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Identification and geographic distribution of genetic groups of *Erysiphe necator* in Chilean vineyards

C. ARAYA^{1),3)}, I. M. ROSALES²⁾, M. A. MÉNDEZ³⁾ and F. DELMOTTE⁴⁾¹⁾ INIA, CRI La Platina, Santa Rosa, La Pintana, Santiago, Chile²⁾ Departamento Ciencias Vegetales, Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile³⁾ Laboratorio de Genética y Evolución, Facultad de Ciencias, Universidad de Chile⁴⁾ INRA, UMR1065 Santé et Agroécologie du Vignoble, Villenave d'Ormon, France

Summary

The grapevine powdery mildew, caused by the biotrophic fungus *Erysiphe necator*, is one of the most important diseases of this crop in Chile. We converted existing single nucleotide polymorphisms in β -tubulin, rDNA intergenic spacer region 1 and eburicol 14- α -demethylase into three cleaved amplified polymorphic sequences (CAPS). These CAPS markers were used for the genetic characterization of *Erysiphe necator* isolates collected on clusters of *Vitis vinifera* cv. 'Cabernet Sauvignon' in 23 vineyards across Chile. Among the 105 Chilean isolates analyzed, 103 revealed to belong to genetic group B and 2 to genetic group A. These results correspond to the first report of the presence of genetic groups A and B of *E. necator* in Chile.

Key words: grapevine powdery mildew, plant-pathogen, *Vitis vinifera*.

Introduction

Vitis vinifera L. is the main fruit crop grown in Chile, where table grapes and wines are among the most important agricultural products. However, production is affected by grapevine powdery mildew, caused by *Erysiphe necator*, a biotrophic, haploid, and heterothallic fungus. *E. necator* is a species native to North America that was introduced into Europe and Australia during the 19th century (BREWER and MILGROOM 2010). In Chile, the pathogen was first reported in 1860, which coincides with the arrival of European cultivars of *V. vinifera* to Chilean vineyards (DEL POZO 2004).

Studies of the genetic diversity in *E. necator* have shown the existence of two groups, A and B, which are strongly differentiated genetically but possess little variability within each group (DÉLYE *et al.* 1997a; PÉROS *et al.* 2005). Groups A and B are found in Europe and Australia, and only group B has been described in western USA. Genetic diversity of *E. necator* in the eastern USA was found to be much greater than in Europe and Australia, further confirming that North America is the native area of this pathogen (BREWER and MILGROOM 2010). Group A decreases early in the season while group B is presented all the time (MONTARRY *et al.* 2008). Moreover, damage on leaves

and clusters is always more severe when the epidemic is initiated by group B isolates (MONTARRY *et al.* 2009).

E. necator is presented in Chile for over a century. However, the presence of flagshoots has not been reported and cleistothecia were first detected only in 2004. Despite the importance of this disease in Chile, the prevalence of the two genetic groups (A, B) is unknown.

In a recent study, MONTARRY *et al.* (2009) converted an existing Single Nucleotide Polymorphism (SNP) at position 209 in the β -tubulin gene into a Cleaved Amplified Polymorphic Sequence (CAPS). While two other SNPs between group A and B have been described, one in the eburicol 14- α -demethylase gene (EnC14-2; position 1587) and the other, in the rDNA intergenic spacer region 1 (EnITS1; position 64), these polymorphisms remain to be converted into CAPS markers for the inference of *E. necator* genetic groups.

Therefore, the objectives of the present study were (i) to convert existing SNPs in the EnC14-2 and the EnITS1 region into CAPS markers and (ii) to identify the genetic groups (A, B) of *E. necator* in Chilean vineyards using these 3 CAPS markers.

Material and Methods

Sample collection: A total of 105 samples from grape clusters naturally infected with *E. necator* were collected from 23 vineyards in summer of 2009 to 2011. These were collected on the *V. vinifera* cv. 'Cabernet Sauvignon' between 27-31 stages on the Eichhorn-Lorenz scale as modified by Coombe. No information was available on the fungicide treatments used in the vineyards sampled.

***E. necator* isolates:** Plants of the susceptible table grape 'Thompson Seedless' were grown for establishment of co-cultures of *E. necator* and grape leaves as described by PÉROS *et al.* (2005). A single spore culture was obtained as described by DÉLYE *et al.* (1997a). Fungal tissue was collected using clear nail polish.

Molecular characterization: DNA extraction from fungal tissue was performed as described by MONTARRY *et al.* (2009). Three CAPS markers were used in this study (i) EnTub, previously developed by MONTARRY *et al.* (2009), (ii) EnC14-2, and (iii) EnITS (DÉLYE *et al.* 1997b, AMRANI and CORIO-COSTET 2006). The last two were converted into CAPS here. To create each marker, pairs

of primers were designed to amplify a DNA fragment that encompasses the targeted SNP (Tab. 1).

PCR and cleaving reactions were performed using the method defined by MONTARRY *et al.* (2009) (Tab. 1). A panel of 10 reference isolates, comprising French isolates from group A (n = 5) and B (n = 5), was used to assess the reliability of the CAPS makers.

Results and Discussion

From the 105 *E. necator* samples, 103 belonged to group B and 2 to group A. The molecular characterization was perfectly congruent among the 3 CAPS markers, i.e., isolates were assigned to the same genetic group by each of the markers used (Tab. 2). The two A isolates identified in

the study were found in samples from the Coquimbo region of Chile. All 105 samples yielded a PCR amplicon for EnTub and EnITS markers. Only 83 isolates (79 %) yielded a successful amplification for EnC14-2 marker (Tab. 2). The higher rate of PCR failure found with EnC14-2 could result from a non-optimal design of primers or from the presence of undetected nucleotidic variability in the gene sequence. A previous study reported PCR failures of this gene for some *E. necator* isolates, indicating that the amplification of this region might be sensitive to the quality and the quantity of DNA samples (DÉLYE *et al.* 1997b). Therefore, our data show that EnTub and EnITS CAPS markers are the most suitable for easy, fast and reliable identification of genetic groups of *E. necator*.

The proportion of genetic groups of *E. necator* found in Chile is in line with the results from European vine-

Table 1

Characteristics of the CAPS markers used for identifying genetic groups (A or B) of *E. necator*

Marker name	Primer sequences 5'-3'	Size (bp)	Enzyme	Size of fragments (bp)	
				Group A	Group B
EnC14-2	F:CATGCCCATCTTCAGATCAT R:CTGACCCAAAAATCCGAAG	880	<i>TspRI</i>	300, 580	880
EnTab	F:GAGATCGTAAGCTTGACAC R:GCACGAGGAACGTATTTGT	449	<i>AccI</i>	449	281, 168
EnITS	F:CAGAGCGAGAGGCTCAGC R:AAAGGTGGGTGTTCTGGC	543	<i>TaqI</i>	15, 42, 53, 59, 175, 196	42, 53, 59, 175, 214

Table 2

Genetic group assignment using 3 CAPS markers in Chilean populations of *E. necator*

Region	Location	Date of collection		n	EnTub		EnITS		EnC14-2	
		Season	Season		A	B	A	B	A	B
		2009-2010	2010-2011							
Coquimbo	C. Exp. Vicuña	12-17-09	12-13-09	27	2	25	2	25	22	
	Vicuña	-	01-20-11	3		3		3	3	
Valparaíso	Pitama	01-07-10	12-22-10	12		12		12	10	
	Hijuelas	-	02-03-11	1		1		1	1	
	Panquehue	-	02-03-11	3		3		3	2	
	San Esteban	-	02-03-11	3		3		3	2	
Metropolitana	San Bernardo	-	01-19-11	2		2		2	2	
	Isla de Maipo	-	01-28-11	2		2		2	2	
L. B. O'Higgins	Quinta de Tilcoco	01-05-10	12-28-10	12		12		12	12	
	Los Boldos	-	01-12-11	3		3		3	1	
	Barrales	-	01-12-11	2		2		2	0	
	Pelequén	-	01-19-11	2		2		2	2	
	Codegua	-	01-19-11	3		3		3	1	
Maule	Panguilemo	-	01-12-11	4		4		4	4	
	San Rafael	01-13-10	-	7		7		7	7	
	Duao	01-08-10	01-11-11	5		5		5	4	
	Río Claro	-	01-12-11	1		1		1	1	
	Longaví	-	01-26-11	2		2		2	1	
	Yerbas Buenas	-	01-26-11	2		2		2	1	
Biobío	Quillón	-	01-25-11	2		2		2	1	
	Bulnes	-	01-25-11	3		3		3	2	
	Chillán-1	-	01-25-11	2		2		2	1	
	Chillán-2	-	01-25-11	2		2		2	1	
Total				105	2	103	2	103	83	

yards: The frequency of group A isolates usually does not exceed 5 %, though they can be predominant in flagshoot symptoms in Mediterranean vineyards (MIAZZI *et al.* 2003, BOUSCAUT and CORIO-COSTET 2007). The low incidence of group A is not related to grape cultivar, since all samples came from 'Cabernet Sauvignon'. The impact of fungicide treatments can not be assessed because this information was not available for the samples. However, the small proportion of group A isolates could result from the sampling material, since group A isolates have been shown to be very low on the cluster (MONTARRY *et al.* 2009) and all samples of *E. necator* in this study were collected from grapevine clusters. An alternate hypothesis is that group A prevalence remains low throughout the season due to unidentified ecological factors.

Conclusion

This is the first report of the presence of genetic groups A and B of *E. necator* in Chile. The CAPS markers described here, combined with the recently described species-specific SSRs (FRENKEL *et al.* 2012) will increase our capacity to study the genetic structure of *E. necator* populations in Chilean vineyards. Further investigation of the genetic structure of *E. necator* populations in Chile are required to assess prevalence of groups A and B on both leaves and clusters throughout the season in order to fully understand the contribution of each group to powdery mildew epidemics.

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