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Fusarium Infection in Wheat, Aggressiveness and Changes in Grain Quality: A Review

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Abstract

Fusarium Head Blight, one of the most devasting diseases on small-grain cereals, has caused severe epidemics worldwide, altering yield and quality parameters of grains and contaminating them with fungal toxins. The aggressiveness of *Fusarium* spp. could be ascribed to different mechanisms, such as the production and release of extracellular plant-cell-wall-degrading enzymes and proteases which are crucial in the processes of fungal colonization and disease establishment. Once infection is established, mycotoxins are released, and they interfere with the metabolism of the host. Wheat grains damaged by *Fusarium graminearum* present changes in their cell structure and in the composition of carbohydrate, protein and starch granules of the endosperm, which modify their physical and chemical properties, altering the desired quality characteristics for baked goods. The protein content of wheat is one of the main determinants of its commercial value since the industrial quality of the grain depends on the concentration and type of its proteins. To evaluate the quality and end use of flour, laboratories and industries use different techniques to analyze its protein content. Since yield and quality of wheat grains depend on the genetic of cultivars, environment and level of infection in the harvested grains, this review focuses on the main events related to the infection by *Fusarium* and the evaluation of the disease from grains and flour. Special attention to changes in the protein fraction are discusses, due to its direct relationship with the commercial value.

Keywords: Wheat; *Fusarium graminearum*; Aggressiveness; Quality parameters; Gluten proteins

Introduction

Cereals have been a basic agricultural product since ancient times because of their nutritional properties, moderate cost and ability to achieve immediate satiation. Wheat–together with corn and rice–is one of the three cereals with the greatest production worldwide and it is also the most widely consumed throughout western civilization. However, crop losses due to fungal contamination represent a significant problem worldwide [1]. *Fusarium* Head Blight (FHB) is one of the most devastating diseases of small-grain cereals. Severe epidemics have occurred, altering yield and quality of grains, as it is manifested in their weight loss, carbohydrate and protein composition changes and the presence of fungal toxins [2,3]. The main etiologic agents of the FHB are *Fusarium graminearum, F. culmorum, Microdochium nivales var nivale, M. nivales var majus, F. avenaceum* and *F. poae*. The dominant fungal agent associated with this disease on wheat is the firstly mentioned *Fusarium graminearum* (Schwabe), the anamorph of *Gibberella zeae* (Schw.) Petch. [4,5]. The damage caused by *Fusarium* spp on the host depends on its aggressiveness, which is defined as the quantity of disease induced by a pathogenic isolate on a susceptible host [6]. Differences in the synthesis of enzymes and mycotoxins might be responsible for the great variability in aggressiveness observed within *Fusarium* spp populations [7]. The mycotoxin most associated with this disease is deoxynivalenol (DON), which has several adverse effects on animal and human health, such as gastrointestinal disorders and severe depression of the immune system, promoting the development of secondary infections [8-10]. Although there is a considerable amount of bibliographic antecedents about the FHB disease, a review that includes from the primary mechanisms of colonization of the fungus on the wheat, to its effects in terms of loss of quality and its evaluation–with special attention in the protein fraction due to its implication in the commercial value–, means a significant contribution to the understanding of one of the diseases that causes the greatest economic losses in crops.

Disease establishment and progression

FHB is considered a floral and monocyclic disease. The generalized model for the progression of the infection involves the entry of the pathogen through an exposed anther followed by the penetration of the ovary and the successive infections of the floral bracts [11]. The hyphae develop on the outer surface of the flowers and glumes, allowing the fungus to make growth extensions toward the stomata and other sensitive inflorescence sites [12]. Then, the fungus grows both inter- and intracellularly in the ovary and bracts and spreads from one spikelet to the other along the rachis. Finally, the growth of fungal hyphae during the later stages of the infection is accompanied by a disintegration of the organelles of the host cell along with a degeneration of the cytoplasm and subsequent collapse of some of the parenchymal cells. These drastic effects result from the action of the enzymes and the mycotoxins, with both playing an essential role in the fungal pathogenicity [13]. The production and release of extracellular plant-cell-wall-degrading enzymes (CWDEs) is crucial in the processes of fungal colonization and disease establishment. A reduced secretion of enzymes might retard both the growth of the fungus on the host surface and the overall infection process, thus giving the host more time for a defensive response [14,15]. Once infection is established, mycotoxins are released, which interfere with the metabolism of the host [16].

Fusarium aggressiveness

The aggressiveness of a pathogen is the quantity of disease induced by a pathogenic isolate on a susceptible host [17]. The aggressiveness of *Fusarium* spp. can be attributed to the production of extracellular enzymes among other mechanisms [2]. One of the early extracellular enzymes secreted by fungal pathogens during infection, are the pectic enzymes which are often required for full virulence because the hydrolytic activity softens the cell walls, thus enabling the success of further infection steps and the spread of the mycelium into the inner tissues of the plant [18,19]. Another group of relevant enzymes are the proteases, which act at an early stage of infection to degrade the

structural proteins of the cell walls in order to invade the host. At a later stage these enzymes are responsible for the degradation of the grain´s storage proteins and thus have the greatest influence on quality changes of the grains [20-22]. Several authors demonstrated that *F. graminearum* and *F. culmorum* produced CWDEs such as cellulases, xylanases and pectinases in wheat spikes at early stages of infection and observed alterations in the cell wall components of the infected host tissue. Those studies suggested that the CWDEs produced by this phytopathogen, after initially enabling tissue penetration, facilitated the consequent establishment of the disease and a rapidly ensuing colonization of the wheat spikes [13,14,23]. Different studies have tried to relate *F. graminearum* aggressiveness with the variability in its genetic structure, but the results obtained were quite variable within the same location or among different geographical areas [24-26]. Besides, the effects of climatic conditions–mainly temperature and relative humidity–are crucial on the magnitude of the disease. Thus variations in climatic conditions among growing seasons will be reflected in the degree of infection on crops [5,27,28]. Cumagun et al. [29] estimated that the isolate/ environment interaction accounted for one third of the variability for aggressiveness, measured as disease severity.

Chemical and physical alterations in infected grains

The alterations in the composition of infected grains are caused by enzymatic degradation of the cell walls and storage substances and contamination with mycotoxins by the fungus. In this regard, the effect of infection by *Fusarium* spp. on grain-cell structure and composition has been well documented by several authors. Different histochemical studies have demonstrated that grains damaged by *Fusarium* spp. show significant changes in the cellular structure as well as in the composition of carbohydrate, protein, and starch granules of the endosperm reserve [30-32]. These authors observed that moderate *F. graminearum* infection caused significant compositional changes in the carbohydrates, lipids, and proteins of American hard red spring wheat. Besides, some researchers determined the existence of cavities and furrows in the endosperm of the starch granules, which is related to the action of amylase enzymes [33,34], along with protein degradation by proteases from *Fusarium* spp. [20]. Pekkarinen et al. [35,36] characterized endoproteases synthesized by *Fusarium* species that degrade grain proteins during infection and studied proteins that could inhibit those enzymes to achieve reduction of changes in the quality of flours. Bechtel et al. [37] also observed that *F. graminearum* was capable of destroying starch granules, storage proteins, and cell walls during the invasion of wheat grains. Furthermore, *Fusarium* Protease continued to hydrolyzed endosperm proteins during dough mixing and fermentation, which resulted in weaker dough and a decreased loaf volume. The nature and concentration of the proteins in wheat are some of the main determinants of its commercial value. Therefore, FHB produces both a reduction in the yield and an alteration in the quality of the grains. Yield losses are caused by a decrease in the number, size and weight of the grains [38,39], while the diminution in grain quality is brought about when the components are altered by the pathogen [32,40].

Evaluation of damaged grains

Fusarium spp aggressiveness is related to the ability to cause damage to crops. The aggressiveness is often separated into elementary quantitative traits of the pathogen life cycle such as infection efficiency, sporulation rate, infectious period or lesion size and capacity for toxin production [2]. Its effect on wheat is mainly estimated by disease severity, measured as the percentage of symptomatic spikelets per spike at different time intervals after inoculation [41]. The disease severity is considered sometimes as a composite variable resulting from the integrated effect of infection efficiency and lesion size. The infection efficiency is defined as the probability that a spore deposited on a receptive host surface produces a lesion in the absence of competitive interactions. While, lesion size is generally defined as the area where there is fungal growth [42]. An outbreak of FHB is often accompanied by mycotoxin contamination, such as the very stable trichothecene deoxynivalenol (DON), which is not degraded during storage, milling, processing or during treatment at high temperatures [43]. The presence of DON is of crucial importance as it is considered as a contamination marker, thus its presence is monitored and quantified according to current regulations for the international marketing of cereals [8-10]. The production of mycotoxins is often considered as an aggressiveness component, however, the relationship between disease severity and mycotoxin production is not clear. Some studies did not show any relationship between aggressiveness (measured as disease severity) and toxin production [44], whereas other authors found that DON-toxin concentrations in grains were closely correlated with disease severity [29,45,46]. In spite of these contradictory reports, it is generally accepted that the accumulation of DON in kernel would also require a successful infection and colonization of the host [47]. Additionally, according to the results obtained by Prange et al. [48] high levels of infection accompanied with DON contents did not necessarily deteriorate baking quality. Moreover, the effect of different levels of infection of *Fusarium* spp. on grain yield, DON content and wheat baking quality has been analyzed in different works from crops grown under different environmental conditions [49-52]. PCR assay for the estimation of the fungal biomass, measurement of the area under a disease progress curve, a thousand kernel weight among others [6,53]. In addition, different standard techniques are used to measure the technological quality parameters of the flours obtained from the wheat grains, which can be altered according to the degree of infection observed, such as crude protein by Kjeldahl method, Zeleny sedimentation index, ash content, wet gluten, falling number, loaf volume, shape features of bread (height and diameter), as well as by means of traditional and new systems of rheological measurements [52,54-56]. It is frequently observed that increasing intensity of *Fusarium* spp. infestation worsened the rheological quality and led to the decrease of most of the evaluated baking parameters. PaPouškoVá et al. [54] observed that higher infection grade was coincided with higher the DON content. However, the crude protein content and wet gluten content were not significantly affected by the *Fusarium* spp. infection, while Zeleny sedimentation and falling number showed distinctively decreased values in the infected samples. Furthermore, Dexter et al. [57] also noted that grain samples damaged by *Fusarium*, exhibited weak dough properties and unsatisfactory baking quality. Suciu et al. [58] detected a decrease in flour yield, an increase in ash content, a darker colour, an unpleasant odour, variation in the protein content and/or wet gluten after *Fusarium* infection.

Quality traits of wheat

The yield and quality of wheat depend on both the genotype and the environmental conditions such as availability of nitrogen and water, the soil type, the temperature and the organisms that interact with the crops [59,60]. Any alteration, such as hydric stress or extreme temperature conditions, will cause changes in the grain composition. Nitrogen fertilization is an appropriate management strategy to

enhance the protein content of wheat, as indicated by the increase in the percentage of protein and by the by the profile of proteins present among the different types of proteins present, whose nature and interaction can improve the quality of the raw material. An essential characteristic for consideration within this context is the ability of a cultivar to fix nitrogen along with the management practices to be used. The use of the most suitable cultivar, in the best environmental conditions, with the most appropriate form of management, in order to attain the highest possible yield and quality per unit, is the challenge to be met [61].

Importance of proteins in wheat

The protein content of wheat is one of the main determinants of the commercial value since the industrial quality of the grains depend on the concentration and type of their proteins which in turn are a function of the variety of wheat, the environmental conditions, and the level of infection present in the grains before and after harvest [21,62,63]. The choice of which wheat genotype to sow is determined by the end use of the product along with the technological or commercial destination [64]. According to the texture of the endosperm, the wheat is classified as either hard or soft. Hard wheats give a generally higher flour yield and a higher percentage of protein than soft wheat's. Durum-wheat flours are destined for baked products that must withstand severe conditions depending on the type of product and the industrial processing involved [65].

Proteins are the major constituent of the wheat flour after carbohydrates and have been traditionally classified into albumins, globulins, gliadins, and glutenins as a reflection of their solubility in different solvents. The storage proteins, gliadin and glutenin, are the main constituents of gluten, a viscoelastic network necessary to support the rest of the chemical components of the wheat, primarily carbohydrates, and retain the gas produced during fermentation [66,67]. The functionality of gluten is one of the main parameters that govern the quality of flour and consequently the breadmaking potential of the wheat [16]. The gliadins were classified as ω, α, β and γ in relation to their electrophoretic mobilities, with the first of these having the highest molecular mass–ranging from 50 to 65 kD [68-70]. These proteins have little or no resistance to extension and appear to be responsible for the coherence and viscosity of the dough. After subsequent studies enabling a more complete understanding of their composition, the glutenins were grouped according to their sulphur content [71]. Accordingly, the gluten proteins can also be classified as: sulphur-rich or sulphur-poor. In the gluten network, the elasticity is determined by the intermolecular disulphide bonds between glutenin molecules; whereas the viscosity is determined by the monomeric fraction of gliadins, with the latter having only intramolecular disulphide secondary structures. The glutenins are a heterogeneous group of proteins, whose molecular weight ranges from about 100,000 to several million, with an average of about 3 million. The glutenin subunits are formed by high- and low- molecular weight species (HMWG and LMWG respectively) [72,73] linked by disulphide bonds. The gliadin-glutenin molar ratio and the size of glutenin polymers, determine the rheological properties of the dough. HMWG subunits are responsible for the fingerprint of wheat cultivars since the various combinations of these protein species form a unique pattern for each variety [74,75]. These subunits, furthermore, have been the most studied in relation to the elasticity of the dough and its baking performance since, along with the LMWG subunits and gliadins, the molecular associations among the larger subunits are responsible for the formation of the viscoelastic network of the gluten occurring in the presence of water and upon mechanical work [76]. Various studies have been focused on changes in the gluten storage protein and its fractions gliadins and glutenins as a result of infection by *Fusarium*, being frequently reported a reduction of glutenin amount parallel to an increase in gliadin quantity after infection in grains, while the amount of albumins and globulins remained at the same level according to analysis by chromatographic techniques [77,78]. Furthermore, electrophoretic techniques revealed destruction of protein compounds in infected wheat samples according to some qualitative changes in the protein spectra; however, no significant correlation was proved between the changes in proteins, deoxynivalenol content and damaged grains [79].

One approach used to help understand how wheat-protein composition relates to the functional properties of flour, is to survey a large number of individual wheat samples and examine how well different quantitative variations in the protein composition correlate with a range of quality parameters. Although high correlations do not necessarily signify cause-and-effect relationships, this procedure is useful in conjunction with other independent approaches for providing insight [80].

The retention of CO_2 during fermentation and subsequent baking of the bread depends on the quality of the constituent proteins. The proteolytic activity that is present in infected grains leads to the breakdown of protein structure, resulting in dough's with low cohesive properties and high extensibility, with little retention of the gas liberated during fermentation: the end result is a reduced loaf volume and a poor bread texture [81].

Protein measurement

Different methodologies can be employed for analyzing the protein composition of wheat flours; all have different levels of sensitivity and/or accuracy. The type of measurement of choice depends on the purpose, the experience of the analyst, and the time available for obtaining the result. Analysis by sodium-dodecylsulphate-polyacrylamide-gel electrophoresis (SDS-PAGE) is the technique most widely used worldwide for identifying the subunits of glutenin and gliadin through the differential electrophoretic mobility of the subunits [40,82-84]. Otherwise, the analysis by high-performance liquid chromatography (HPLC) of cereal proteins facilitated the establishment of a precise relationship between measurements of protein composition and the quality parameters [85-87]. Changes in the profile of the reserve proteins within a grain product through proteolytic activity have been analyzed efficiently by the size-exclusion form of HPLC in numerous studies [20,40,48,88-91]. Cuniberti et al. [91], for example, analyzed the protein composition-functionality relationship for a set of Argentine wheats using this methodology. The reliability of size-exclusion HPLC and the small amount of sample required for the determination make this technology an ideal tool for the early detection of promising genetic material in plant-breeding programs [88,89]. The advent of capillary electrophoresis allowed the introduction of an alternative to the conventional electrophoresis and HPLC, the two most commonly used protein-analytical methods. Colombo et al. [92] optimized the conditions for the separation of gliadins through the implementation of capillary electrophoresis and assessed the ability of this technique to discriminate between different cultivars. As an added advantage, only small amounts of sample are required, and sample preparation is simple. Moreover, this technique involves straightforward protocols for capillary column cleaning and maintenance. These characteristics enable the analysis of many samples

in a relatively short time. In this regard Brzozowski et al. [22] analyzed the changes in the storage proteins of wheat –and mainly the gliadins– resulting from the activity of the proteases of *Fusarium* spp. by means of capillary electrophoresis. In addition, a direct detection of changes in the constituent proteins can be conducted by spectrophotometry. A fast method of determining a diminution in wheat bread making quality as a result of excessive thermal treatment during drying, where the extracted proteins are stained with Coomassie Brilliant Blue G 250 and measured at 595 nm was proposed by Tosi et al. [93].

Alterations in the wheat proteins due to fungal proteases, were detected indirectly by traditional rheological instruments such as an alveograph (measuring the extensibility of the gluten network), a farinograph (evaluating the variation of the consistency of the dough), or an extensograph (detecting a modification in the extensibility) [90-93]. Although, these methods are reliably used to evaluate the gluten strength and quality at the same time they are time-consuming, labor-intensive and require large sample sizes. Therefore, relatively new rheological methodologies, which in a single measurement evaluate different rheological characteristics, in short time and with small samples, have been designed and launched into the market under the names Mixolab and GlutoPeak-Test. These methodologies show good correlations with traditional measurements. The Mixolab technique was developed by Chopin Technologies Company, and launched into the market at the AACC annual meeting in 2005. It allows a complex analysis of flour recording the mechanical changes during mixing and heating, which simulates the mechanical work as well as the thermal conditions that might be expected during the baking process [94]. The Mixolab test provides in a single step information about the water absorption capacity and kneading stability, as well as the gelatinization temperature, amylolytic activity, or starch gelling [95,96]. The high sensitivity of Mixolab system for monitoring the changes in rheological characteristics, has allowed observing that increasing contamination with *Fusarium* spp. worsened the rheological quality, with the subsequent negative effects on proteins and starch in the grain. Further, high correlations were found between Mixolab characteristics and the standard technological parameters such as farinograph, extensograph, amylograph, Zeleny sedimentation index, falling number, loaf volume and the shape features of the bread [54]. The GlutoPeak-Test (GPT) has been proposed for the quick evaluation of wheat baking quality by measuring the aggregation behavior of gluten [97]. During the test, the gluten of the flour forms a network and develops a resistance against the mixing paddle. The resistance is measured as torque and demonstrated as a torque-time curve [98,99]. Moreover, the GPT is able to clearly distinguish wheat flours quality according to measurements of aggregation behavior of gluten upon addition of water and high-speed mixing [100]. The use of multivariate statistics demonstrated that GPT indices were significantly correlated with the total protein content and with many of the conventional parameters which are currently used for flour characterization. Additionally, this technique has the advantages of f requiring few minutes of analysis (less than 10 min) and a small amount of sample (9g), properties of great interest along the value chain [98,99,101].

Discussion

Since, the yield and quality of the wheat grains depend not only on the genetic of cultivars, but also on the environment and the level of infection present in the harvested grains, the analysis of the process of primary infection and consequent plant-pathogen interaction will provide the basis for further research. The different approaches to the disease, according to the stages of FHB and its consequences tend to the interpretation and design of new control strategies. The grain protein content, as an indirect parameter for baking quality, is globally still the main criterion, because gluten proteins are recognized as the most important components governing bread-making. The functionality and versatility of flour is associated with the capacity of its storage proteins–gliadins and glutenins–to form gluten. It is generally accepted that wheat flour functionality is related to the absence or presence of specific protein types and subunits. One example is the composition of high-molecular-weight glutenin subunits (HMW-GS), which are the basis for a quality score system used until now [82]. Although each, wheat flour can organize its storage proteins into a viscoelastic network, its characteristics can greatly differ according to genotype and environmental conditions. Therefore, different classes of wheat are suited for different types of products to deliver certain functional attributes. Different criteria are used to evaluate the technological quality of the flours and therefore their destination in the wheat production chain. Both traditional and novel methods recently released to the market are highly reliable, being the size of the sample, the measurement time and its industriousness decisive criteria in the choice of the methodology to be used. Besides, based on the results published up to now, the data obtained between both types of methodologies present compatibility according to the adequate statistical tools.

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