Quantitative PCR (qPCR) based method for predicting high levels of *Fusarium* toxins in barley samples

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The *Fusarium sambucinum* species complex is a main contaminant of small-grain cereals, including barley. Species belonging to this group are able to produce mycotoxins, among them are the type B trichothecenes (deoxynivalenol/DON and nivalenol/NIV), zearalenone (ZEN) and the "emerging" *Fusarium* toxins, enniatins (ENNs). Due to their significance, alternative methods for their screening have been reported in the literature. Notably, the quantitative PCR technique (qPCR) shows potential in this regard, through the quantification of key genes that are part of the biosynthetic pathway of these toxins. The aim of this study was to validate a qPCR method as a rapid test to predict mycotoxins in barley samples, by quantifying key biosynthetic genes of DON and its acetylated forms (15-acetyl deoxynivalenol/ 15-ADON and 3-acetyl deoxynivalenol/ 3-ADON) (*TRI12*), NIV (*TRI12*), ZEN (*ZEB1*) and ENNs (*ESYN1*) and correlate each one of them with the mycotoxin levels determined by LC-MS (liquid chromatography coupled with mass spectrometry). Thirty-five barley samples grown in Brazil were selected and *EF1-α* was used as reference gene. Pearson correlation analysis was performed using Prism software version 9. The validation of the qPCR method showed adequate results. Positive correlation between *TRI12* and DON was observed, as well as between the concentrations of *ESYN1* and enniatins. However, there was no correlation between *TRI12* and NIV, as well as between *ZEB1* and ZEN, due to the low levels of these mycotoxins in the samples. These results demonstrate the use of qPCR for predicting mycotoxins in highly contaminated grains, especially for those where the contamination levels are above the limits established by the government agencies. Nevertheless, further studies are necessary in order to

determine a better correlation between lower levels of mycotoxins and a target mycotoxin biosynthetic gene. Therefore, the use of gene expression data may be a strategy for a more reliable correlation.

Key words: mycotoxins, *Fusarium*, contamination.