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Short Communication

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Detection of a Specific Transposon in *Erysiphe necator* from Grapevines in France

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Abstract

The biotrophic fungus, *Erysiphe necator* the causal agent of the grape powdery mildew, has two genetic groups A and B in European and Australian vineyards. A strain of group A was used to isolate a DNA sequence that exhibits high sequence homology to RNaseH of a non-LTR (long tandem repeat) retrotransposon of *Glomerella cingulata*. PCR primers were designed and tested for their specificity to genetic group A of *E. necator*. This molecular tool is more efficient and sensitive than nested PCR based on polymorphism in the CYP_{51} and β-tubulin genes.

Introduction

Grape powdery mildew caused by *Erysiphe necator* is one of the most widespread diseases of grapevine (*Vitis vinifera*) worldwide. *Erysiphe necator* may overwinter in two distinct forms: as resting mycelium within dormant buds (flag shoot symptom) and/or on the bark of grapevines as cleistothecia (Sall and Wrysinski, 1982; Gadoury and Pearson, 1988).

In European and Australian vineyards, two genetic groups of *E. necator*, A and B, have been distinguished (Délye et al., 1999; Stummer et al., 2000). We distinguished groups A and B of *E. necator* by an allele-specific PCR method using nucleotide polymorphism (or SNPs) in CYP_{51} or β-tubulin genes (Délye et al., 1999; Amrani and Corio-Costet, 2006). Furthermore, SCAR (sequence characterized amplified region) or RAPD (random amplified polymorphism DNA) primers for the two genetic groups of *E. necator* have been designed by European groups leading to the amplification of short fragments of unknown sequences (Cortesi et al., 2005; Hajjeh et al., 2005; Nunez et al., 2006; Peros et al., 2006). Rapid, sensitive and specific detection of the two genetic groups for population dynamics in field-scale studies are needed. Such a tool should be derived from the use of combined markers. The objectives of this study based on previous research were: (i) to identify a specific marker for group A based on transposon variation within *E. necator* and (ii) to assess the specificity of the marker for group A using samples collected from different cultivars and locations in France.

Materials and Methods

Collection and samples

Twenty-four single spore isolates of *E. necator* from the collection of INRA-Bordeaux (one from Greece, 23 from France) and 575 samples of powdery mildew colonies collected from four grape varieties in four French vineyards were used in molecular marker development (Table 1, Fig. 1). Vineyard samples were collected from flag shoots or leaves with sparse spot symptoms from May to the end of June in 2000. A nested PCR assay based on polymorphism in the CYP_{51} gene (Délye et al., 1999) was used to characterize each isolate as belonging to either genetic group A or B, or to a mixture of A and B isolates. The 575 samples were used to test the relative frequencies of groups A and B at each location (Table 1).

Marker development

DNA was extracted from spores and mycelia (24 single spores) or from infected plant tissue (575 field spot samples) and the CYP_{51} sequences amplified according to methods described previously (Délye et al., 1995, 1999). After cloning and sequencing (Amrani and Corio-Costet, 2006) of a specific fragment of group A single spore (2B17, Aude, France, 2000), the DNA sequence was compared with those from other fungi using the BLAST program (NCBI, website: http://www.ncbi.nlm.nih.gov/Blast/). Specific primers were designed, TransA (5′-GCACAGGAAAACGCGGTTCTTT-3′) and RtransA (5′-CCCTTTTAAGAGTATGCGAGCTG-3′) for amplification of a DNA fragment (594-bp). For PCR, 20 μl of reaction mixture contained 67 mM Tris–HCl (pH 8.8), 16 mM $(NH_4)_2SO_4$, 15 mM $MgCl_2$, 0.01% Tween-20, 300 μM each dNTP, approximately 10–50 ng of template DNA, 0.1 U of

Table 1
Relative frequencies of group A and B of *Erysiphe necator* characterized by CYP_{51} nested PCR, from samples collected on leaves from different cultivars in four French vineyards

Department	Grapevine cultivars	Number of samples	Date of sampling	Genetic group identified (%)	
				A	B
Gironde	Merlot	100	June 25	0.5	99.5
Aude	Chardonnay	48	June 17	2.1	97.9
Aude	Carignan[a]	89	May 16	34.8	65.2
Pyrénées/orientales	Carignan[a]	135	May 25	87.4	12.6
Pyrénées/orientales	Grenache[a]	37	June 6	45.9	54.1
Hérault	Carignan[a]	103	May 20	79.6	20.4
Hérault	Chardonnay	22	June 18	4.5	95.5
Hérault	Grenache[a]	41	June 26	41.5	58.5

[a]More than one flag shoot per five rootstock (from two to 15).

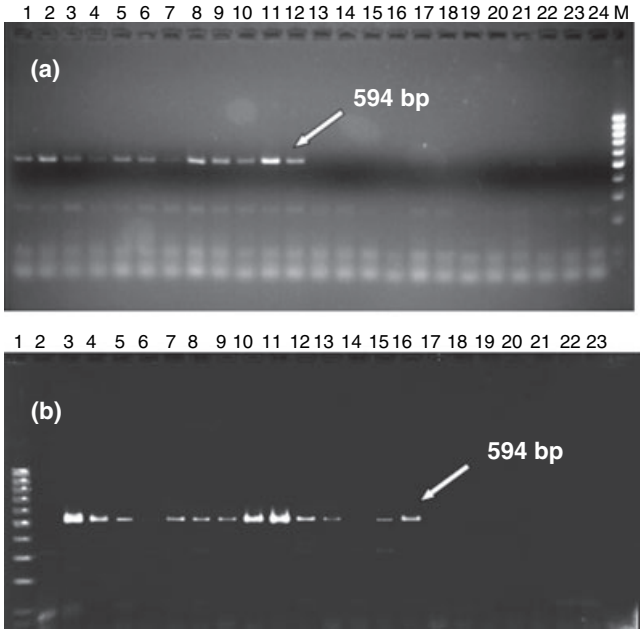

Fig. 1 (a) PCR products obtained by using transposon primers from DNA of 12 strains A, lanes 1–12; B1-01, B2-01, B2-02, B2-10, B3-02, B7-02, 2B17 (Aude, 2000, France), CC50, CC12 (Gironde, 1999, France), CPH3 (Pyrénées-Orientales, 2000, France), FMO23 (Hérault, 1998, France), AOG11 (Greece, 1999), and 12 strains B from collection, B1-13, B1-14, B2-12, B5-08, B8-08, 2 BG (Aude, 2000, France), BE3, BE5, SC15 (Gironde, 1999, France), FNI11, FNB12, FMN22 (Hérault, 1998, France). Lane M contained a 100-bp DNA ladder (Generuler TM, Fermentas). (b) PCR products obtained from 21 samples collected on Carignan from Aude vineyard (lanes 3–23). Lanes 3–12: samples first identified as group A by CYP51 nested PCR, lane 13: samples first identified as group A and B, lanes 14–23: samples first identified as group B. Lane 1: molecular weight marker, lane 2: water control (no DNA)

Silverstar DNA polymerase (Eurogentec SA, Seraing, Belgium) and 0.2 μM of each of the primers TransA and RtransA. PCR was performed in 34 cycles, using a Crocodile III thermal cycler (Appligene, Oncor, Ilkirch, France), each cycle consisting of 1 min of denaturation at 95°C, 1 min of annealing at 68.3°C and 1 min of extension at 72°C. Amplified fragments were separated by electrophoresis in 1% agarose gels stained with ethidium bromide. The negative control reaction contained no template DNA for the detection of contaminant DNA. No PCR products were detected for *V. vinifera*, *Botrytis cinerea*, *Alternaria alternata*, *Eutypa lata*, *Plasmopara viticola* and *Phaeomoniella chlamydospora*.

Results

Grapevine cultivars in different vineyards sampled for *E. necator* exhibited variation in the frequency of isolates belonging to groups A when samples were collected from epidemic onset until the end of June (Table 1). Group A was detected at a low frequency (0.5–4.5%) on Merlot in Gironde and on Chardonnay in Aude and Hérault. In contrast, group A was recovered frequently (34.8–87.4% of samples) on Carignan and Grenache from Aude, Pyrénées-Orientales and Hérault.

Amplification of *E. necator* DNA from genetic group A using specific primers TransA and RtransA resulted in a major product of 594 bp (Fig. 1). The DNA sequence amplified and cloned from total DNA (GENBANK accession number DQ025531) was 89.4% similar to a non-LTR retrotransposon reverse transcriptase (RNaseH) of *Glomerella cingulata* (GENBANK accession number AAA85636). This result suggests that the *E. necator* sequence identified here might correspond to transposon reverse transcriptase. The sensitivity of the transposon marker relative to CYP_{51} PCR was tested using different dilutions of DNA, from 1/2 to 1/500, which showed that the direct transposon PCR (one step) was 10 times more sensitive than the CYP_{51} nested PCR (two steps). When primers Trans A and RtransA were applied to DNA from 24 single-spore isolates of *E. necator*, the 594 bp product was amplified only in isolates of group A (Fig. 1a) as determined by the CYP_{51} assay (data not shown). The transposon marker was also applied to 23 of 575 samples collected in 2000 that were characterized with the CYP_{51} tool as belonging to group A (10 samples), a mixture of groups A and B (one sample) or to group B (10 samples, Fig. 1b). All samples identified as group A or as a mixture of group A and B exhibited the specific 594-bp band. No PCR products were amplified in samples belonging to group B, except for two samples (lanes 15 and 16, Fig. 1b). These two samples were assayed a second time with CYP_{51} markers, to confirm their group B identity (data not shown). When direct transposon PCR was applied to

all 575 samples collected from four vineyards, all samples first identified as belonging to group A with the CYP_{51} markers, also tested positive for the presence of putative transposon. However, the 594-bp product was also amplified in 18% of samples designated group B using CYP_{51} marker.

Discussion

Transposon-PCR is a more efficient and sensitive marker than CYP_{51} nested PCR for characterizing genetic groups of *E. necator* based on the presence or absence of a putative transposon. The sensitiviy of the assay might explain why the putative transposon was detected in 18% of group B isolates from the large field sample, as determined by CYP_{51} nested PCR. The incomplete association of the transposon with group A field isolates might also indicate that the new marker is capable of detecting hybrids between groups A and B. In general, the results provide further support for the existence of at least two genetic groups in *E. necator*. In Bordeaux vineyards (Gironde), observation of asexual overwintering as well as detection of group A was rare. By contrast, in the Mediterranean vineyards (Pyrénées-Orientales, Hérault, Aude), the two genetic groups occur in different ratios depending on cultivar and/or geographic location.

Based on the presence or absence of transposons, studies on *B. cinerea* have described two sympatric species with different ecological requirements (Martinez et al., 2005). Similarly, in studies of two genetically distinct biotypes (A and B) of *Colletotrichum gloesporioides*, a non-LTR retrotransposon (cgt1) dispersed in the genome was shown to be responsible for significant genetic differences between biotypes (He et al., 1996). McClintock (1984) proposed that transposition could be seen as a response to environmental stress and an adaptative response of the genome. In a similar way, we suggest that the specific transposon in *E. necator* may be an adaptative response to environmental pressure. It has further been proposed that the activity of transposable elements may contribute to mutation leading to specialization and race specificity in phytopathogenic fungi (Daboussi and Capy, 2003). We suggest that different life strategies among populations of *E. necator* and potential differences in aggressiveness might partly explain local distributions of group A and that transposable elements could be involved in the evolution of *E. necator*.

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