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Genetic characterisation of *Botrytis cinerea* populations in Eger wine district

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Abstract

Botrytis cinerea (teleomorph: *Botryotinia fuckeliana*) is a cosmopolitan ascomycetous fungus that causes grey mould on a great number of plants by infecting various tissues. In grapevine, the frequent occurrence of *B. cinerea* prior to harvesting results serious losses of fruits and deterioration of wine quality. Information about the populations of plant pathogen fungi is essential for the effective and economic protection. A plant pathogenic fungal population with high level of genetic variation is likely to adapt more rapidly to fungicides or resistant host plants than populations with little or no genetic variations, and information on the level of migration between populations and on the presence or absence of sexual reproduction within a population may indicate how rapidly will novel (fungicide resistant or more pathogenic) genotypes spread between populations. Application of the tools provided by recent advances in population genetics and biology are crucial in gathering those information. Characterization of their genotype was done by analyzing MSB1 minisatellite sequences and by the determination of the presence or absence of transposon elements. In general, sequence analysis revealed a high degree of genetic diversity and the combination of alleles suggests the presence of sexual reproduction in the area, while the disperse distribution of the genotype indicates high migration rate. Based on the absence or presence of two transposable elements *Boty* and *Flipper*, *vacuma*, *transposa* and *flipper* type isolates were identified.

Introduction

Botrytis cinerea (de By.) Pers. (teleomorph: *Botryotinia fuckeliana*, Whetz) is a cosmopolitan ascomycetous fungus that causes grey mould on a great number of plants by infecting various tissues (Coley-Smith et al, 1980). In grapevine, the frequent occurrence of *B. cinerea* prior to harvesting results serious losses of fruits and deterioration of wine quality. *B. cinerea* has been shown to have several variable genetical and physiological traits. Studies on French and Chilean isolates revealed the presence of three intrapopulation: (1) *transposa*, having transposable elements *Boty* and *Flipper*, (2) *vacuma*, having no transposable elements, and (3) *boty* containing transposable element *Boty* alone (Munoz, et al 2002). Characterization of their genotype was done by analyzing MSB1 minisatellite sequences.

Materials and methods

Isolation: One hundred isolates of grapevine berry-infected *B. cinerea* from various locations of the Eger wine district were collected. Individual strains were obtained by single-

spore isolations. Potato dextrose agar (PDA, Scharlau) was used as a base medium. *DNA isolation and detection of transposable elements*: Magnalyser (Roche) was used for the disruption of fungal cells. DNA was isolated with Plant DNA Purification Kit (QuiaGene). Isolates were tested for the presence of *Boty* (648 bp), (Diolez et al. 1994) and *Flipper* (1250 bp), (Levis et al 1997) transposon elements with a single PCR reaction (Munoz et al. 2002). *MSB1 amplification and sequencing*: PCR reactions were performed using Pwo Master (Roche). Primers and conditions for MSB1 were as described by Giraud et al (1998). Sequencing were done by MWG-biotech, Ebersberg, Germany.

Results and discussion

Isolates containing only the *Flipper* transposon element showed the highest ratio (60%) of them. This genotype is absent in France and Chile (Munoz 2002). The *Transposa* elements containing 32%, and there are 8% *vacuma* among the isolates. Sequence analysis of minisatellite (MSB1) showed polymorphism in all the tree DNA fragments. In general, sequence analysis revealed a high degree of genetic diversity, with no widespread of clonal lineages. The analysis of MSB1 sequences indicated tree bigger groups. Parsimony phylogenetic analysis of MSB1 sequences indicates only one closely related group (clone) of *B. cinerea*. We could not find any geographical preference of the different genotypes groups. The combination of alleles suggests the presence of sexual reproduction in the area, while the disperse distribution of the genotype indicates high migration rate.

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