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Development of SSR markers and assessment of polymorphism by High Resolution Melting Analysis in populations of chestnut nut rot agent *Gnomoniopsis castanea*

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DEVELOPMENT OF SSR MARKERS AND ASSESSMENT OF POLYMORPHISM BY HIGH RESOLUTION MELTING ANALYSIS IN POPULATIONS OF CHESTNUT NUT ROT AGENT *GNOMONIOPSIS CASTANEA*. F. Sillo, G. Lione,

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In the complex phytosanitary situation of European chestnut, a relevant role is played by the recently described chestnut nut rot agent *Gnomoniopsis castanea*. In order to elucidate the epidemiology of this pathogen through a population genetic study, five SSR loci were isolated starting from four genomic libraries enriched in SSR sequences prepared and screened through the Microsatellite Amplified Library (MAL) method. To assess their polymorphism, a recently developed analysis called High Resolution Melting Analysis (HRMA) was used on 132 *G. castanea* isolates, collected in Italy, France and Switzerland. This approach allowed to distinguish different alleles based not only on repeat number but also on melting temperature differences among amplicons. Based on HRMA results, isolates were grouped in different clusters, each representing an allelic variant. Clusterization was confirmed by sequencing and alignment of the representative alleles. These results indicate that HRMA is an efficient, rapid and sensitive alternative to traditional electrophoresis-based method for SSR markers. It also allows to detect polymorphisms present in the SSR flanking regions, thus allowing to discriminate a larger number of haplotypes in fungal genetic population studies.