Trichothecene genotypes of toxigenic *Fusarium* species associated with wheat head blight in western Canada

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Abstract

In this study, infected wheat stem and grain samples were collected from three field plots in Alberta (Lethbridge, Lacombe and Beaverlodge) and one field plot in Saskatchewan (Scott) to characterize the major *Fusarium* species associated with FHB in western Canada as well as the associated trichothecene genotype. A *Fusarium*-selective medium was used to isolate *Fusarium* species and the translation elongation factor 1 alpha (*TEF1α*) gene sequence was used for molecular identification of the isolates. A total of 263 *Fusarium* isolates belonging to nine *Fusarium* species were recovered. PCR assay based on the *Tri5* gene (encoding trichodiene synthase) was used to screen for trichothecene-producing species, and the results showed that, 132 *Fusarium* isolates (46.4%) were found to amplify *Tri5* gene (*Tri* +). Trichothecene genotyping using two multiplex PCR assays based on the *Tri*3 and *Tri*12 genes encoding for Trichothecene 15-O-acetyltransferase and Trichothecene efflux pump, respectively, showed that the 3ADON trichothecene is the most dominant genotype in all trichothecene producing *Fusarium* species tested during this study followed by type-A trichothecene (29.5%), while 15ADON was found to be the least trichothecene genotype observed (2.5%). A phylogenetic analysis of selected *Fusarium* isolates based on the *TEF1α* and the *Tri5* gene sequences showed that, trichothecene genotype differences are not well correlated with the species evolutionary relationships of FHB-associated *Fusarium* species. The results presented here extend the previous knowledge about the adaptive evolution within trichothecene genes.

Methodology

Sampling and isolation of *Fusarium* pathogens:

Wheat stem and grain samples were air dried and plated in 9-cm petri plate containing PCNB medium (5 seeds or 3 nodes /plate). From each location, 240 seeds and 144 nodes were examined for the presence of *Fusarium*. Plates were incubated at 24 ºC under 12-hours photoperiods for 5 days and growing cultures were purified on PDA medium for DNA extraction and molecular characterization.

DNA extraction, PCR amplification and phylogeny:

DNA was extracted from *Fusarium* isolates using DNeasy Plant Mini Kit (Qiagen), the primers EF1/EF2 (**O'Donnell et al 1998**) were used to amplify the *TEF1α* gene, and the primers Tri5-1/Tri5-2 (**Niessen and Vogel 1998**) were used to amplify *Tri5* gene for sequencing and molecular characterization of *Fusarium* isolates. Trichothecene genotype was determined by multiplex PCR based on both *Tri3* and *Tri12* genes according to **Ward et al 2007**. *TEF1α* and *Tri5* sequences were analyzed phylogenetically using the PhyML 3.0 software (**Guindon et al., 2010**) and phylogenetic trees were reconstructed with iTOL online tool (**Letunic and Bork , 2016**).

Conclusion

***F. graminearum* is not the main causal agent for FHB in western Canada, and more concern should be given to the other *Fusarium* species involved in FHB complex (e.g. *F. poae*, *F. culmorum* and *F. avenaceum*). Nearly, half of the tested *Fusarium* isolates (46.4%) were found to produce trichothecene mycotoxins, with the more virulent 3ADON representing the most dominant trichothecene genotype.**

Results

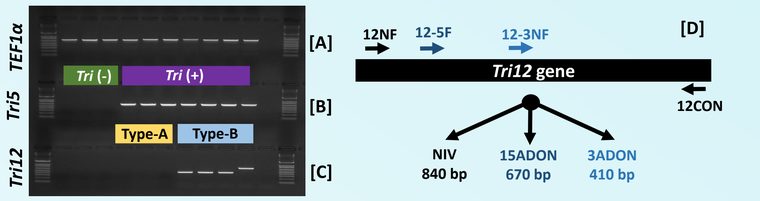


Figure 1: PCR amplification of *TEF1α* [A], *Tri5* gene [B], and *Tri12* multiplex PCR for trichothecene genotyping [C]. *Tri12* gene was amplified by multiplex PCR using four primers (12NF, 12-15F, 12-3F and 12CON) in order to determine the type B trichothecene genotype in *Fusarium* isolates. Three different genotypes can be identified based on the amplicon size: NIV, 15ADON and 3ADON **[D]**.

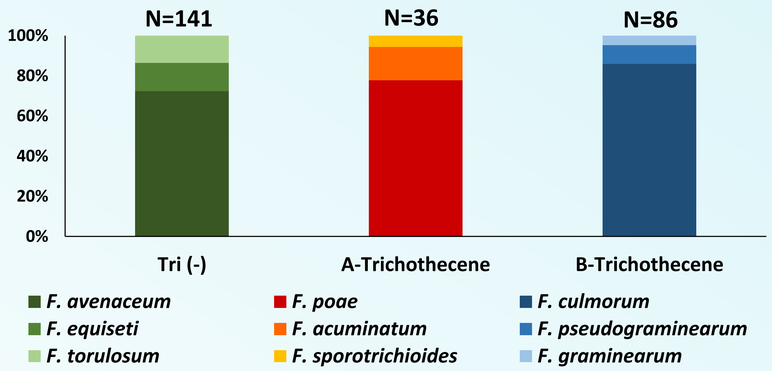


Figure 2: Trichothecene genotypes among the tested *Fusarium* species. 46.4% of the tested *Fusarium* isolates were found to be trichothecene-producers (i.e amplify *Tri5* gene*). F. culmorum* is the most dominant species with all isolates having the 3ADON genotype.

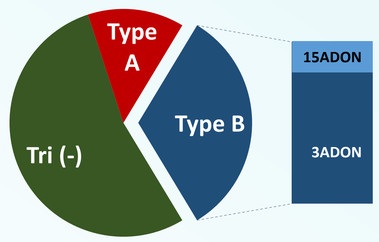
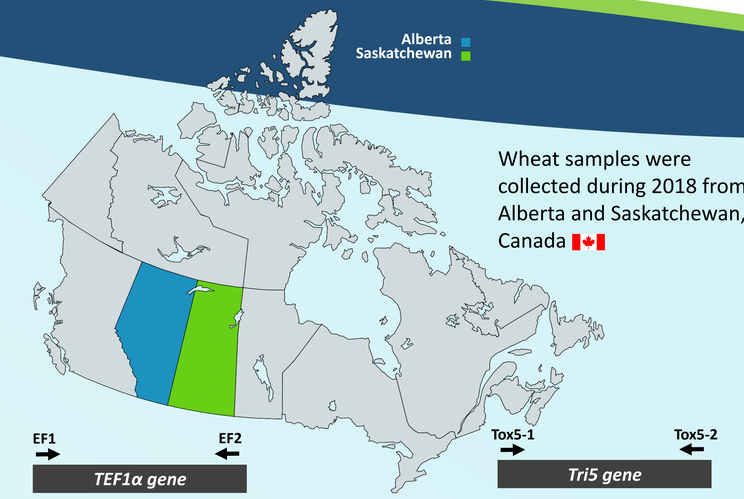


Figure 3: Trichothecene genotype frequencies. 32.7% of the tested *Fusarium* species are DON-producers , with the more toxic 3ADON genotype dominating type-B trichothecenes. The 15ADON genotype only recorded in *F. graminearum* isolates.



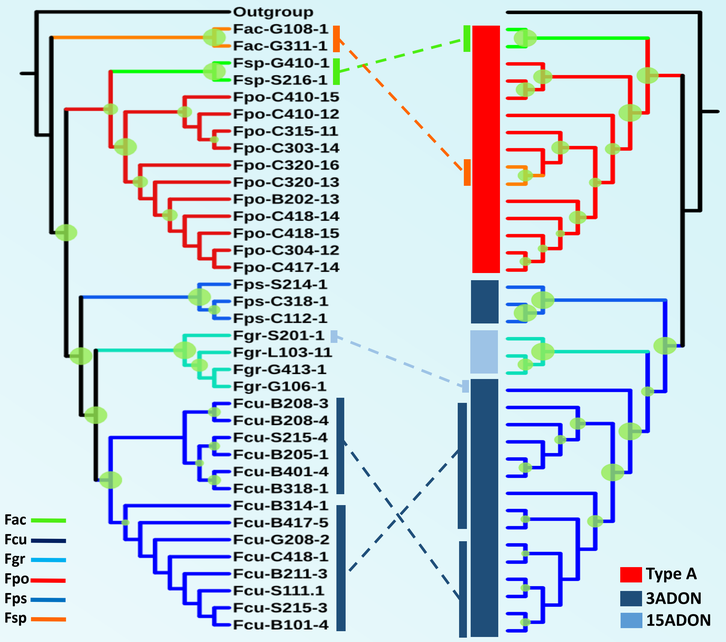


Figure 4: Phylogenetic relationships between trichothecene genes evolution (based on *Tri5* sequences) and their corresponding *Fusarium* species evolution (based on *TEF1α* sequences. The tree topology is based on neighbour joining analysis and the circles at the nodes represent the boot strap support values (100-1000). The trees showed that, trichothecene genotype differences are not well correlated with the species evolutionary relationships.

References

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