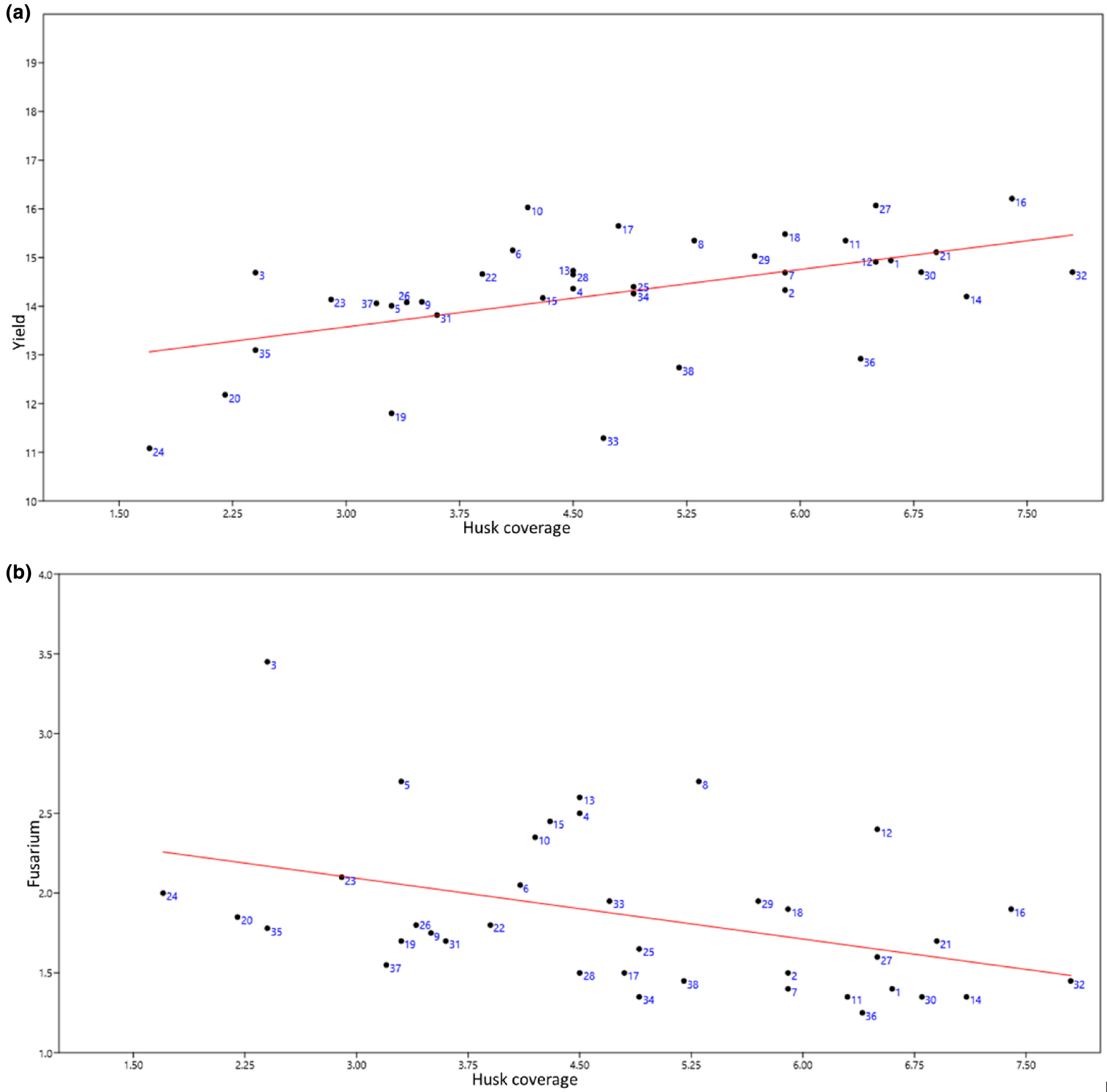

FIGURE 6 Regression plots considering the data collected in Vicenza. (a) Regression plot between the husk coverage and yield ($r = 0.51207$; $p = 0.0010146$). (b) Regression plot between the husk coverage and Fusarium ($r = -0.41492$; $p = 0.0095913$). In red the regression line.

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 involved in its regulation, to select maize varieties with enhanced resistance to fungi. 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.

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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 available from the corresponding author upon reasonable request.

ORCID

Andrea Magarini https://orcid.org/0000-0003-1604-3921
Federico Colombo https://orcid.org/0000-0001-7795-6279

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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