Toward molecular differentiation of common bunt and dwarf bunt fungi

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Bunt disease of grasses is characterized by the formation of black, powdery teliospores, which partially or completely replace the ovary and thus the seed of the host plant. The causal agents of these diseases all belong to the smut fungal genus *Tilletia*. Some 140 species of *Tilletia* (Ustilaginomycotina, Basidiomycota) are described from their Poaceae hosts. Three of these species, *T. caries*, *T. controversa* and *T. laevis*, cause bunt diseases of wheat. *T. caries* and *T. laevis* are the causal agents of common bunt and *T. controversa* causes dwarf bunt. Common bunt is present worldwide in wheat-growing areas whereas dwarf bunt is restricted geographically and is an important quarantine species in several countries.

The three species are morphologically distinct and also differ significantly in infection biology. Therefore, differentiation of species is currently done based on morphological and physiological features. However, this is very time-consuming and requires expert knowledge. The main aim of this study is to develop a robust, quick and reliable molecular method for the differentiation of *T. caries*, *T. controversa*, and *T. laevis.*

Initially, analyses of standard phylogenetic markers (nuc rDNA LSU, ITS, rpb2, tef1α, β-tub) were performed to find polymorphies between the species. However, using these gene regions very little variability could be found in total, and the few differences observed were neither consistent within nor between species. Subsequently, random detection of polymorphism was explored using ISSR-PCR (inter simple sequence repeats). ISSR-PCR products were sequenced, however, obtained sequences did not show sufficient polymorphism to differentiate the species. The results of our phylogenetic studies confirm that these three bunt species are very closely related, and cannot be differentiated with standard phylogenetic markers, thus even questioning their species rank or suggesting that these species have split only recently.

Because of the failure of these two approaches and because no more genetic information was publicly available, whole-genome sequencing was initiated using Single-molecule real-time (SMRT) sequencing developed by Pacific Bioscience (PacBio®).

In the meantime genome sequences of *T. caries and T. controversa* have been published (Illumina data) which are currently under investigation for potential genomic regions that can be used for differentiation of the three species. As a result one promising gene region has so far been identified. The specificity of this region is currently tested using 70 specimens collected from different geographical region, collected between 1933 and 2016.

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186

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