REAL-TIME PCR DETECTION AND QUANTIFICATION OF *FUSARIUM GRAMINEARUM* FROM WHEAT SAMPLES OF A SUPERMARKET IN MEXICO CITY

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Introduction. In México, wheat is the second most important cereal crop of Mexican food. One of the fungi with the highest incidence in crops is *Fusarium graminearum*, which is the causal agent of blight or scab in wheat and its toxicity is recognized as one of the major problems of agriculture. *F. graminearum* produces two highly relevant mycotoxins (MT) in terms of food security, deoxynivalenol (DON) and zearalenone (ZEN) (1). The consumption of these toxins can cause nausea, diarrhea, vomiting, skin irritation, and feed refusal (2). In México there have been few studies related to the detection of this mycotoxins (3,4). Preventing MT fungal formation is considered the most effective measure for reducing the level of these in food, as a consequence; it is necessary the identification and control of pollutant mycobiota. Analysis of MT is generally complicated as a MT purification is required, which strongly reduces the analyte concentration. Then, rapid and simple methods for detection and quantification of *F. graminearum* are required. An option can be the search for genes involved in the production of MT. The premise is that if the gene is present, there is the possibility that the grain is contaminated with MT.

This project aimed to develop a rapid, specific and sensitive method based on the detection and quantification of MT-producing fungi in wheat by real-time PCR (qPCR).

Methods. Five wheat samples from market "Central de Abastos" in México City and five wheat samples artificially contaminated with *F. graminearum* were analyzed. Sampling was according to (EC) No 401/2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs (5). Primers were designed for identification of MT genes (poliketide synthase (*pks4*), trichothecene (*tri5*)) and endogenous genes (actin (*act*) and β-tubulin (*βtub*)). DNA extraction was done from mycelia of *F. graminearum* and wheat according to González-Osnaya, 2011 (4) and with FastID kit. qPCR was done by SYBR Green chemistry in a 7500 system (Life technologies) and master mix (MM) from different providers were evaluated. qPCR results were compared with plate counting in PDA agar method.

Results. There are several critical steps that need to be considered when establishing a qPCR-based method for DNA quantification. We observed that the method described by González-Osnaya, 2011 (4) is less useful for DNA extraction from fungi spores than FastID kit, which works well for mycelia, spores and wheat DNA. Primer design and MM are very important for obtention of a single peak in dissociation curves, meaning no dimer primer formation and strong specificity (Fig. 1). The quantification results are presented in Table 1.

Fig.1 Amplification (A) and Dissociation (B) curves of *tri5* gene from different *F. graminearum* DNA concentrations by qPCR with SYBR Green.

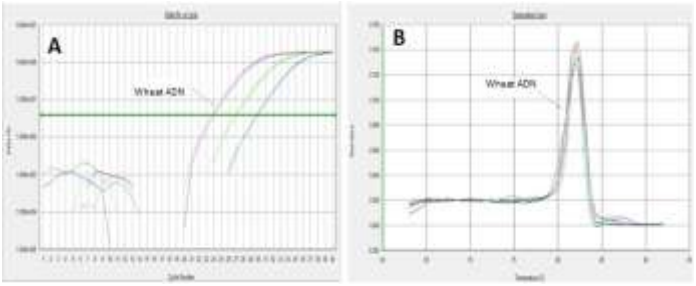

Table 1. Plate count of fungi and qPCR quantificaction of *pks4* and *tri5* genes of *F. graminearum* in commercial wheat samples.

	Plate counting	Gen *tri5*	Gen *pks4*
Sample	UFC of Fungi*/g of wheat	Concentration (%)	Concentration (%)
A	2885	Negativo	Negativo
B	25	Negativo	Negativo
C	25	0.007	Negativo
D	15	0.035	Negativo
E	1440	0.016	Negativo

*None of observed colonies had the *F. graminearum* morphology.
Results are the average of three different samples.

Conclusions. A rapid, specific and sensitive method based on the detection and quantification of MT-producing fungi in wheat by qPCR was standardized, which will detect and quantify *F. graminearum* on wheat samples with a detection limit (LD) of 0.1 pg of *F. graminearum* per nanogram of ADN extracted.

Although many fungi colonies on agar were counted, especially in two wheat samples (A and E), none showed the morphology of *F. graminearum*. However, qPCR data indicated that some of them had a low concentration *F. graminearum.* The detection and quantification of both MT genes is important because we have not observed *F. graminearum* by using *pks4* gene, while it can be detected in low concentration in three samples by using tri5 gene.

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