DISTRIBUTION OF TRANSPOSONS IN *BOTRYTIS CINEREA* ISOLATES COLLECTED FROM THE WINE REGIONS OF EGER AND TOKAJ, HUNGARY

Kálmán Z. Váczy[1] – Levente Karaffa[2] – Erzsébet Fekete[2] – György J. Kövics[3] – Lajos Gál[1] – Erzsébet Sándor[3]

[1]Research Institute for Viticulture and Enology, Eger, Hungary
[2]Department of Genetics and Applied Microbiology, Faculty of Science, University of Debrecen, Debrecen, Hungary
[3]Department of Plant Protection, Faculty of Agriculture, University of Debrecen, Debrecen, Hungary

Botryotinia fuckeliana (de Bary ex de Bary) Whetzel (anamorph: *Botrytis cinerea* Pers.:Fr.) is a cosmopolitan ascomycetous fungus that causes grey mould on a great number of plants in the temperate zone worldwide by infecting various tissues (Jarvis, 1980). In grapevine, the frequent occurrence of *B. cinerea* prior harvesting results in serious losses of fruits and deterioration of wine quality. This is also the case in Eger, a major Hungarian wine region in the North-Eastern part of the country, where *B. cinerea* is considered to the third most important grapevine pathogen after downy mildew (*Plasmopara viticola* /Berk. and Curt ex de Bary/ Berl. and de Toni) and powdery mildew [*Erysiphe necator* Schwein. var. *necator* (syn.: *Uncinula necator* /Schwein./ Burrill var. *necator*)], with an estimated annual loss of up to 15-20 %. In contrast, some 100 km eastwards in the Tokaj wine region, *B. cinerea* is also responsible for the phenomenon called 'pourriture noble' (noble rot). Under certain unique environmental conditions, mycelia growing on the surface of the uninjured, healthy berry drains water (but no substrates) via the fine infection hyphae. As a consequence, the concentration of all the soluble compounds within the berry significantly increase (Jarvis, 1980). Such berries yield the sweet, special quality wine called „aszu".

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Transposable elements (TEs) are fragments of DNA that can insert into new chromosomal locations and often make duplicate copies of themselves in the process (Feschottes et al., 2002). TEs were first discovered in maize (McClintock, 1984) and later have been found in several eukaryotic, eubacteria and archaea genome. In addition to the wide array of 'hosts', the variety of transposons described also increased considerably (Finnegan, 1989). Fungal transposons were first identified in the yeast *Saccharomyces cerevisiae* (Boeke, 1989), though the first indirect evidence for their

presence in filamentous fungi arose years earlier from conventional genetic studies with *Ascobolus immersus* mutants (Decaris et al., 1978).
There are two main classes of TEs (Finnegan, 1989). Class I elements are related to retroviruses and they transpose through the reverse synthesis of DNA from template RNA, while class II elements move in the genome through direct DNA to DNA transposition without an RNA intermediary. Class I elements are known as retrotransposons and include TEs with or without 'long terminal repeated sequences' (LTRs). Retroelements have been found in a number of fungal species such as *Alternaria alternata* (Kaneko et al., 2000), *Ascobolus immersus* (Goyon et al., 1996), *Aspergillus fumigatus* (Neuvéglise et al., 1996), *Aspergillus nidulans* (Nielsen et al., 2001), *Neurospora crassa* (Kinsey and Helber, 1989). DNA transposons are also widespread and have been described among others in *Agaricus bisporus* (Sonnenberg et al., 1999), *Ascobolus immersus* (Colot and Rissignol, 1995), *Aspergillus niger* (Glayzer et al., 1995), *Magnaporthe grisea* (Kachroo et al., 1994), *Nectria haematococca* (Enkerli et al., 1997), *Neurospora crassa* (Yeadon, 1995), and *Podospora anserina* (Hamann et al., 2000).
B. cinerea has been shown to possess two transposons. *Boty* is a class I LTR-retro-transposon (Diolez et al., 1995), while *Flipper* is a class II element (Levis et al., 1997). In this paper we will show that at least four genotypes of isolates related to the presence or absence of these transposons occur in the Eger wine region. Potential significance of this finding is discussed.

Materials and Methods

Field strains of *B. cinerea* were collected from various locations of the Eger and Tokaj wine districts. They were isolated from infected berries between 2003 and 2004 during the vintage period (September-October). Fungal strains from both wine regions are numbered by the chronology of collection, irrespective to the local provenance.
DNA was extracted from aerial mycelium of *B. cinerea* with Plant DNA Purification Kit (QuiaGene). Transposons were detected with PCR reactions described at Munoz et al., 2002. Presence or absence of the two transposons was confirmed by agarose gel electrophoresis (Figure 1) using standard protocols (Sambrook et al., 1989).

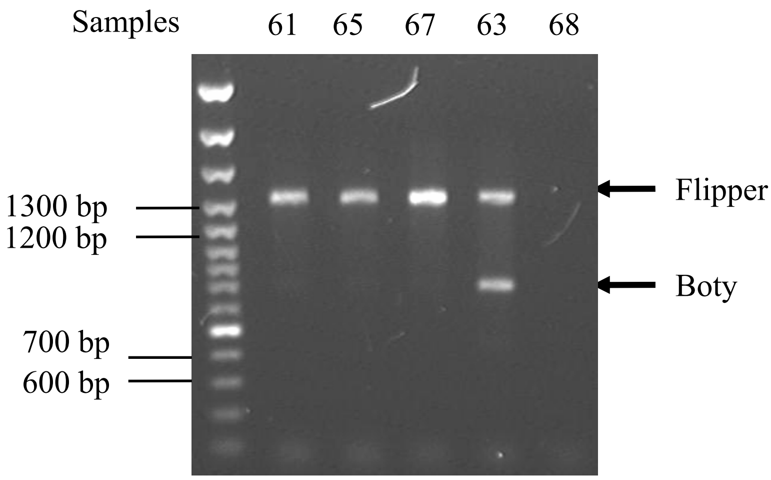

Figure 1. Gel electrophoresis picture of transposon elements

Results

B. cinerea has been shown to possess a highly versatile genome reflected in a considerable metabolic flexibility. Depending on the actual environmental conditions (Martinez et al., 2003), the fungus is able to act both as a saprophyte and a pathogen, and has developed resistance to most of the fungicides used to control it (Faretra and Pollastro, 1991; Leroux et al., 1999).

Studies on French and Chilean *B. cinerea* isolates have revealed the presence of three distinct intrapopulation: (1) transposa, having transposable elements *Boty* and *Flipper*, (2) vacuma, having none of the two and (3) boty containing the transposable element *Boty* alone (Munoz et al., 2002). The flipper intrapopulation, containing the transposable element *Flipper* alone has thus far only been described in two separate isolates from the United Kingdom and France (Albertini et al., 2002).

Boty and *Flipper* are both specific for *B. cinerea*. While proteins encoded by transposable elements are supposed to be used exclusively for the purpose of their own reproduction without any interference with the metabolism of the host organism, a more rapid biomass formation in the vacuma intrapopulation relative to boty and transposa was observed (Martinez et al., 2003). It should also be noted that the level of fungicide resistance significantly differed in transposa and in vacuma-type *B. cinerea* populations in French isolates (Albertini et al., 2002). It is not yet known whether the two events are related to each other, and if they are then it is a cause or a consequence of the altered transposon pattern.

In the framework of this project 68 and 17 *B. cinerea* isolates have been collected from the Eger and Tokaj wine regions, respectively. To the best of our knowledge, this is the first Central-Eastern European collection of its kind, and only the fourth worldwide. Two French studies from the Champagne and Bordeaux wine regions, respectively analysed a collection of 259 (Giraud et al., 1997) and 121 (Martinez et al., 2003) isolates, while a Chilean study was based on 69 cases. Distribution of the *Flipper* and *Boty* transposons in the Hungarian isolates are markedly different to those in French and Chilean collections (Table 1).

Table 1. Distribution of transposons in *Botrytis cinere*a isolates collected from the Eger and Tokaj vine regions, Hungary, and percentage of the transposa, vacuma, flipper and boty intrapopulations in other countries as found in the literature

Type of strain[a]	No. of isolates[b]		Percentage		Percentage		
	Eger	Tokaj	Eger	Tokaj	Chile[(c)]	France[(d)]	France[(e)]
transposa	12	0	17.64	0	79.71	75.00	61.34
flipper	51	17	75.00	100	0.00	0.00	0.00
boty	1	0	1.47	0	11.59	0.00	0.00
vacuma	4	0	5.89	0	8.70	25.00	38.66
All	68	17	100	100	100	100	100

[a] transposa isolates contain both the *Boty* and *Flipper* transposable elements. Flipper and boty are isolates containing *Flipper* or *Boty*, respectively, while vacuma are isolates without *Boty* and *Flipper*.
[b] all the isolates as well as viticultural and geographical details of their provenance are available from the first author of this paper upon request.
[c] Munoz et al.,2002; [d] Giraud et al., 1997; [e] Martinez et at., 2003

While genotype transposa is clearly prevalent in the French and Chilean samples with some two-third of the isolates containing both transposons, the percentage of this particular intrapopulation was less than 18 percent in Eger and zero percent in Tokaj. While genotype boty was present in over 10 percent of the Chilean collection, we found only one single strain in Eger (and none in Tokaj) that carried the *Boty* transposon alone. We note, while the French studies indicated the presence of boty genotype in their collections, no defined values were provided in either case.

Distribution of the genotype vacuma ranged between 8 and 38 percent in the literature. Our investigations yielded only a handful of vacuma isolates in Eger and none in Tokaj.

The most striking observation in our study is the appearance of the genotype flipper, an intrapopulation of *B. cinerea* hitherto considered extremely rare. However, this genotype is obviously prevalent in the two Hungarian wine regions studied, with 75 percent of the isolates containing only flipper transposon in Eger and all in Tokaj. None of the three collections cited in this paper have reported on the appearance of this genotype.

Transposons are highly mobile genetic elements while *B. cinerea* is a truly cosmopolitan fungus. Comparative analysis of transposon distributions in *B. cinerea* isolates collected from all around the world may thus be a worthy method to study fungal population genetics.

There are no tested hypothesises on the physiological role of transposons. Mobility of transposons including those in the filamentous fungi *Magnaporthe grisea* (Ikeda et al., 2001) and *Fusarium oxysporum* (Mes et al., 2000) were reported to increase during certain stress conditions such as substrate deficiency, drought, heat and exposure to γ-radiation. It remains to be tested whether stress conditions will influence *B. cinerea* transposons in anyway.

Discussion

This study showed that all of the four transposon-related genotypes of *B. cinerea* ever described in the literature exists in the Eger wine region. Most noteworthly, genotype flipper, considered extremely rare elsewhere in the world is apparently dominant both in the Eger and Tokaj wine regions. In fact in Tokaj, flipper was the only transposon found. It remains to evaluate whether *B. cinerea* genotypes defined over transposon distribution are relevant to the role the fungus plays in viticulture and enology.

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References

Albertini, C., Thebaud, G., Fournier, E. and Leroux, P. (2002): Eburicol 14*α*-demethylase gene (*CYP51*) polymorphism and speciation in *Botrytis cinerea.* Mycol. Res. 106:1171-1178.

Boeke, J.D. (1989): Transposable elements in *Saccharomyces cerevisiae*, In: Mobile DNA, Berg, D.E. and Howe, M. (Eds) ASM Press, Washington DC, pp. 335-374.

Colot, V. and Rossignol, J.L. (1995): Isolation of the *Ascobolus immersus* spore color gene b2 and study in single cells of gene silencing by methylation induced premeiotically. Genetics 141:1299-1314.

Decaris, B., Francou, F., Lefort, C. and Rizet, G. (1978): Unstable ascospore color mutants of *Ascobolus immerses*. Mol. Gen. Genet. 162:69-81.

Diolez, A., Marches, F., Fortini, D., and Brygoo, Y. (1995): *Boty*, a long-terminal-repeat retroelement in the phytopathogenic fungus *Botrytis cinerea*. Appl. Environ. Microbiol. 61:103-108.

Enkerli, J., Bhatt, G. and Covert, S.F. (1997): *Nht1*, a transposable element cloned from a dispensable chromosome in *Nectria haematococca*. Mol. Plant-Microbe Interact. 10:742-749.

Faretra, F. and Pollastro, S. (1991): Genetic Basis of resistance to benzimidazole and dicarboximide fungicides in *Botryotinia fuckeliana* (*Botrytis cinerea*). Mycol. Res. 95: 943-951.

Feschottes, C., Zhang, X. and Wessler, S.R. (2002): Miniature inverted-repeat transposable elements and their relationship to established DNA transposons, In: Mobile DNA II, .Craig NL, Craigie, R., Gellert, M. and Lambowitz, A.M. (Eds) ASM Press, Washington DC, pp.1147-1158.

Finnegan, D.J. (1989): Eukaryotic transposable elements and genome evolution. Trends Genet. 5: 103-107.

Giraud, T., Fortini, D., Levis, C., Leroux, P. and Brygoo Y. (1997): RFLP markers show genetic recombination in *Botryotinia fuckeliana* (*Botrytis cinerea*) and transposable elements reveal two sympatric species, Mol. Biol. Evol. 14: 1177-1185.

Glayzer, D.C., Roberts, I.N., Archer, D.B. and Oliver, R.P. (1995): The isolation of *ant1*, a transposable element from *Aspergillus niger*, Mol. Gen. Genet. 249: 432-438.

Goyon, C., Rossignol, J.L. and Faugeron, G. (1996) :Native DNA repeats and methylation in *Ascobolus*. Nucleic Acids Res. 24: 3348-3356.

Hamann, A., Felle, F. and Osiewacz, H.D. (2000): The degenerate DNA transposon *pat* and repeat-induced point mutation (RIP) in *Podospora anserina*. Mol. Gen. Genet. 263: 1061-1069.

Ikeda, K., Nakayashiki, H., Takagi, M., Tosa, Y. and Mayama, S. (2001): Heat shock, coper sulfate and oxidative stress activate the retrotransposon MAGGY resident in the plant pathogenic fungus *Magnaporthe grisea*. Mol. Genet. Genomics 266: 318-325.

Jarvis, W.R. (1980): Taxonomy. In: The Biology of *Botrytis*. Coley-Smith, J.R., Verhoeff, K. and Jarvis W.R. (Eds) Academic Press, London pp. 1-18.

Kachroo, P., Leong, A.S. and Chattoo, B.B. (1994): *Pot2*, an inverted repeat transposon from the rice blast fungus *Magnaporthe grisea*. Mol. Gen. Genet. 245: 339-348.

Kaneko, I., Tanaka, A. and Tsuge, T. (2000): REAL, an LTR retrotransposon from the plant pathogenic fungus *Alternaria alternata*. Mol. Gen. Genet. 263: 625-634.

Kinsey, J.A. and Helber, J. (1989): Isolation of a transposable element from *Neurospora crassa*, Proc. Natl. Acad. Sci. USA 86: 1929-1933.

Leroux, P., Chapeland, F., Desbrosses, D. and Gredt, M. (1999): Patterns of cross resistance to fungicides in *Botrytinia fuckeliana* isolates from French vineyards. Crop Protection 18: 687-697.

Levis, C., Fortini, D. and Brygoo, Y. (1997): Flipper, a mobile Fot1-like transposable element in *Botrytis cinerea*. Mol. Gen. Genet. 254: 674-680.

Martinez, F., Blancard, D., Lecomte, P. Levis, C., Dubos, B. and Fermaud, M. (2003): Phenotypic differences between *vacuma* and *transposa* subpopulations of *Botrytis cinerea.* Eur. J. Plant Pathol. 109: 479-488.

McClintock, B. (1984): The significance of responses of the genome to challenge. Science 226: 792-801.

Mes, J.J., Haring, M.A.and Cornelissen, B.J. (2000):*Foxy*: An active family of short interspersed nuclear elements from *Fusarium oxysporum.* Mol. Gen. Genet. 263: 271-280.

Munoz, G., Hinrichsen, P., Brygoo, Y. and Giraud, T. (2002): Genetic characterisation of *Botrytis cinerea* populations in Chile. Mycol. Res. 106: 594-601.

Neuvéglise, C., Sarfati, J., Latgé, J.P. and Paris, S. (1996): *Afut1*, a retrotransposon-like element from *Aspergillus fumigatus*. Nucleic Acids Res. 24: 1428-1434.

Nielsen, M.L., Hermansen, T.D. and Aleksenko, A. (2001): A family of DNA repeats in *Aspergillus nidulans* has assimilated degenerated retrotransposons. Mol. Genet. Genomics 265: 883-887.

Sambrook, J., Fritsch, E. and Maniatis T. (1989): Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

Sonnenberg, A.S., Baars, J.J., Mikosch, T.S., Schaap, P.J. and VanGriensven, L.J. (1999): *Abr*1, a transposon-like element in the genome of the cultivated mushroom *Agaricus bisporus* (Lange) Imbach. Appl. Environ. Microbiol. 65: 3347-3353.

Yeadon, P.J. and Catcheside, D.E. (1995): *Guest*: A 98pb inverted repeat transposable element in *Neurospora crassa*. Mol. Gen. Genet. 247: 105-109.

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K.Z. Váczy[1] , L. Karaffa[2] , E. Fekete[2] , G.J. Kövics[3] , L. Gál[1] and E. Sándor[3]

[1]Research Institute for Viticulture and Enology, Eger, Hungary
[2]Department of Genetics and Applied Microbiology, Faculty of Science, University of Debrecen, Debrecen, Hungary
[3]Department of Plant Protection, Faculty of Agriculture, University of Debrecen, Debrecen, Hungary

Summary

Analysis of the distribution of the transposable genetic elements Boty and Flipper in *Botrytis cinerea* (grey mould) isolates collected from the Eger and Tokaj wine regions, North-Eastern Hungary is presented. We demonstrate the prevalence of a rare intrapopulation called Flipper, and discuss the differences among *B. cinerea* populations isolated from Western European and South American wine regions.