

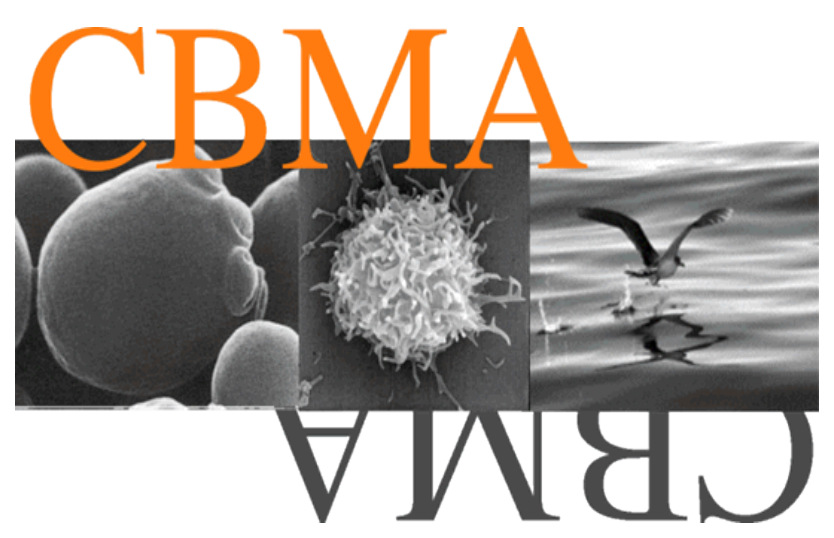
Phenotypic Differentiation and Genetic Diversity of *Saccharomyces cerevisiae* Strains from Vineyards of the Azores Archipelago

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INTRODUCTION

This work aims to evaluate the diversity of *Saccharomyces cerevisiae* strains from vineyards of the Azores Archipelago and to estimate the degree of phenotypic and genetic differentiation among geographically isolated islands.

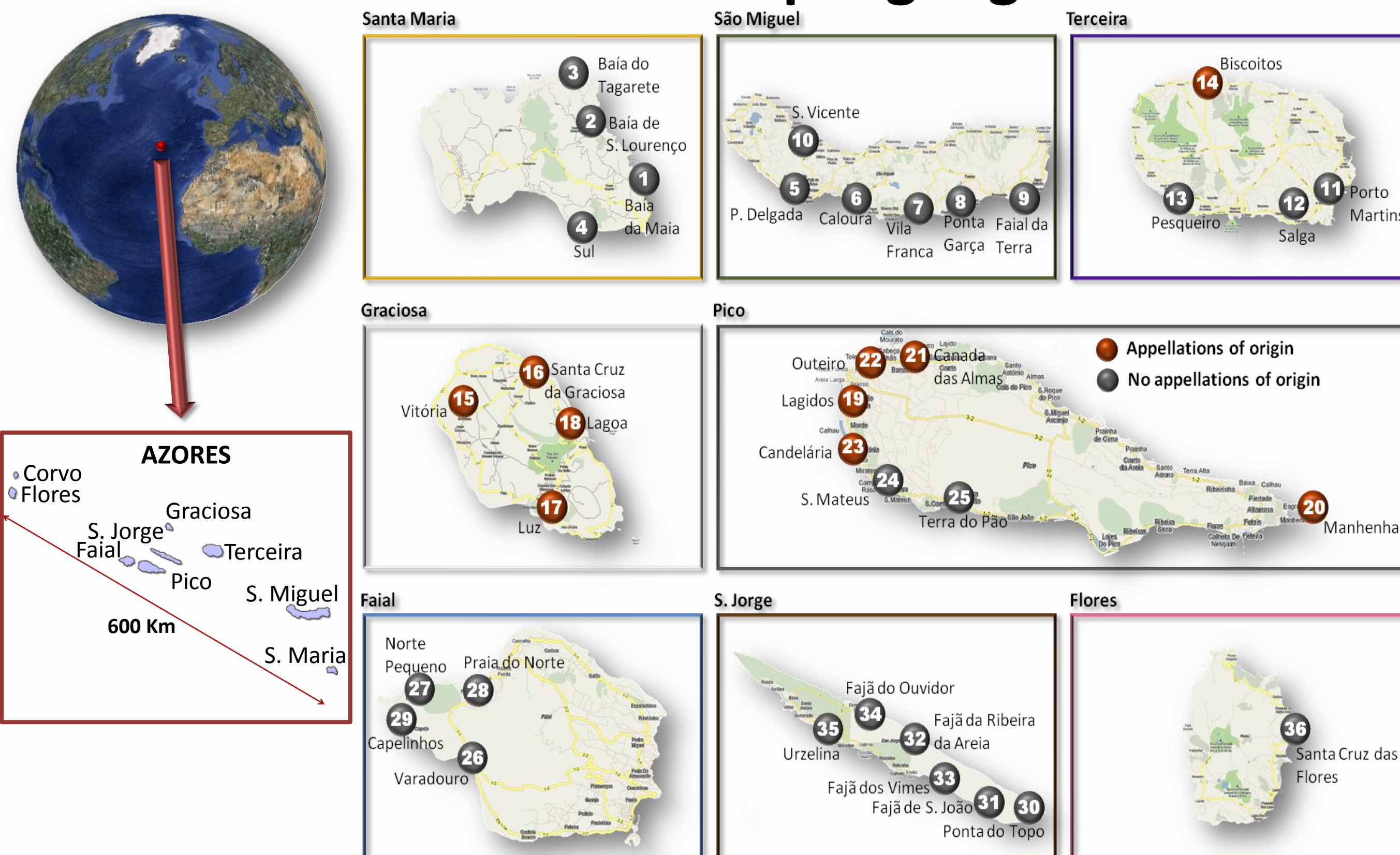
On the Archipelago there are several wine producing regions (including three appellations of origin) and also extensive areas of abandoned vineyards. The Azorean *terroirs*, correspond to very particular ecosystems whose fermentative flora has not yet been characterized.

MATERIAL AND METHODS

Sampling

During the harvests of 2009 and 2010, 163 grape samples were collected from 36 locations on eight islands of the archipelago. The grape samples belonged to traditional (TV) and hybrid (HV) varieties, including vineyards in appellations of origin, abandoned vineyards (AV) and non-abandoned vineyards (NAV).

Sampling regions



Number of grape samples

Type of vineyard	Grape variety	Year	S. Maria	S. Miguel	Terceira	Graciosa	Pico	Faial	S. Jorge	Flores	Total
Non-abandoned vineyards	Arinto	2009			3	2	7				14
		2010			3	1	7		2		13
	Terrantez	2009		2			1				3
		2010		2			1				3
	Verdelho	2009		3	2	4	4				13
		2010		2	2	3	4				11
Hybrid varieties	2009	4	4	4	4	8	4	5	1	34	
	2010	3	4	3	4	8	4	5		31	
Abandoned vineyards	Hybrid varieties	2009	4	3	4	7	2	4		24	
		2010	4	2	2	6	1	4		17	
Total			15	17	22	22	53	11	22	1	163

Yeast Isolation

From each sample (2 kg grapes), 500 ml of must was obtained and fermented. The evolution of fermentation was followed daily and when the weight loss was about 65-70 g/L, aliquots of serial dilutions (10^{-1} - 10^{-5}) were spread on plates containing YPD medium. After two days of incubation at 30 °C, 30 colonies were collected from one of the five dilutions containing between 30 and 300 colonies.

Molecular Identification

The *S. cerevisiae* strains were differentiated by the comparison of polymorphic patterns that were obtained from interdelta sequences amplification by PCR [1, 2].

Phenotypic Characterization

From each strain a dense cell suspension was prepared ($A_{640}=10$) and 1 μ L was inoculated in duplicate in plates containing different culture media as outlined below. Cell growth was evaluated after incubation at 25 °C – 27 °C for 2 - 3 days.

Phenotypic test	Culture medium
A. Killer phenotype	Malt Extract Agar (MEA) supplemented with 0.03% (w/v) methylene blue; Inoculation on a layer of a sensitive (CECT 1415) or killer (CECT 1182) reference strain
B. Ethanol resistance	MEA supplemented with 8% (v/v) ethanol
C. Combined ethanol and SO ₂ resistance	MEA supplemented with 8% (v/v) ethanol and 100 ppm SO ₂
D. SO ₂ resistance	Estimated from comparison of tests B and C
E. β -glucosidase activity	Esculin Glycerol Agar (EGA) medium [3]
F. H ₂ S Production	BIGGY Medium (Difco)

Genetic Diversity

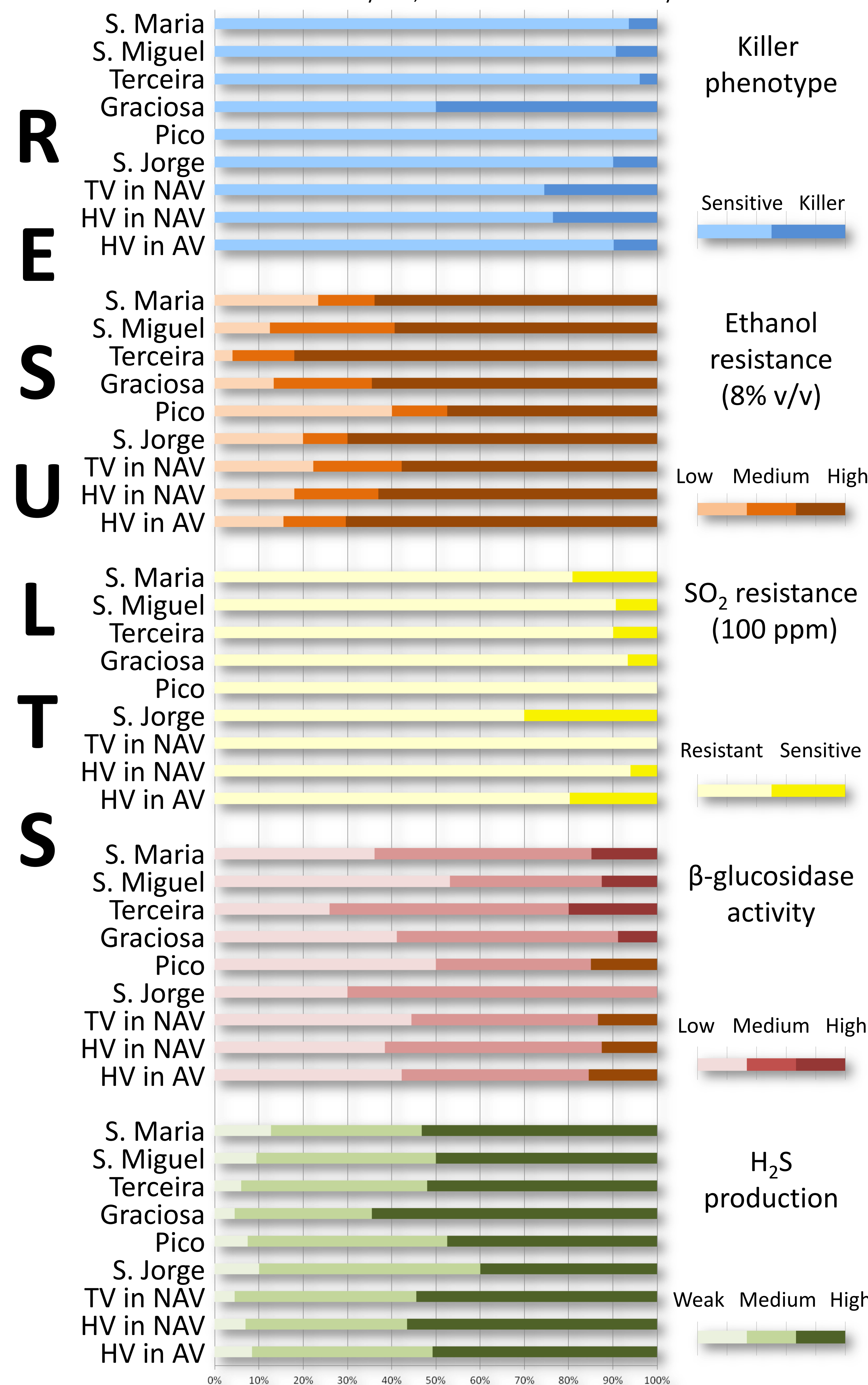
	S. Maria	S. Miguel	Terceira	Graciosa	Pico	Faial	S. Jorge	Flores
N.º of <i>S. cerevisiae</i> strains/sample	47	33	49	103	40	2	10	0
N.º of samples fermented by strains of <i>S. cerevisiae</i>	7.8	3.0	4.9	6.4	2.1	2	1.3	0.0

The highest numbers of *S. cerevisiae* strains per sample were found in Santa Maria and Graciosa islands.

Phenotypic Differentiation

Strains showing each phenotype (%)

TV - traditional grape varieties; HV - hybrid grape varieties;
AV - abandoned vineyards; NAV - non-abandoned vineyards.



Killer activity clearly predominated among the *S. cerevisiae* strains from Graciosa Island (50%). This could be related to a higher competition between strains due to the largest area of contiguous vineyards sampled (regions 15-18). When all islands were compared, more than twice strains showing killer phenotype was observed in non-abandoned vineyards (NAV) in comparison with the abandoned vineyards (AV). This might be associated with a higher competition between strains in vineyards that are cultivated.

The highest percentage of ethanol resistant strains was found on Terceira Island (82%), whereas this value ranged between 48% and 70% in Pico and S. Jorge, respectively. Global analysis showed that strains from hybrid varieties (HV) tend to be more ethanol resistant (67% to 70%), in comparison with strains from traditional varieties (TV) (58%). This characteristic was not correlated with the sugar content of the samples (172 g/L and 162 g/L in grapes from traditional and hybrid varieties, respectively).

The lowest percentages of strains showing SO₂ resistance were observed in S. Maria and S. Jorge islands, where viticulture is very traditional without SO₂ addition. Comparison of all islands showed that all strains from traditional varieties (TV) were resistant to SO₂. This trait might become predominant with the regular SO₂ usage in wineries that are often located close to non-abandoned vineyards (NAV).

The percentage of strains showing strong β -glucosidase activity ranged between 0% (S. Jorge) and 20% (Terceira). No differences were observed between abandoned (AV) and non-abandoned vineyards (NAV) when the comparison was done across all islands.

S. cerevisiae strains from Graciosa island were the highest H₂S producers. This might be associated with the predominance of the killer phenotype on this island. Further investigation is underway. The strains from abandoned vineyards (AV) showed a higher H₂S production than the ones from non-abandoned vineyards (NAV).

Conclusions

A total of 271 *S. cerevisiae* strains was obtained from seven islands of the Azores Archipelago. The highest genetic diversity was found in samples from the islands S. Maria (7.8 strains/sample) and Graciosa (6.4 strains/sample).

The phenotypic comparison of the isolates showed that some characteristics might be under selection, depending on environmental conditions such as the extension of cultivated vineyards, strains diversity, type of vineyard (abandoned or non-abandoned), grape variety and enological practices.

- Strains with Killer phenotype predominate in cultivated vineyards, where strains diversity is rather high.

- Ethanol resistance tends to be higher among strains obtained from hybrid grape varieties.

- A lower SO₂ resistance of strains from islands where this preservative is rarely used indicates that this compound may be a driver of positive selection.

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