



# Looking for Fusarium Resistance in Oats: An Update

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**Abstract:** In recent years, an increase of interest has arisen in oats due to their unique health-related properties. Fusarium Head Blight (FHB) is recognized as a major threat to oat production and safety. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) assesses the risks of the presence of *Fusarium*-produced mycotoxins in foods and the tolerable intake level. This paper summarizes updates on *Fusarium* resistance in oats, describing the advances in phenotyping strategies and diagnostics methods and discussing the role of the infection process of the microbiome and bioactive compounds peculiar to oats. A special emphasis has been placed on the presentation of new genetic, genomic, and biotechnological knowledge and tools available today and their perspectives on breeding programs aiming to develop FHB-resistant genotypes.

**Keywords:** oats; *Fusarium*; resistance; mycotoxins; phenotyping; avenanthramides; genomic resource; GWAS; CRISPR/Cas9; next generation breeding



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## 1. Introduction

Oats rank fifth in the world's cereal production, with more than 23 million tons. About 60% of such production is concentrated in European continents, but other continents are heavily involved, such as America and Australia [1]. This crop is used chiefly as livestock feed: oats are appreciated for the good hay, the excellent grazing, the good silage, and the grains that, rich in fats and proteins, are a valuable, energy-rich source for all kinds of animals [2]. Oats have even a long history of use as human food, recently rediscovered and strengthened [3]. The high value of oat grains in human nutrition, which is unique among cereals, is widely recognized, as reviewed by Morcia et al. [4]. It is based on the high contents of lysine-rich proteins, polyunsaturated fatty acids, dietary fiber, and anti-inflammatory phenolic compounds, such as avenanthramides, peculiar to oats [5].

Some fungal species can grow and produce mycotoxins in oats, which constitutes a major hazard to cereal quality for animal and human consumption, apart from being of great economic concern to cereal producers and the grain processing industry [6]. In particular, Fusarium Head Blight (FHB) is recognized as a major threat to oat production and safety. The disease is caused by different species belonging to the genus *Fusarium*. These fungal plant pathogens are producers of mycotoxins, secondary metabolites that are chemically stable and survive food and feed processing, posing a potential risk to human and animal health [7–9]. In contrast to the situation in wheat and barley, Tekauz et al. [10] stated that FHB is typically not visible in an oat field and that visual in-crop severity is not a reliable indicator of the damage to mature seed. Despite panicle symptoms not being obvious and seed and test weights of the harvested grain being satisfactory, high levels of mycotoxins can be detected.

The infection of agricultural products for human nutrition with *Fusarium* spp. and the resulting mycotoxin contamination is of high concern for international (FAO/WHO 2001)

and European authorities [6]. Deoxynivalenol (DON), zearalenone (ZEN), and fumonisin B1 are three of the five most important mycotoxins on a European and world scale. The European Scientific Committee on Food has set tolerable daily intake (TDI) values for DON to  $1 \mu\text{g}\cdot\text{kg}^{-1}$  bodyweight per day and for the sum of T-2 and HT-2 toxin to  $0.06 \mu\text{g}\cdot\text{kg}^{-1}$  bodyweight per day.

The infection of agricultural products for human consumption with *Fusarium* spp. and the consequent mycotoxin contamination is of high concern for international authorities.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) assesses the risks for the presence of mycotoxins in foods and the tolerable intake level (of body weight per day) Health-Based Guidance Values (DON  $1.0 \mu\text{g}\cdot\text{kg}^{-1}$ , ZEN  $0.5 \mu\text{g}\cdot\text{kg}^{-1}$ , T-2 and HT-2  $0.06 \mu\text{g}\cdot\text{kg}^{-1}$ , Fumonisin  $2.0 \mu\text{g}\cdot\text{kg}^{-1}$ ) [11] and provides advice on risk management and recommendations that are used by governments and by the Codex Alimentarius Commission to establish international maximum levels of contamination in food [12].

European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain (CONTAM) established a TDI for DON, ZEN, T-2 and HT-2, Fumonisin, and Nivalenol (NIV) (Table 1).

**Table 1.** Maximum level for DON and ZEN in cereals and cereals products with particular attention to oats (Commission Regulation (EU) 2023/915 of 25 April 2023) [13], indicative levels for the sum of T-2 and HT-2 (Commission Recommendation 2013/165/EU) [14] and TDI (EFSA CONTAM—Panel EFSA Panel on Contaminants in the Food Chain). \* ND = Non-determined.

Mycotoxin	TDI $\mu\text{g}\cdot\text{kg}^{-1}$ Bodyweight per Day	Food	Maximum Level ( $\mu\text{g}\cdot\text{kg}^{-1}$ )	Indicative Level ( $\mu\text{g}\cdot\text{kg}^{-1}$ )
DON	1.0 (EFSA Journal 2017;15(9):4718) [15]	Unprocessed durum wheat grains oat grains	1750	
		Cereals placed on the market for the final consumer, cereal flour, semolina, bran and germ as final product placed on the market for the final consumer except milling products of maize	750	
		Bread, pastries, biscuits, cereal snacks and breakfast cereals	500	
		Baby food and processed cereal-based food for infants and young children	200	
ZEN	0.25 (EFSA Journal 2011;9(6):2197 2016;14(4):4425) [16,17]	Unprocessed cereal grains except maize grains	100	
		Cereals placed on the market for the final consumer, cereal flour, bran and germ as final product placed on the market for the final consumer except maize	75	
		Bread, pastries, biscuits, cereal snacks and breakfast cereals except maize	50	
		Baby food and processed cereal-based food for infants and young children	20	
T-2, HT-2	0.02 (EFSA Journal 2017;15(1):4655 2017;15(8):4972) [18,19]	Unprocessed cereals: oats (with husk)		1000
		Cereal grains for direct human consumption: oats		200
		Oat bran and flaked oats		200
		Oat milling products other than oat bran and flaked oats		100
		Breakfast cereals including formed cereal flakes		75
		Bread (including small bakery wares), pastries, biscuits, cereal snacks, pasta		25
		Cereal-based foods for infants and young children		15
Fumonisin B1	1.0 (EFSA Journal 2018;16(2):5172) [20]	Oat grains and oat products	ND *	ND *
NIV	1.2 (EFSA Journal 2017;15(4):4751) [21]	Oat grains and oat products	ND *	ND *

Many countries through their competent institutes (European Commission (EC), Food and Drug Administration of United States (U.S. FDA), Public Health Agency of Canada (PHAC), Russian Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing (Rospotrebnadzor), etc.) have set maximum levels of mycotoxins for some cereals and their derivatives to protect consumers from the harmful effects of mycotoxins.

In particular, the EC has issued the most complete and detailed regulations to set the maximum level or indicative level of mycotoxins in food, also for oats and their derived products at a rigorous level that is reasonably achievable through good agricultural, processing, and storage practices, taking into account the risk related to the consumption of the food (Table 1).

Because of the relevance of mycotoxins for the quality of oat and oat products, several strategies have been proposed to counteract the problem. Mielniczuk et al. [22] recently reviewed the methods proposed to control *Fusarium* cereal head diseases and grain contamination with mycotoxins before and after harvest. Good Agricultural Practices are the pillar for controlling *Fusarium* in the field: agronomic practices, together with chemical, physical, and biological treatments, can be of great help in inhibiting *Fusarium* development and reducing mycotoxins in grain. An ecologically friendly strategy is the development of varieties genetically resistant to *Fusarium* ssp. and toxin accumulation.

Hautsalo et al. [23] reviewed the resistance to FHB in oats, focusing on the techniques used in phenotyping and on the resistance source found. This review is an important reference for the knowledge collected up to 2017/2018 on the topic. Schematically, these are the points reported by the authors about resistance to *Fusarium graminearum* in oats:

- I. *Fusarium* resistance in oats is a complex, quantitative trait. Five types of resistance mechanisms are known:
  - resistance to the initial infection (type I): is due to earliness that ensures the avoidance or to plant morphologies and chemical compounds that act as barriers against the fungus;
  - resistance to the spread of the infection (type II): plant morphologies, chemical compounds, and detoxification mechanisms that limit the spread of the fungus;
  - resistance to kernel infection (type III): kernel morphologies, chemical barriers, defense proteins, induced resistance mechanisms to limit kernel infection and late blight;
  - tolerance (type IV): antioxidant and detoxification strategies to compensate for infection;
  - toxin accumulation (type V): toxin modification and transportation to reduce the toxin content
- II. Inoculation methods for screening *Fusarium*-resistant plants.  
 Spawn and spray inoculation protocols have been developed and applied to oats accessions grown in nurseries, greenhouses, or growth chambers.
- III. Phenotyping the FHB resistance.

Visible symptoms are not reliable for phenotyping. Field resistance can be due to avoidance; therefore, plant height and earliness are used as covariates in data analysis. PCR and real-time PCR assays are available to identify and quantify *F. graminearum*. Freezing blotter test and germination capacity can measure the kernel infection. DON quantification can be conducted with a panel of different analytical strategies.

The authors concluded that finding a resistance source for breeding is challenging in oats. Several sources of partial resistance have been identified, but to speed up resistance breeding, smart methods to quantify resistance are needed, together with high-throughput phenotyping and genomic selection.

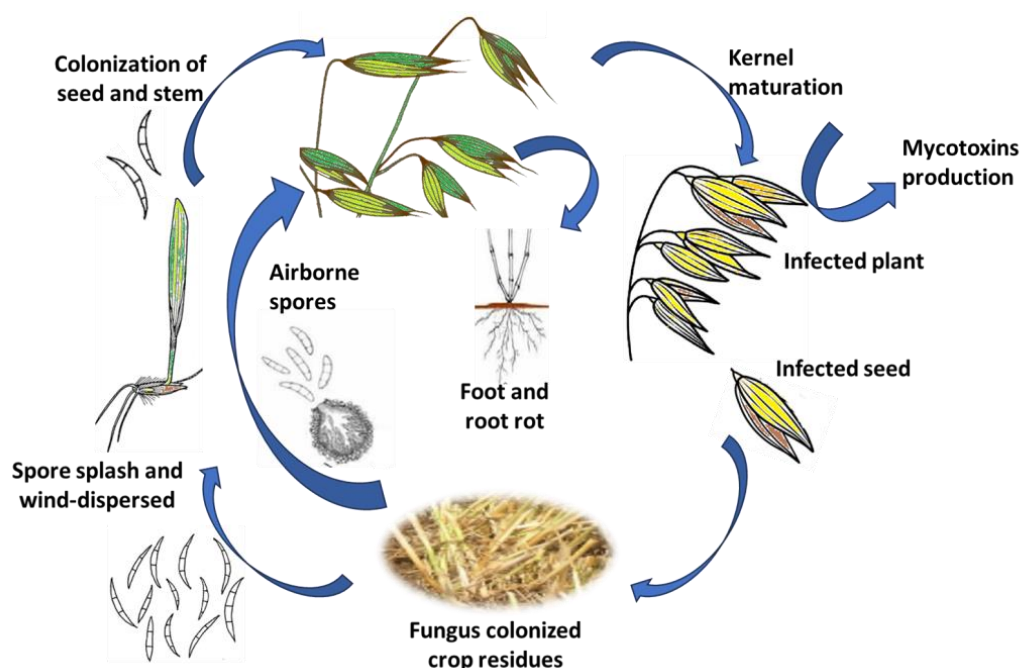
Starting from this pillar review, an update of the subsequent literature on *Fusarium* resistance in oats, along with an overview of the *Fusarium* species found to be of greatest relevance to this crop, will be presented.

In order to address these topics, the following actions were conducted:

- The available literature of the last 10 years was screened based on the keywords “Oats”, “*Fusarium*”, “FHB”, “mycotoxins”, “*Fusarium* resistance”, “Oats genomics”;
- The articles resulted from the search were examined carefully for their content and for their novelty in comparison with the already published reviews on the topic;
- In total, more than 100 articles were finally selected for this update.

## 2. Epidemiology of *Fusarium* in Oats

In oats, FHB, the most important cereal disease worldwide, is caused by a set of several *Fusarium* species with different lifestyles and different types of mycotoxins produced. The most important *Fusarium* species in oats include *F. graminearum*, *F. poae*, *F. langsethiae*, *F. avenaceum*, and *F. culmorum* [24]. *Fusarium* species can survive the winter as saprotrophs in crop residues, and in the spring and summer, their spores can spread to nearby plants by splashing or wind. When the spores land and germinate on the host plant tissues, the fungal infection begins. The germ tubes develop into hyphae, can directly infiltrate plant tissue, or enter through wounds and natural openings. In oats, anthers seem to be important during the early phases of infection. As a result, it seems that oats genotypes that extrude their anthers have a decreased risk of FHB compared to genotypes that retain them. The fungus invades the surfaces of the palea, lemma, and caryopses. At every stage of plant growth, *Fusarium* can cause a broad range of illnesses, such as foot and root rot and the generation of chalky, shriveled kernels with a low germination rate [25]. Figure 1 shows the schematic disease cycle of FHB in oats.



**Figure 1.** Disease cycle of FHB in oats.

Straw and other contaminated crop residues are natural sources of *Fusarium* inoculum. Plowing is one of the agronomic and cultural activities that reduces the accumulation of crop residues while also significantly lowering the inoculum level. Several investigations revealed that oats harvested from harrowed fields had a higher concentration of *Fusarium* infections and mycotoxins than oats collected from plowed fields and that the risk of *F. langsethiae* and HT-2 + T-2 toxin contamination in oats decreased with increasing tillage intensity [26]. In addition to tillage, other agronomic practices such as crop rotation, increasing soil organic matter, and microbial/biological activity levels are linked to the lower presence of *Fusarium* and its mycotoxins in cereals. On the other hand, high inputs of mineral nitrogen fertilizer and the use of specific fungicides and herbicides may raise

infection risks, increasing the susceptibility of cereal plants to *Fusarium* diseases [27,28]. Overuse of nitrogen extends the plant's vegetative period, resulting in an extended period of plant exposure to pathogens; the cellular wall becomes thinner and more susceptible to fungal invasion [29].

The *Fusarium* genus contains fungi that are particularly adept at colonizing a variety of environments with varying temperatures, humidity levels, and soil composition. Defining the temperature requirements of the different species involved in the FHB complex is necessary for understanding the variability of the fungal communities causing the disease in different areas and years, for developing weather-driven disease prediction models for FHB, and for predicting the types and amounts of toxic compounds in kernels [30]. Furthermore, the epidemiology of *Fusarium*, which is constantly evolving due to climate change, could lead to a dramatic increase in *Fusarium* infection phenomena worldwide [31]. As reviewed by Kos et al. [32], scientists have been warning about global warming more frequently over the past ten years, tying it to the growth of mycotoxin-producing molds in various parts of the world. The development of toxicogenic molds and the generation of their secondary metabolites may be significantly impacted in the future by more pronounced climate change, which may also affect host-pathogen interactions. For example, the frequent increase of contamination by *F. graminearum*, a species capable of producing various toxic mycotoxins observed in Northern and Central Europe, is becoming a major concern. *F. culmorum* was once thought to be the primary DON producer in Northern Europe, but *F. graminearum* has since supplanted it as the most significant species. The findings of different investigations support the discovery of a shift from *F. culmorum* to *F. graminearum* in FHB-infected oats in Europe [33]. To better understand the epidemiology of fusariosis, several investigations have been conducted on oats, particularly in Europe and North America (Table 2).

As proof of this, Karlsson et al. [34] analyzed the dynamics of *Fusarium* spp. and their mycotoxins production in Swedish cereals for 16 years (from 2004 to 2018). During this analyzed 16-year period, the level of NIV and HT-2/T-2 toxins in spring oats were higher than in the other cereals. NIV presence was associated with *F. poae* and the HT-2/T-2 toxins with *F. langsethiae*. *F. graminearum* dominated *F. culmorum* for fourteen out of 16 years and was identified as the principal DON and ZEN producer.

In southeast Norway, a high quantity of *Fusarium* mycotoxins has been recorded in oat grain. *F. langsethiae* and *F. graminearum* are identified as the major HT-2/T-2 and DON toxins producers, respectively, while *F. avenaceum* was the principal producer of enniatins (ENN) and beauvericin (BEA) in Norwegian oats [26].

Recently, Gil-Serna et al. [35] found HT-2/T-2 toxins accumulation in oat grains in Spanish oats. They discovered *F. langsethiae*, one of the major T-2/HT-2 trichothecenes producers in northern latitudes, for the first time in Southern European oats. Similar results are obtained by Morcia et al. [36]; *F. langsethiae* was identified as the principal producer of HT-2/T-2 toxins in Italian barley. The presence of this fungus can be strongly influenced by agronomic practices (organic or conventional), preceding crops, tillage, oat variety, and meteorological conditions, but above all, climate change plays an important role in the shift range of *F. langsethiae* growth. In addition, *F. poae*, *F. tricinctum*, and *F. cerealis* were the most often discovered *Fusarium* species in Spanish oats as B trichothecene producers.

De Colli et al. [37] revealed for the first time the co-occurrence of numerous mycotoxins, including the emerging and masked mycotoxins, in Irish unprocessed oats sampling during 2015–2016, and they discovered that T-2/HT-2 were the most often detected mycotoxins.

Canada ranks among the top producers and exporters of high-quality oats, contributing 15% to total global output and over 60% to exports [38]. Also, in Canada, FHB is the principal fungal disease that causes important economic losses for oats and other cereals production. In Ontario, from 2001 to 2017, the predominant *Fusarium* species identified in oat kernels was *F. poae*, which represented 68% of the pathogen population [39]. Similar results were obtained in the province of Manitoba from 2016 to 2018; *F. poae* was the main *Fusarium* species affecting oats, followed by *F. graminearum*, *F. sporotrichioides*, *F. avenaceum*, and *F. culmorum* [40].



A mycological investigation conducted in 2018–2019 on wheat, barley, and oat grains grown in the Urals and West Siberia found the presence of 16 species of *Fusarium* fungi. The most common species were *F. sporotrichioides*, *F. avenaceum*, *F. poae*, and *F. anguioides*. In the grain mycobiota of cereal crops, *F. graminearum* and its mycotoxin DON are frequently found in both the West Siberian and Ural regions. There have been new discoveries of rare fungal species in Russia’s Asian territory: *F. langsethiae* and *F. sibiricum*, which are mostly known for their type A trichothecene mycotoxins, were discovered in the Kurgan and Kemerovo regions, respectively. Furthermore, fumonisin-producing *F. globosum* was found in the Omsk and Altai Krai regions. However, compared to oat grain samples, wheat and barley grain samples had a greater diversity of *Fusarium* species [41].

**Table 2.** Principal Mycotoxins and *Fusarium* species isolated from oats grains in the last years.

Region	Fungus	Mycotoxins	References
Northern and Central Europe	<i>F. graminearum</i> , <i>F. culmorum</i>	DON	[33]
Swedish	<i>F. poae</i> , <i>F. langsethiae</i> , <i>F. graminearum</i>	NIV, HT-2/T2, DON, ZEN	[34]
southeast Norway	<i>F. langsethiae</i> , <i>F. graminearum</i> , <i>F. avenaceum</i>	HT-2/T2, DON, ENNs, BEA	[26]
Spain	<i>F. langsethiae</i> , <i>F. poae</i> , <i>F. tricinctum</i> , <i>F. cerealis</i>	HT-2/T-2, B trichothecenes	[35]
Ireland	<i>Fusarium</i> spp.	T-2/HT-2	[37]
Canada	<i>F. poae</i> , <i>F. graminearum</i> , <i>F. sporotrichioides</i> , <i>F. avenaceum</i> , <i>F. culmorum</i>	-	[39,40]
Urals and West Siberia	<i>F. graminearum</i> , <i>F. langsethiae</i> , <i>F. sibiricum</i> , <i>F. sporotrichioides</i> , <i>F. avenaceum</i> , <i>F. poae</i> , <i>F. anguioides</i>	DON, A-trichothecene	[41]
South-Western Siberian	<i>F. sibiricum</i> , <i>F. globosum</i>	fumonisin	[41]

### 3. Genetic Resources as Valuable Sources of FHB Resistance

Due to decades of work by botanists and geneticists, extensive collections of oat genetic resources are now accessible globally in a number of private and public collections and Gene Banks. As a result, with more than 130,000 accessions available, oat germplasm is among the top 10 genetic resource collections held by GeneBanks [42]. De Carvalho et al. [43] report that 116 significant GeneBanks presently preserve the genetic resources for oats. The largest collections of oat landraces are found in Germany (Leibniz Institute of Plant Genetics and Crop Plant Resources), the Russian Federation (Federal Research Center N. I. Vavilov All-Russian Institute of Plant Genetic Resources, VIR), the United States (NSGC), and Canada (Plant Gene Resources of Canada, PGRC, at the Saskatoon Research and Development Centre, Saskatoon, Saskatchewan). *Avena strigosa*, *A. abyssinica*, *A. brevis*, and *A. nuda* are examples of marginally cultivated oats that are included in the collections, along with their wild relatives and genetic stocks with specific features. Several core collections, which involve cutting the number of accessions to around 10% of the total genotypes in a larger collection, have been established with the aim of collecting the higher diversity in a limited pool of genotypes. GeneBank information systems have been established, enabling researchers and stakeholders to view and access the GeneBank assets (e.g., GRIN-CA/GRIN-Global-CA, GRIN-USA, and EURISCO).

Oat genetic resources collections can be screened to identify potential sources of disease resistance as a key strategy for the development of new oat cultivars.

### 4. Phenotyping for *Fusarium* Resistance Using Artificial Infection and Field Trials

Screening for FHB resistance can be a complex procedure itself, as reviewed by Hautsalo et al. [23]. In the last five years, some studies have focused on the optimization of such phenotyping activity as a starting point for an effective selection of resistance. A key point has been underlined by Hautsalo et al. [44]: “Considering that the resistance response

of an oat genotype is a sum of several mechanisms acting simultaneously, methods for studying each of the contributing mechanisms separately are needed". Moreover, accurate identification of FHB symptoms is essential for phenotyping, but the symptoms of FHB are cryptic, causing errors in scoring the disease during trials.

Essential for the purposes of phenotyping for resistance is the artificial infection, widely used in place of natural infection, linked to countless environmental variations. However, the inoculation methods have different effectiveness depending on the species of *Fusarium* considered and on the environmental humidity.

Slikova et al. [45] evaluated the impact of three different methods of spraying *F. culmorum* and *F. graminearum* spores on mycotoxin accumulation in grains of different *Avena* species. The mycotoxin accumulations gradually increased from the spray inoculation ( $0.68 \text{ mg} \cdot \text{kg}^{-1}$ ), spray + polyethylene (PE) bag cover 24 h ( $2.75 \text{ mg} \cdot \text{kg}^{-1}$ ), and spray + PE bag/48 h ( $9.46 \text{ mg} \cdot \text{kg}^{-1}$ ) methods.

Tekle et al. [46] reported that the artificial infection after spawn inoculation is more efficient than the spray procedure because it mimics the natural infection process and accounts for both passive and active resistance. The perithecia present in the inoculum at different developmental stages release ascospores over several days, and this ensures that they cover the long flowering period of oats. Even after successful infection, the visual scoring for resistance is not feasible for oats. The authors measured DON accumulations together with germination capacities in pluriannual nursery experiments to identify resistant genotypes.

After spray and spawn, even the point inoculation method has been developed in small-grain cereals. The role of this method has been evaluated by Hautsalo et al. [44]. Two genotypes with different traits that could reflect resistance to FHB in a greenhouse environment were tested. Both spray and point inoculations were used in the experiments. In the case of point inoculation, a droplet of *F. graminearum* inoculum was inserted between the palea and lemma of the primary oat floret. When spray-inoculated, the two varieties show different resistance levels against the initial infection. On the contrary, after point-inoculation, both varieties cannot resist the fungus growth and spread within spikelets. The point inoculations can, therefore, be useful to evaluate the aggressiveness of different *Fusarium* isolates and comparisons of oat responses to these.

Hautsalo et al. [24], after a complex trial carried out on eight greenhouse and 13 field experiments in 406 oat accessions inoculated with DON-producing *Fusarium*, concluded that the ranking for susceptibility, based on DON quantification, obtained in the greenhouse was significantly different from that obtained in the field. They concluded that the data obtained after field experiments are more informative for farmers and breeders. The days to maturity and the plant height, characteristics that are fully expressed on the field and involved in the escape, can, in fact, impact the *Fusarium* infections and DON in the field, as observed for other cereals [47].

Of particular concern in oats is the asymptomatic infection caused by *F. langsethiae*, a pathogen capable of producing the highly toxic T-2 and HT-2 mycotoxins. Such T-2 and HT-2 producers require different inoculation strategies than DON producers.

The success of inoculation of *F. langsethiae* in oats is strictly linked to a specific susceptibility window: oats were more susceptible to *F. langsethiae* at mid-flowering than at heading or beginning of flowering [48], as demonstrated by spray fungal inoculations at different growth stages.

Aamot et al. [49] added new evidence on the plant developmental stage critical for *F. langsethiae* infection. From heading forward, they demonstrated that oats were susceptible to *F. langsethiae*/HT-2 + T-2 and that inoculating oats during grain filling and ripening led to high levels of HT-2 + T-2 in the harvested grain. This suggests that it may be difficult to consistently manage *F. langsethiae*/HT-2 + T-2 in oats since late infections might easily offset the benefits of agricultural control techniques used earlier in the season (such as fungicide sprays during blooming). A number of additional trichothecene A metabolites were also produced in reasonably large amounts as a result of late infections. However, they are not

currently covered by the proposed EU legislation despite presumably contributing to the total toxicity and posing a risk to the safety of food and feed.

A substantial impact of the humidity duration on the success of NIV, T-2, and HT-2 producers was not seen in the majority of the experiments: *F. poae* and *F. langsethiae* are associated with drier conditions as opposed to wet situations [50–52]. However, Medina and Magan [53] found that the optimum water activity was  $>0.98$  and that no growth occurred at a water activity of 0.9 or below when investigating the effect of water activity on FL growth in vitro. Additionally, Xu et al. [54] found a positive association between T-2 and HT-2 accumulation on oat grain samples collected in the UK and warm, rainy weather during May and dry conditions afterward. In a glasshouse experiment, bagging oat shoots for six days following spray inoculation led to a successful *F. langsethiae* infection, according to Divon et al. [55]. The study conducted by Schöneberg et al. [49], however, suggests that a period of 4 h with 99% RH is sufficient for *F. poae* or *F. langsethiae* infection and subsequent toxin generation. As a result, during anthesis, high humidity combined with relatively low temperatures could raise the danger of NIV or T-2/and HT-2 contamination in oats. This is consistent with the result that the *F. graminearum* ascospores germinated more readily in humid, lower (15 °C) temperatures than in hotter, less humid settings [56].

Focusing on field-based phenotyping, an extensive study on the impact of climate, weather, tillage, and cereal intensity has been conducted by Kaukoranta et al. [57]. After analyzing data collected from 804 field trials in the temporal window from 2003 to 2014, infections by *F. langsethiae* led to the buildup of T-2 and HT-2, which occurred sooner and over a considerably shorter length of time than the infections by *F. graminearum* that resulted in the accumulation of DON, according to the reactions to high humidity and temperature. Seasons with humid and cool weather one to two weeks prior to anthesis and warm weather during other stages were favorable for T-2 and HT-2 contamination.

## 5. Phenotyping Strategies and Diagnostics Methods

The presence of mycotoxigenic *Fusarium* in oats, in particular *F. langsethiae* and *F. poae*, represents a food safety problem. The early detection of *Fusarium* fungi can be crucial to prevent more extensive field damage and mycotoxin contamination, preventing risks to human and animal health. Trichothecenes are the major cause of contamination in European oats [35]. *F. langsethiae* and other *Fusarium* T-2 and HT-2 producers have been isolated from oats without visible symptoms. Therefore, innovative and rapid technologies are needed to intervene effectively to reduce mycotoxins [58].

Fungi detection can be carried out using various techniques that are quicker than traditional ones, which involve the isolation of specific cultures [59].

Molecular techniques based on Polymerase Chain Reaction (PCR) allow the specific identification of fungal DNA, evolving over the years towards increasingly sensitive and rapid quantitative techniques [33,49].

Real-time quantitative PCR (qPCR), single and multiplex, is a DNA-based technique currently most applied to detect fungal species in infected plants and derived products.

A TaqMan-based qPCR method targeting the *Fusarium*-specific elongation region was developed to quantify in oat all *Fusarium* species simultaneously and then characterize them with a new metabarcoding method [33]. DNA sequencing can be used to identify *Fusarium* species responsible for contamination, and a metagenomic approach in oats provides a detailed view of the microbial community present [35]. Metabarcoding and metagenomic are two approaches used to analyze microbial DNA to describe the diversity and composition of fungal communities without the need to cultivate individual species. Metagenomics aids in the comparison of fungal compositions and allows for the analysis and comparison of the community's collective genomes, while metabarcoding is used to determine the composition of the microbial community by amplifying particular fungal DNA regions (barcodes) and then sequencing them using high-throughput sequencing techniques. These techniques facilitate the rapid and thorough analysis of intricate fungal communities, offering insights into ecological dynamics and environmental health.



An innovative and sensitive chip digital PCR, a third-generation PCR technology [60], was applied by Morcia et al. [61] to detect *F. graminearum*, *F. culmorum*, *F. sporotrichioides*, *F. poae*, and *F. avenaceum* in wheat and oat grains and compared to a qPCR approach.

Optical methods applied to oat samples have also been found to be effective for the detection and monitoring of *Fusarium* contamination and are able to provide rapid and objective information, such as photoluminescent optical diagnostics [62,63].

Spectroscopic approaches, such as FTIR in the mid-IR region, Raman, and luminescence methods, have been used by some authors to distinguish infected grains of oats. By chemometric analysis, it is possible to identify spectral characteristics used as infection markers. Luminescence studies revealed the presence of chlorophyll characteristic peaks absent in healthy oat grains [64].

Different analytical techniques are used to detect mycotoxins, dangerous secondary metabolites in cereals: thin layer chromatography (TLC) and high-pressure liquid chromatography (HPLC) techniques with different detectors, liquid chromatography–tandem mass spectrometry (LC-MS/MS), liquid chromatography high-resolution mass spectrometry (LC-HRMS), gas chromatography–tandem mass spectrometry (GC-MS/MS), immunochemical methods lateral flow device (LFDs), enzyme-linked immunosorbent assay technology (ELISA), surface-enhanced Raman Spectroscopy (SERS), fluorescence polarization immunoassay (FPIA), electronic nose [65–67]. Innovative approaches are used for multiple mycotoxins detection in cereal and products. A fast multi-toxin assay based on reflective phantom interface (RPI) technology was developed to identify and quantify DON, ZEN, T-2, and HT-2 toxins in wheat [68], while Lattanzio et al. [69] used LC-HRMS to detect the presence of T-2 and HT-2 toxins and their principal glucosyl derivatives in barley and malt.

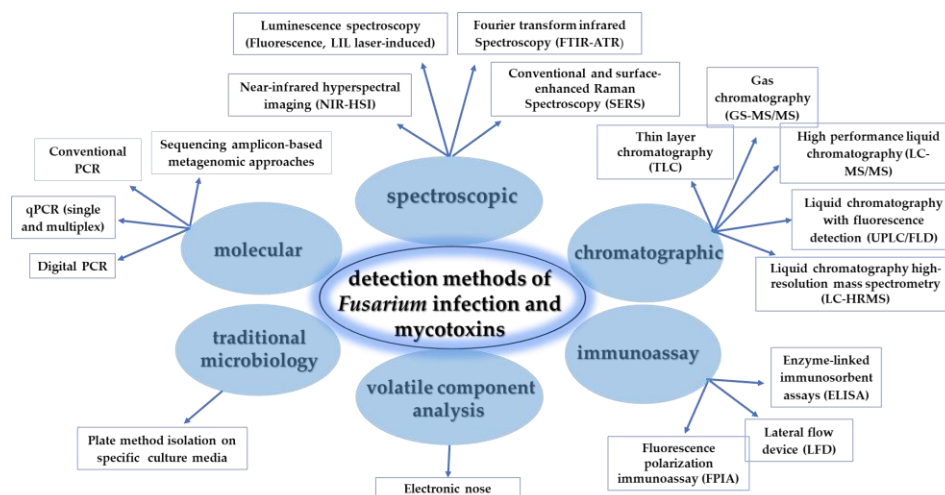
Gil-Serna et al. [35] analyzed oat seeds to study a new distribution of mycotoxins in southern Europe following climate change. They used Ultra-high-performance liquid chromatography–tandem mass spectrometry (UHPLC-MS/MS) to detect and quantify several toxins such as T-2 and HT-2, NIV, DON, ZEN, Fumonisin B1 and B2, etc. Tarazona et al. [70] investigated the natural occurrence of mycotoxins in Spanish oat grains samples using ultra-high performance liquid chromatography–electrospray ionization tandem mass spectrometry (UPLC–(ESI+)–MS/MS) multiple reactions monitoring chromatograms of mycotoxins.

Enzyme-linked immunosorbent assay technology (ELISA) is often used for screening in cereals due to the rapidity, sensitivity, and specificity of detection [67,71].

Some authors have compared rapid diagnostic tests, an enzyme immunoassay for T-2 and HT-2, and some immunochromatographic tests (lateral flow device—LFD) with LC-MS/MS in oats samples. LFD tests are particularly suitable for on-site use. The results obtained showed that they are reliable and accurate methods, and there is no significant difference between the methods evaluated [67].

ELISA method and ultra-performance liquid chromatography with fluorescence detection (UPLC/FLD) method were compared to detect T-2 and HT-2 mycotoxins in cereals, including oats. The correlation between the two analytical approaches showed good results, confirming the possibility of complementary use in the official control [71].

Near-infrared hyperspectral imaging (NIR-HSI) has shown to be a promising technique for the quantification of mycotoxins in cereals as it is rapid and cheaper compared to conventional techniques (Figure 2). It was applied to oats naturally contaminated with T-2 and HT-2. Hyperspectral images and the ELISA method, as a reference, with the use of multivariate tools, have allowed the development of a prediction model for these mycotoxins [72].



**Figure 2.** Main techniques used to detect *Fusarium* and mycotoxins in cereals.

## 6. Involvement of Peculiar Oat Compounds in FHB

Over time, various peculiar compounds produced by oats have been investigated for their possible contribution to oat protection against pathogens.

In 2011, Doeblert et al. [73] hypothesized that a chemical component of oats might contribute to FHB resistance. In their results, a hexane-soluble chemical in oat flour appears to inhibit *F. graminearum* growth and might contribute to oats' resistance to FHB. Oxygenated fatty acids, including hydroxy, dihydroxy, and epoxy fatty acids, were identified in the hexane extracts and were suggested as candidates for causing the inhibition.

Cell walls are dynamic structures [74]. A broad range of hydrolytic enzymes produced by *F. graminearum*, acting as cell wall degrading enzymes (CWDEs), e.g., xylanases, have been investigated for their role in the FHB infection process [75] and described as effectors [76]. Plant inhibitor proteins directed toward fungal CWDEs are considered part of the defense mechanisms to counteract microbial pathogens [77]. Physical, chemical, or biological stress factors are detected by the plant that is able to react by cell surface hardening and related tolerance or resistance [78]. Oat cell walls are rich in  $\beta$ -glucan, a water-soluble dietary fiber present in the non-starchy fraction of polysaccharides [4].

In order to limit the penetration of the pathogen, the plant cell wall undergoes a rapid reinforcement in which the accumulation of  $\beta$ -(1-3)-glucan [79,80],  $\beta$ -D-glucans [81], and proteins is observed in the area between the cell wall and the cell membrane [82].  $\beta$ -D-glucans form a gel layer in the cell wall that may act as a defensive wall protecting the cell from invasion by fungi and, at the same time, also provide a pattern of signals to the immunity system [83]. The direct protective involvement of oat  $\beta$ -D-glucans during FHB infection was investigated by Havrlentová et al. [84]. The content of  $\beta$ -D-glucans in oat grains is 2–7% and is influenced by genetic and/or environmental factors, and high levels of this cell walls polysaccharide are observed in naked grains of cultivated oat. This work studied the relationship between the content of  $\beta$ -D-glucans in oat grain and the infection with *F. graminearum* and *F. culmorum* obtained by artificial inoculation in samples belonging to hulled or naked cultivated oats and wild oats. The authors concluded that according to the results and statistical evaluation, naked oats better reacted to the artificial inoculation by accumulating lower levels of both DON and pathogen DNA, suggesting a direct protective role of  $\beta$ -D-glucans during FHB infection.

Avenanthramides, which are unique to oats, are a group of phenolic alkaloids with antioxidant properties found in oat tissue, including grain [85].

Oat produces avenanthramides phytoalexins, also in response to infection by pathogens or treatment with plant immunity elicitors, such as partially deacetylated chitin [86] and butylated hydroxytoluene [87,88]. Moreover, the co-treatment of germinating oats with methyl jasmonate (MeJA) and abscisic acid (ABA) demonstrated a strong correlation be-

tween enhancement of all three avenanthramides (Avn A, B, and C) production and the MeJA and ABA exposure. In the study conducted by Wise et al. [85], avenanthramide accumulation in the oat grain was associated with crown rust fungal disease. They showed that in most of the cultivars evaluated, the extent of their accumulation also correlated with their genetic disease resistance; avenanthramide accumulation in the grain was, with noted exceptions, highest in those cultivars showing the greatest genetic resistance to crown rust. No studies correlating avenanthramides content in oats with genetic FHB resistance have been carried out to date, and this remains an interesting open field of investigation.

Correlations between the content of certain bioactive compounds and the resistance to diseases suggest that breeding programs aimed at raising the levels of health-benefiting components in cereal grain might concurrently allow the development of cultivars adapted to unfavorable environmental conditions [89].

## 7. Genotyping Tools in Oats

Compared to other cereals, oat has lagged behind in the genetic and genomic studies attributed to its large and complex genomes, estimated in the range of 4.12 Gb to 12.6 Gb. [90,91].

GrainGenes, the global centralized repository for curated, peer-reviewed datasets of small grain cereals, including oats, collects genomic data sets as well as genetic, germplasm, and phenotypic datasets for oats. For oat geneticists and breeders in the public and private sectors globally since 1992, GrainGenes has been a valuable source of data and knowledge [92]. Several genetic maps have been developed in oats, and a number of different marker sets have been proposed [93]. A 6K marker chip based on an Infinium design is commercially available.

As reviewed by Yao et al. [94], 117 new map sets containing 762 maps, 21,376 markers, and 785 new QTLs are now included in the GrainGenes database. The list of maps can be found via the ‘Search: Genetic Maps at GrainGenes’ link on the homepage or at <https://wheat.pw.usda.gov/GG3/node/876#oats6x>, accessed on 10 January 2024. Moreover, about 300 oat germplasm records that link to T3 (<https://triticeaetoolbox.org>) and GRIN (<https://www.ars-grin.gov>) records were added.

Maughan et al. [95] produced 2019 the first reference-quality, whole-genome reference assemblies for *A<sub>s</sub>*- and *C<sub>p</sub>*-genomes, using *A. atlantica* and *A. eriantha*. Using the long-read PacBio technology, PepsiCo announced in 2021 that it has finished assembling the 21 chromosomes of the North American oat variety OT3098 as part of a public-private partnership project. The data are hosted on the USDA Agricultural Research Service’s GrainGenes website at <https://wheat.pw.usda.gov/jb/?data=/ggds/oat-ot3098-pepsico>, accessed on 1 January 2024, and the datasets can be downloaded at [https://wheat.pw.usda.gov/GG3/graingenex\\_downloads/oat-ot3098-pepsico](https://wheat.pw.usda.gov/GG3/graingenex_downloads/oat-ot3098-pepsico), accessed on 1 January 2024. A high-quality reference genome of *A. sativa* and of its diploid progenitors *A. longiglumis* and *A. insularis* was completed in 2022 by Kamal et al. [96]. The international PanOat consortium is now working on a pan-genome deriving from the sequence assemblies of 30 different oat genotypes [97].

## 8. Mapping *Fusarium* Resistance

In oats, genome-wide association studies (GWAS) have been reported previously for agronomic and quality-related traits [98,99], but the first study to identify genomic loci that contribute to variation in mycotoxin levels in *F. langsethiae*-inoculated oats was carried out in 2020 by Isidro-Sanchez et al. [91]. They carried out field tests over the course of two years, inoculating 190 spring oat cultivars with a combination of three isolates of the causal agent of disease. Mycotoxins were determined using liquid chromatography-tandem mass spectrometry. 16,863 genotyping by sequencing markers were used for genotyping the different varieties. T-2 + HT-2 mycotoxin accumulation was linked to 5 SNPs in the linkage group Mr06 by genome-wide association studies. The correlation between the markers was excellent, and just one QTL was found. The marker avgbs\_6K\_95238.1 was

shown to be associated with genes whose zinc-finger proteins and lipase, lipase-like, or lipase precursor mRNA sequences are similar. It has been demonstrated in the past that these areas significantly boost resistance to *Fusarium* species. The authors concluded that the *Mr06* Linkage group plays an important role in *F. langsethiae* resistance.

Haikka et al. [100] evaluated North European germplasms and breeding lines for DON and agronomic traits and compared two strategies, GWAS and genomic prediction, for their potential application. DON content, *F. graminearum* DNA content (relative to oat DNA) evaluated using real-time quantitative polymerase chain reaction (qPCR), *Fusarium*-infected kernels, and germination capacity were resistance-related parameters. The majority of the plant material included in the study are breeding lines, along with a few cultivars and exotic accessions. On the resistance-related and gathered agronomic traits, GWAS and genomic prediction, enabling genomic selection, were carried out. There were significant genetic relationships between variables linked to resistance: DON concentration had a positive correlation with qFUSG (defined as the relative amount of *F. graminearum* fungal DNA (pg) per oat DNA (100 ng) measured by qPCR) of 0.60 and a negative correlation with germination capacity of 0.63. With the data at hand, the authors were unable to discover any conclusive relationships between markers and characteristics associated with resistance. On the other hand, some resistance-related features in genomic prediction demonstrated good accuracy.

Khairullina et al. [101], in order to better understand the molecular mechanisms behind oat's resistance to *Fusarium*, have characterized the first oat genes encoding UDP-glucosyltransferases (UGT), enzymes capable of inactivating various trichothecenes. Further research should disclose their (redundant) significance in *Fusarium* infections and the buildup of mycotoxins and their masked forms, using both transgenic/edited plants and recombinant UGT proteins. The authors concluded that future research could employ the two identified UGT genes as markers for identifying and breeding FHB-resistant oat germplasm, which should eventually lead to a finished product with reduced mycotoxin levels.

The Recombinant Inbred Lines (RIL) obtained from a “Buffalo” × “Tardis” cross, in which “Buffalo” is the susceptible variety, has been used by Stancic [102] to perform an association analysis on the relationship between the content of HT-2 + T-2 in the harvested oat grain and the DNA of *F. langsethiae*. Height and flowering time typically co-localized with QTL associated with *F. langsethiae* DNA and HT-2 + T-2. On chromosome 18D, the linkage group Mrg04 comprised markers linked to height, flowering period, *F. langsethiae* DNA and HT-2 + T-2. This linkage group has already been identified as the location of the Dw6 dwarfing gene [103]. Another linkage group was discovered that was connected to the HT-2 + T-2 and flowering time of *F. langsethiae* DNA but not to height (Mrg20).

Stancic's study [102] was followed by the in-depth analysis carried out by Blackshaw-Crosby [104]. As “Tardis” Mrg04 and Mrg21 alleles were introgressed into the “Buffalo” background genome, decreases in HT2 + T2 concentrations were seen as compared to the Near Isogenic Lines (NIL) with the original parent lines. While Mrg21's influence was dependent on the planting season, Mrg04's impact was constant across all experiments. Although the Mrg21 QTL exhibited a less significant impact than the Mrg04 QTL, fall-seeded plots with “Buffalo” alleles introgressed into the “Tardis” background showed a decrease in HT-2 + T-2. The HT-2 + T-2 concentration was not affected by the introduction of Mrg20 from “Tardis” into “Buffalo”, and Mrg20 from “Buffalo” was introduced into “Tardis” with variable results between years.

Willforss et al. [105] carried out the first proteogenomic study to understand the molecular response of oats when exposed to FHB. The proteomes of resistant and susceptible cultivars were compared, and candidate proteins of interest were identified and linked to protein sequence variants. The authors developed an R-Shiny-based interface for interactive exploration of the dataset using univariate and multivariate statistics. Quantitative protein differences between Belinda and Argamak varieties were found. Argamak-resistant variety synthesizes eighteen specific peptides during infection, among them several lipoxigenases.



## 9. Progress in Understanding Plant-Pathogen Interactions, Genetic Engineering of R Protein, and Their Importance in the Future Development of FHB-Resistant Oats

Knowledge about the fascinating plant immunity system is constantly expanding. Plants possess a two-tiered innate immune system: the basal immunity (or Pattern-Triggered Immunity, PTI) and the highly specific immunity (also called R-genes-based resistance or Effector-Triggered Immunity, ETI). Recently, a study performed by Yuan et al. showed similarities in downstream defense outputs between PTI and ETI [106]. In the same year, Ngou et al. [107] stated immune pathways activated by cell-surface and intracellular receptors in plants mutually potentiate to activate strong defenses against pathogens. A work by Pruitt et al. [108] explained that the effective defense against host-adapted microbial pathogens relies on mutual potentiation of immunity by both PTI and ETI components. Reshaping our understanding of plant immunity has broad implications for crop improvement [108].

Plant disease resistance genes (R genes) encode proteins that detect pathogens. R genes have been widely investigated, although the *modus operandi* of most cloned R genes is still unknown [109]. R protein can induce immune responses via nine mechanisms [110], and several resistomes have been characterized. The study carried out in 2022 by Förderer et al. [111] on Sr35 wheat resistosome structure defined common principles of immune receptor channels and demonstrated proof of principle for structure-based engineering of Nucleotide binding and Leucine Rich-repeat receptors (NLRs) for crop improvement. Indeed, the knowledge of R protein structure and function is now applicable to R gene engineering in order to expand their recognition abilities. An example of this is the work published by Maidment et al. in 2023 [112], exposing how the effector target-guided modification of an integrated domain expands the disease resistance profile of a rice NLR immune receptor. Moreover, Kourelis et al. [113] published in 2023 a study showing nanobodies raised against defined pathogen moieties and fused to an NLR scaffold derived from a rice-derived receptor, used to create synthetic R proteins holding great promise. Nevertheless, the deployment of new-to-nature genes may be delayed by regulatory concerns [109].

On the other hand, few plant susceptibility genes required for pathogen growth have been identified, although their knockout seems provide strong resistance [94]. In cereals, CRISPR/Cas9-mediated mutations of MLO wheat susceptibility gene mutations led to powdery mildew resistance [114]. Chen et al. [109] summarized new methods emerging for discovering effectors; the consequent characterization of the effector targets can be used as an insight to enlarge the list of identified disease susceptibility genes in the host [115].

The *Fusarium*-oat pathosystem is poorly characterized at present. The knowledge of molecular pathways employed by *Fusarium* species in the early phase of host colonization is essential for the development of novel oat FHB control strategies, encompassing the development of resistance-empowered oat plants. The study of the oat immunity system and its interaction with the biotic and abiotic environment, and in particular, the identification of oat resistance genes, is essential to breeding programs aimed to enhance host resistance against FHB.

## 10. Influence of Microbiological Environment on FHB Oat Resistance

To optimize the use of microbial agents to control FHB, it is essential to understand how *Fusarium* employs effector proteins to modulate the microbiome composition and promote disease development in the host [116]. Specialized metabolites produced by the *Fusarium* spp., acting as effectors, can have antimicrobial effects [117,118] in order to compete with other microbes colonizing the host [119]. DON is phytotoxic and considered a virulence factor of fungal pathogens, and DON resistance is a component of FHB resistance [120]. This mycotoxin can act as a non-proteinaceous effector [121], and at the same time, it can influence the microenvironment by altering the pH, nutrient availability, or other factors, creating conditions conducive to microbial growth. Abid et al. [122] studied the fate of DON and its impact on the soil microflora and soil fauna.



Numerous beneficial fungal and bacterial microorganisms possess the ability to detoxify mycotoxin. The toxin biodegradation mechanism is the process by which the secondary metabolites and enzymes produced by microorganisms break down and eliminate the hazardous group of mycotoxin compounds while generating less toxic or non-toxic breakdown products. For example, the bacterium *Eggerthella* sp. DII-9, arising from chicken intestines, is capable of promoting de-epoxidation of trichothecenes mycotoxins such as DON, T-2, and HT-2 [123]. Hassan and colleagues demonstrated that *Bacillus megaterium* has significant *Fusarium* mycotoxins biodegrading capacity and, for this reason, can be considered a decontaminating agent in the food industry [124].

The use of microorganisms as a detoxification method is regarded as a promising strategy and is considered specific, efficient, and environment-friendly. Microorganisms are able to biotransform or biodegrade mycotoxins, thus increasing yield [125]. Noel et al. [126] found that endophytic fungi *Alternaria destruens*, *F. commune*, and *F. oxysporum* increased wheat seed weight and reduced *F. graminearum* DON accumulation in wheat.

A microbiome perspective on FHB had been conducted by Karlsson et al. in 2021. The knowledge on the composition of the cereal microbiome under different environmental and agricultural conditions is growing, and studies are ongoing to plainly link microbiome structure to FHB suppression [127]. The importance of the host microbiome in hindering FHB through direct and indirect effects was recently discussed in a review on the state of research to manage FHB by Moonjely et al. [116], including the interesting aspect of microbiome role in defense priming.

The utilization of native and/or applied microorganisms is a promising means of controlling FHB and mycotoxins in plants, and bacteria, fungi, and viruses can be potential biological control agents (BCAs) against *F. graminearum* [116], although the choice of application method of BCAs depends on multiple factors such as the type of agent, formulation, crop stage and weather conditions [128].

Endophytes can be defined as a mix of fungi, bacteria, and other microorganisms that reside within living plant tissues, often boosting plant growth, resistance to abiotic stress, and defense response to pathogen attacks, that recently have been gaining interest as potential BCAs [129]. Studies have shown promising results in wheat [130,131]. Notably, regarding exploitation of endophytes for plant protection against FHB, the study conducted by Wang et al. [132] showed that their use could be extended to transformed single strains expressing plant resistance genes.

The exploiting of BCAs for FHB control has been reviewed by Petrucci et al., including their recent use in oat [133]. A study was carried out aimed at the isolation and identification of fungal strains associated with green oat spikelets and exhibiting biocontrol activity against *F. graminearum* infection. Among the tested isolates, *Pseudozyma flocculosa* was shown to be the most successful strain. *P. flocculosa* is a basidiomycetous yeast, not pathogenic to plants or animals. Treatment of oats with *P. flocculosa* significantly reduced FHB symptoms, *F. graminearum* biomass, and DON accumulation in oats and induced expression of genes encoding for PR proteins [134]. Also, the potential of fungal BCA *Clonostachys rosea* to reduce FHB and mycotoxin accumulation was examined [135]. The treatment of oat spikelets with *C. rosea* caused a reduction in *Fusarium* DNA and DON content in mature kernels and enhanced both the rate of DON detoxification and expression of DON-detoxifying UGTs. Moreover, significant upregulation of markers of induced resistance was shown, including PR proteins and the WRKY23 transcription factor, indicating that the biocontrol effect of *C. rosea* is attributed to the induction of plant defenses.

Today, the off-target impacts of fungicides on native or applied microbial agents still need to be studied in-depth, also in the context of *F. graminearum* infection, with the aim of achieving the most effective use of fungicides while minimizing losses to ecosystem function [116].

## 11. Perspectives on Biotechnological Tools to Empower Oats against FBH

Based on recent advances in oat genome data, genetic engineering can support both functional studies and provide the next-generation breeding tools for the genetic improvement of oats. One of the main advantages of these techniques is their precision and speed in introducing specific traits into the plant genome. Oat biotechnology has advanced at a similar pace as the rest of cereals, although it lags still behind [91], and progress has been made in oat manipulation.

Tissue culture is an essential tool in both classical breeding, where plant cell culture facilitates the rapid production of double haploid plants, which can be employed to accelerate the process, and in modern breeding techniques, because it enables genetic manipulation and precise genome editing at the cellular level. An overview of oat tissue cultures was presented by Pathi and Sprink in 2023 [136]. This review encompasses their usage in genetic transformation, haploid technology, protoplast technology, and genome editing; also, the need to establish tissue culture and transformation genotype-independent protocols in oats and other important potential challenges are discussed.

In 2022, Mahmoud et al. [90] published the development of an Ac/Ds transposon-based gene tagging system that could facilitate and expedite functional genomic studies in oat, leading to the discovery of genes at Ds insertion sites that showed homology to gibberellin 20-oxidase 3, (1,3;1,4)-beta-D-glucan synthase, and aspartate kinase.

CRISPR genome-editing technology paved a new way for the investigation of gene function, identifying candidate gene sequences for targeted breeding. Moreover, this tool offers precious opportunities for engineering desirable traits in plants with precise genome modification and without the need for transgene integration, leading to mutated germplasm that is easier to commercialize. The engineering of oats using site-specific nucleases has not been extensively explored; also, because oats are hexaploidy, simultaneously mutating three alleles presents a significant challenge [136]. In 2021, Donoso [137] reported the first use of the CRISPR/Cas9 system to study the regulatory role of Thaumatin-like protein 8 in beta-glucan synthesis in oats. To the best of our knowledge, no attempt to use this promising tool to investigate FHB resistance in oats has been published to date. The targeted insertion or exchange of genes using homology-directed repair has not been currently applied in oats.

Successful reduction of FHB via overexpression of an antifungal gene has been observed, such as the nepenthesin 1 overexpression in barley [138], indicating a further opportunity for disease resistance against FHB that can also be exploited in oats in the future. On the basis of the principle that multiple resistance genes can be used for stronger resistance through gene pyramiding [139], the overexpression of multiple genes connected to FHB resistance may permit a broad-spectrum resistance in oats in the future.

## 12. Conclusions

At the conclusion of this update of scientific research regarding the coexistence between mycotoxigenic fungi of the genus *Fusarium* and a crop of growing nutritional interest such as oats, some points can be highlighted:

Epidemiological updates suggest that there is a clear evolution of *Fusarium* populations in oat cultivation, with the emergence of previously marginal species. One of the drivers of these changes is recognized to be climate change.

Beyond the undoubted value of agronomic practices for the containment of FHB caused by widespread or new *Fusarium* species, the availability of genetic resistance is undoubtedly an effective and environmentally sustainable tool. The source of FHB resistance can be potentially found in the worldwide, significant oat genetic resources collections available. However, screening for FHB resistance can be a complex procedure itself, and to overcome this point in the last years, several efforts have been focused on the optimization of phenotyping, including infection strategies and diagnostic methods.

Interestingly, correlations between the content of certain bioactive compounds and the resistance to FHB have been found, suggesting that increasing the content of health-benefiting components in oat grain might concurrently contribute to grain safety.

Oat genomics is making great progress, making genomic sequences, mappings, and genetic materials available to the international oat researchers and breeders' community.

The immediate prospect is to give new strength to the breeding of oats for resistance to FHB, using the many physiological, genetic, genomic, and biotechnological tools developed in recent years. The final goal is to ensure, also through genetics, the safe production of a cereal with peculiar nutritional and health-promoting characteristics.

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