CLONING AND CHARACTERIZATION OF A TRANSPOSABLE ELEMENT FROM TUBER MELANOSPORUM: A NEW TOOL FOR GENOTYPING THE FINEST BLACK TRUFFLE

C. RICCIONI, A. RUBINI, F. PAOLOCCI, S. ARCIONI

National Research Council, Plant Genetics Institute, Perugia division, Via Madonna Alta 130, 06128 Perugia, Italy
claudia.riccioni@irmgpf.pg.cnr.it

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Tuber spp. are ascomycetes that establish ectomycorrhizal symbiosis with shrubs and trees and produce hypogeous ascocarps, known as truffles. The finest species T. melanosporum and T. magnatum, due to their organoleptic properties and the restricted geographic distribution, are highly prized and appreciated worldwide. T. melanosporum shows variation in several traits, including taste and aroma, across its geographical range. Such variation was accounted to environmental factors only, since few SSR-loci and RAPD analyses have not revealed a geographic pattern of genetic diversity, at least among its northernmost populations (Bertault et al. 1998, 2001). Both highly informative markers and extensive sampling are, however, requested to shed light on genetic structure and species dynamics of this truffle species as well as to type samples according to their provenance. On this purpose, we are attempting to develop retrotransposon-based molecular markers (i.e. SSAP, Sequence-Specific Amplification Polymorphism), as highly informative and throughput genetic markers. Therefore, degenerate primers (Pearce et al 1999) designed on the conserved regions of RNaseH genes of plant retrotransposons were used to clone an homologous PCR fragment from T. melanosporum. This PCR fragment was subsequently extended to its 5’ and 3’ ends by a genome-walking strategy and a retroelement with a gag-protease-dUTPase-reverse transcriptase-RnaseH-Integrase arrangement was cloned. The element we have identified appears therefore to be a gypsy-like retrotrasposon. The presence of the dUTPase gene in the POL region has only been found in the basidiomycetes Phanerochaete chrysosporium Ty3/gypsy retrotrasposon (PcGR2), likely as a residual of retroviral ancestral DNA.

This is the first retroelement so far identified in Tuber. Although LTR sequences have not been identified yet, we have exploited the possibility to use our retroelement for testing T. melanosporum genetic variability. To this purpose, 4 PCR primers were designed on the POL region and used in combination with a microsatellite primer (GACA)₄ to assay 20 T. melanosporum ascocarps from different geographic areas. A primer pair combination yielded polymorphic fingerprints. Thus, retroelements could be successfully used to disclose T. melanosporum biodiversity and genotype the finest black truffles according to their origin.

References