COMPARATIVE GENOME HYBRIDIZATION DEMONSTRATES LOSS OF CHROMOSOMES IN MYCOSPHAERELLA GRAMINICOLA ISOLATES

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We report on detailed genetic linkage analyses of the ascomycete *Mycosphaerella graminicola*, the causal agent of septoria tritici blotch of wheat, using 1793 Diversity Arrays Technology markers, 258 AFLP markers and 25 SSR markers that resulted in one of the most dense genetic linkage maps currently available for a fungus. Two mapping populations were generated with isolate IPO323 (originating from Dutch bread wheat) as a common parent in crosses with either IPO94269, A Dutch bread wheat strain, or IPO95052, an Algerian durum wheat strain. In both crosses, we identified dispensable chromosomes that were present in both parental isolates but absent in 15-20 % of the progenies. We demonstrate that these chromosome number polymorphisms (CNPs) are due to aberrations during meiosis and are most probably due to nondisjunction during meiosis II since twin isolates were mitotically stable. We conclude that *M. graminicola* has the highest number of dispensable chromosomes that vary in size from 0.77 to 0.39 Mbp, representing 40 % of the chromosomal complement. Chromosomes loss neither hampered sexual compatibility nor pathogenicity, which underscores the extraordinary genome plasticity of *M. graminicola*.

To further analyze this genome plasticity we developed a Comparative Genomic Hybridization (CGH) NimbleGen whole genome tiling array (http://www.nimblegen.com/) based on the finished genome of *M. graminicola* IPO323 (http://genome.jgi-psf.org/Mycgr1/Mycgr1.home). The detailed genetic maps and karyotypes enable genome wide analyses for variation in DNA copy number and provides a new window to study chromosome polymorphism and translocations in natural isolates. In total, 50 to 75–mer 387,000 probes - based on the IPO323 genome, BAC-ends of isolate IPO94269 and wheat genes - were spotted on the array with a mean probe spacing of 118bp and a maximum stringency of 2. We analyzed the aforementioned parents and two progeny isolates #2133 and #51 from both mapping populations. Hybridization of *M. graminicola* IPO94269, #2133 and #51 displayed substantial CNPs compared to the IPO323 reference genome for the specific missing chromosomes previously demonstrated by karyotyping as indicated by graphical genotyping. Our results reveal widespread deletions between the progeny isolate #2133 and the parental isolate IPO323 and confirm the absence of three complete chromosomes in this progeny isolate. We currently exploit CGH to identify large genome differences such as deletions or translocations to understand genome plasticity and its relation with virulence, host specificity and speciation. Mapped recombination breakpoints provide a window on actual recombination sites. These studies address highly relevant genomic aspects of *M. graminicola* that take place under natural conditions and will eventually enable us to understand the genomic dynamics of this pathogen and its impact on pathogenicity and fungicide resistance.

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