

**ABSTRACT**

This study aims at the characterization of the fermentative yeasts flora of the vineyards from Azores, for biodiversity preservation and the constitution of a *Saccharomyces cerevisiae* strain collection. During the harvest of 2009, 88 grape samples were collected from eight islands of the archipelago, that belonged to traditional (Arinto, Verdelho and Terrantez) and hybrid grape varieties. The sampling plan covered 36 locations, including vineyards in appellations of origin and abandoned vineyards. Forty nine spontaneous fermentations were achieved. About 83% of the musts obtained from traditional varieties finished fermentation, while this value was 47% and 33% for musts from hybrid varieties in non-abandoned and abandoned vineyards, respectively. From the final phase of the fermentations, a total of 1470 isolates was obtained. The identification was performed by restriction fragment length polymorphism analysis (RFLP) of ITS sequences. The *S. cerevisiae* strains were differentiated by the comparison of polymorphic patterns that were obtained from interdelta sequences amplification by PCR. We obtained 660 isolates of non-*Saccharomyces* species and 810 isolates of *S. cerevisiae*, classified in 168 strains. Surprisingly, the percentage of *S. cerevisiae* in fermentations performed with grapes from abandoned vineyards was significantly higher (75%) compared to the values determined for non-abandoned vineyards (56%, for both traditional and hybrid varieties). Contrarily, the number of *S. cerevisiae* strains per fermentation was lower (3-11) in samples from abandoned vineyards compared to non-abandoned vineyards (1-23). The relative proportion of *S. cerevisiae* and non-*Saccharomyces* species was not associated with the grape variety, but rather the type of vineyard. The highest number of *S. cerevisiae* strains per fermentation was observed in samples collected from the Graciosa Island. Our results show that Azorean vineyards have a high genetic diversity of *S. cerevisiae*, even in locations where no human intervention occurs.

**INTRODUCTION**

On the Azores Archipelago there are three appellations of origin on the islands Terceira, Graciosa and Pico. Other wine producing regions and also extensive areas of abandoned vineyards can be found on different islands. The Azorean *terroirs* produce wines with well defined profiles, due to the soil, grape varieties, climatic and cultural characteristics. From an ecological point of view, these *terroirs* correspond to particular habitats, whose fermentative flora has not yet been characterized, both from the perspective of yeast biodiversity and the oenological potential. Traditionally, azorean wines were obtained by spontaneous fermentations by indigenous yeasts species, which may have significantly contributed to the aromatic profiles of wines in a given island or region. However, the current widespread use of commercial yeast may have contributed to the loss of Azorean wines typicality. This study aims to assess and characterize the yeast biodiversity of Azorean vineyards and to constitute a collection of *S. cerevisiae* strains as a resource for future selection and breeding of strains that are most adapted to the characteristics of the wine of Azores.

**MATERIALS AND METHODS**

**Sampling**  
 Sampling year: 2009  
 Sampling locations: 36 locations in eight islands of the archipelago, including vineyards in the 3 appellations of origin and other wine-producing regions.  
 Grape varieties: Arinto (13 samples), Verdelho (13 samples), Terrantez (4 samples), Hybrid varieties (58 samples)

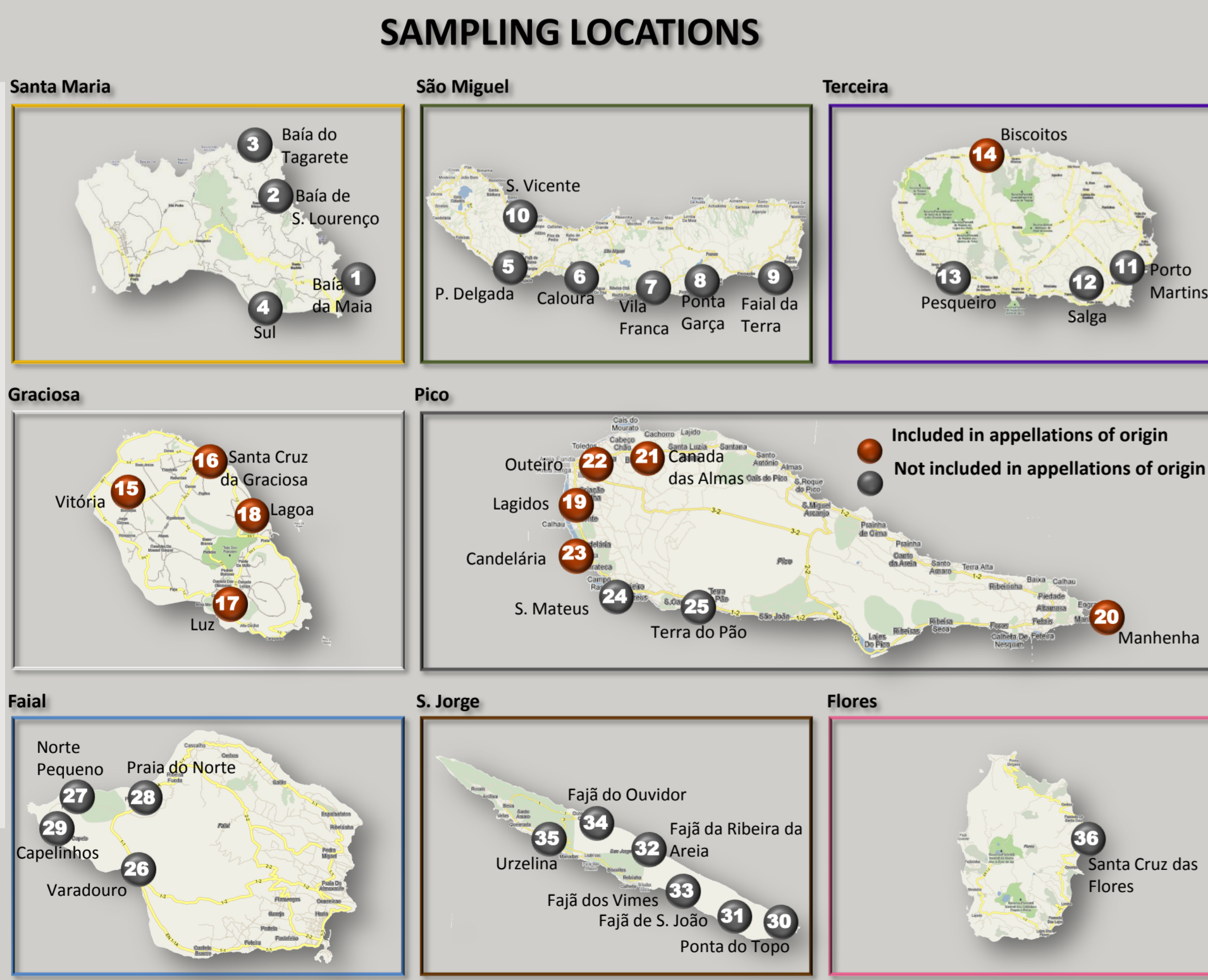
**Type of vineyard:**  
 Non-abandoned  
 Abandoned vineyards (more than 5 years)

**Yeast isolation**  
 From each sample, 500 ml of must was obtained and fermented.

The evolution of fermentation was followed by the weight loss determination. From each fermentation, 30 yeast colonies per must sample were obtained at the stage corresponding to a weight loss of 65-70 g/l.

**Molecular identification**

The species molecular identification was performed by restriction fragment length polymorphism analysis (RFLP) of ITS sequences [1, 2]. The *S. cerevisiae* strains were differentiated by the comparison of polymorphic patterns that were obtained from interdelta sequences amplification by PCR [3, 4]



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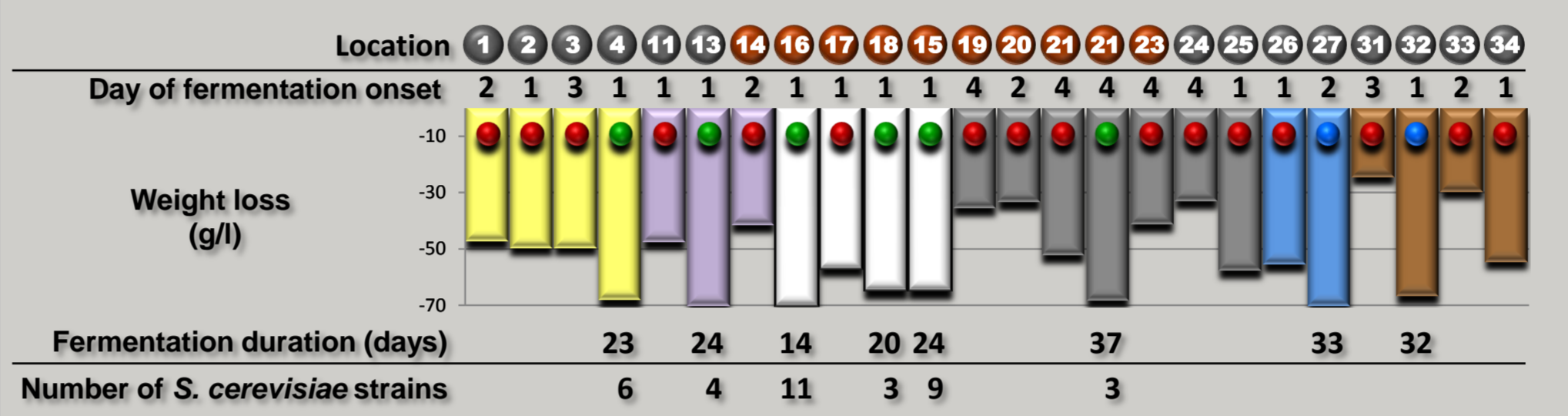
J. Drumonde-Neves<sup>a, b</sup>, M. T. Lima<sup>a</sup>, D. Schuller<sup>b</sup>



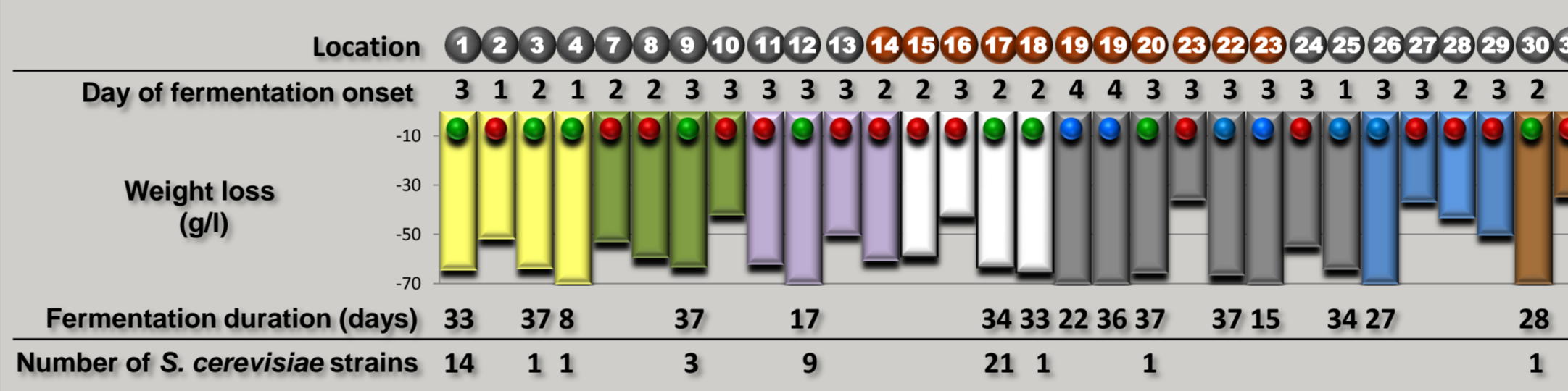
(a) Research Center for Agricultural Technology of Azores - Department of Agricultural Sciences, University of Azores  
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**RESULTS**

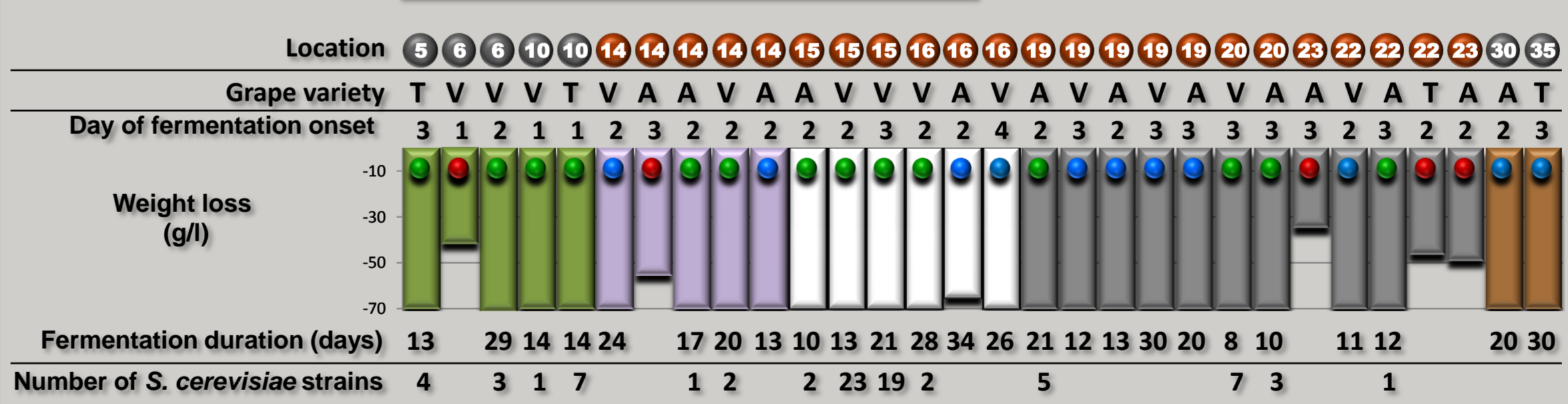
**SAMPLES OF HYBRID VARIETIES FROM ABANDONED VINEYARDS**



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**SAMPLES OF TRADITIONAL VARIETIES**



Traditional grape varieties			
Hybrid varieties from non-abandoned vineyards			
Hybrid varieties from abandoned vineyards			
Number of samples	24	34	30
Number of fermentations	8	16	25
Day of fermentation onset (average)	2,0	2,4	2,3
Duration of the fermentations (average in days)	25,9	29,3	18,6
Finished fermentations (%)	33,3	47,1	83,3
Fermentations completed by <i>S. cerevisiae</i> (%)	75,0	56,3	56,0
Fermentations completed by non- <i>Saccharomyces</i> species (%)	25,0	43,7	44,0
Total number of <i>S. cerevisiae</i> strains	36	53	80
Minimum number of <i>S. cerevisiae</i> strains / sample	3	1	1
Maximum number of <i>S. cerevisiae</i> strains / sample	11	21	23

**CONCLUSIONS**

We found a high genetic diversity of *Saccharomyces cerevisiae* strains in the vineyards of the Azores, regardless the grape variety, type of vineyards, or the geographic origin of the samples.  
 In abandoned vineyards, this species occurred with greater abundance, although with a lower genetic diversity.

**References**

- [1] Esteve-Zaroso B., et al. Int. J. Syst. Bacteriol., 1999, 49:329-337
- [2] Fernandez-Espinar M. T. et al. 2000, Antonie van Leeuwenhoek 78: 87-97
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About 57% of the samples completed the fermentation.  
 Fermentation of traditional varieties lasted, on average, 19 days, whereas this period was 25-29 days for the musts of hybrid grape varieties.  
 More than 83% of the musts obtained from traditional varieties finished fermentation, while this value was 47% and 33% for musts from hybrid varieties in non-abandoned and abandoned vineyards, respectively.  
 From the final fermentative phase, a total of 1470 yeast isolates was obtained. We identified 660 isolates of non-*Saccharomyces* species (such as *Candida zemplinina*, *Hanseniaspora vineae*, *Pichia guilliermondii*) and 810 isolates of *S. cerevisiae*, classified in 168 strains.  
 The percentage of *S. cerevisiae* in fermentations performed with grapes from abandoned vineyards was higher (75%) than the values obtained for non-abandoned vineyards (56%).  
 The number of *S. cerevisiae* strains per fermentation in samples collected from abandoned vineyards was lower (3-11) than in the vineyards in production (1-23). The highest values were observed in samples from the Graciosa Island.  
 The relative proportion of isolates belonging to the species *S. cerevisiae* and non-*Saccharomyces* depended on the type of vineyards, but not the grape variety or on the geographic origin of the samples.

**ABSTRACT**

This study aims at the characterization of the fermentative yeasts flora of the vineyards from Azores, for biodiversity preservation and the constitution of a *Saccharomyces cerevisiae* strain collection. During the harvest of 2009, 88 grape samples were collected from eight islands of the archipelago, that belonged to traditional (Arinto, Verdelho and Terrantez) and hybrid grape varieties. The sampling plan covered 36 locations, including vineyards in appellations of origin and abandoned vineyards. Forty nine spontaneous fermentations were achieved. About 83% of the musts obtained from traditional varieties finished fermentation, while this value was 47% and 33% for musts from hybrid varieties in non-abandoned and abandoned vineyards, respectively. From the final phase of the fermentations, a total of 1470 isolates was obtained. The identification was performed by restriction fragment length polymorphism analysis (RFLP) of ITS sequences. The *S. cerevisiae* strains were differentiated by the comparison of polymorphic patterns that were obtained from interdelta sequences amplification by PCR. We obtained 660 isolates of non-*Saccharomyces* species and 810 isolates of *S. cerevisiae*, classified in 168 strains. Surprisingly, the percentage of *S. cerevisiae* in fermentations performed with grapes from abandoned vineyards was significantly higher (75%) compared to the values determined for non-abandoned vineyards (56%, for both traditional and hybrid varieties). Contrarily, the number of *S. cerevisiae* strains per fermentation was lower (3-11) in samples from abandoned vineyards compared to non-abandoned vineyards (1-23). The relative proportion of *S. cerevisiae* and non-*Saccharomyces* species was not associated with the grape variety, but rather the type of vineyard. The highest number of *S. cerevisiae* strains per fermentation was observed in samples collected from the Graciosa Island. Our results show that Azorean vineyards have a high genetic diversity of *S. cerevisiae*, even in locations where no human intervention occurs.

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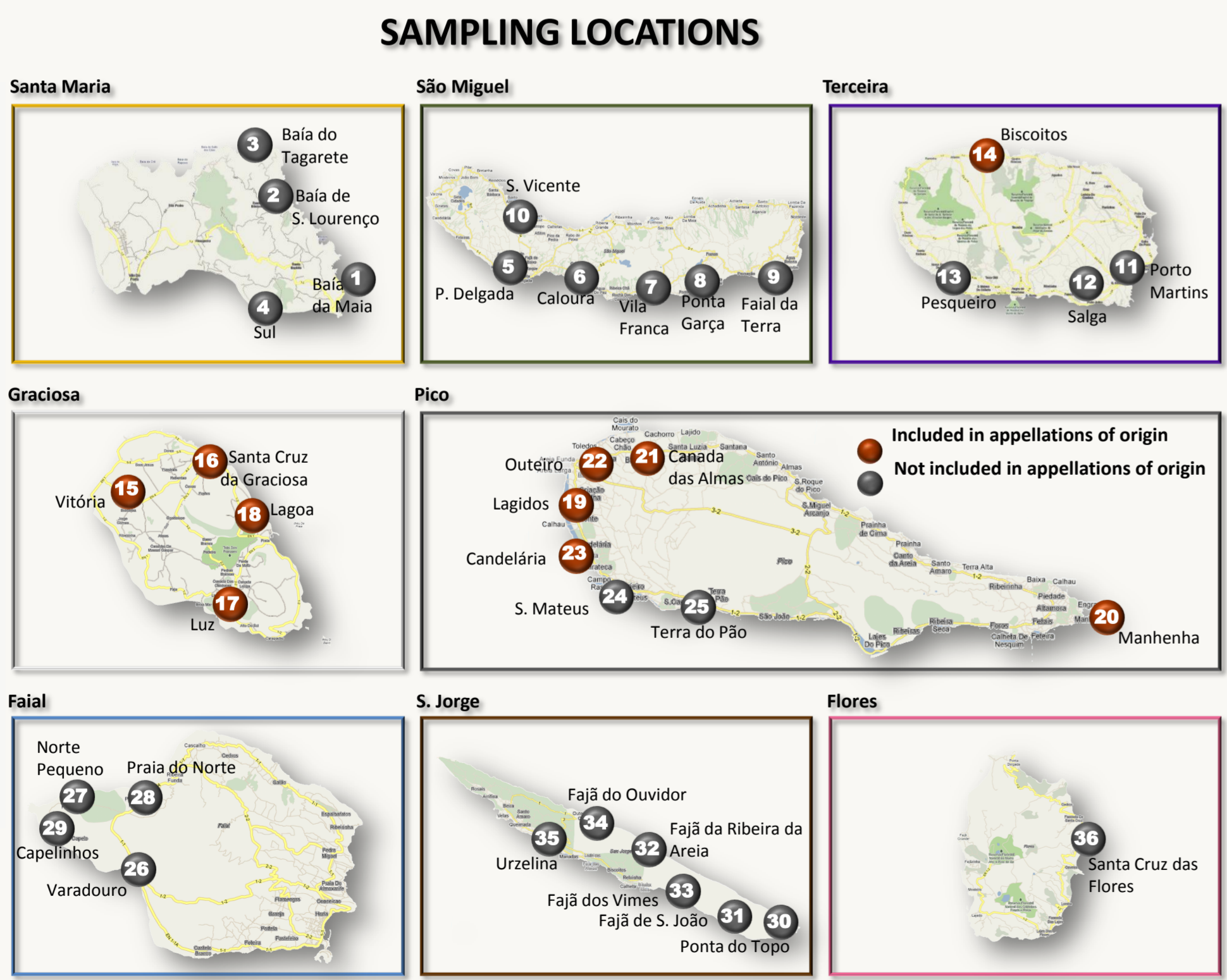
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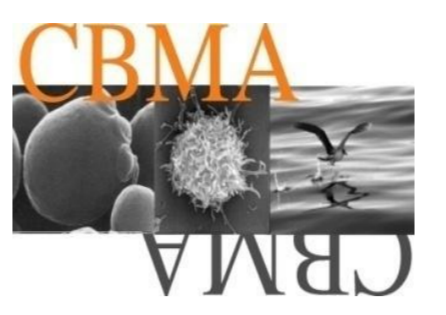
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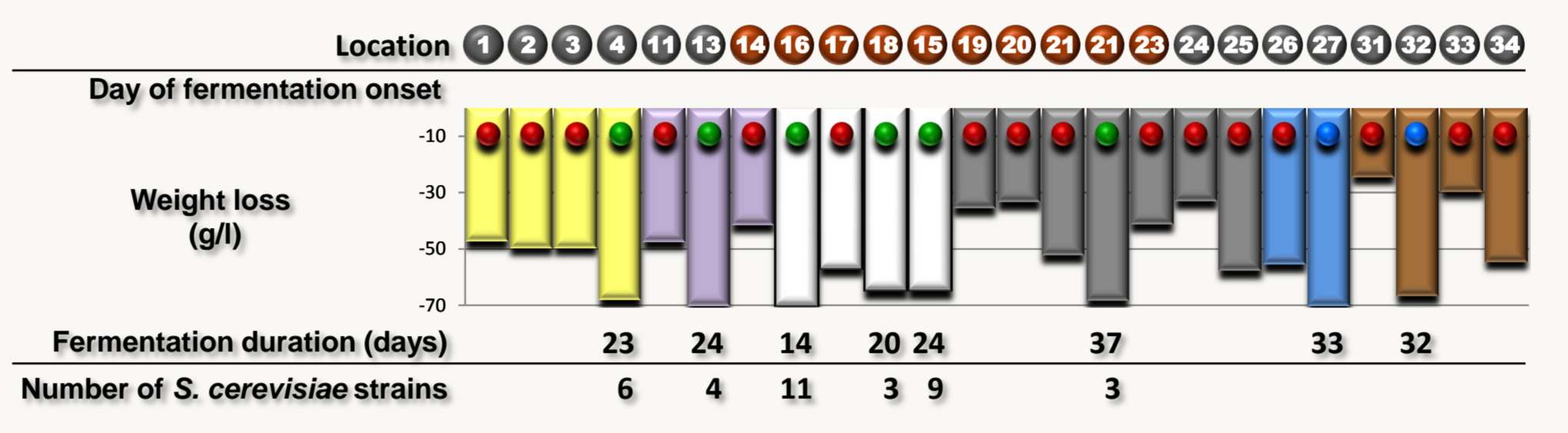
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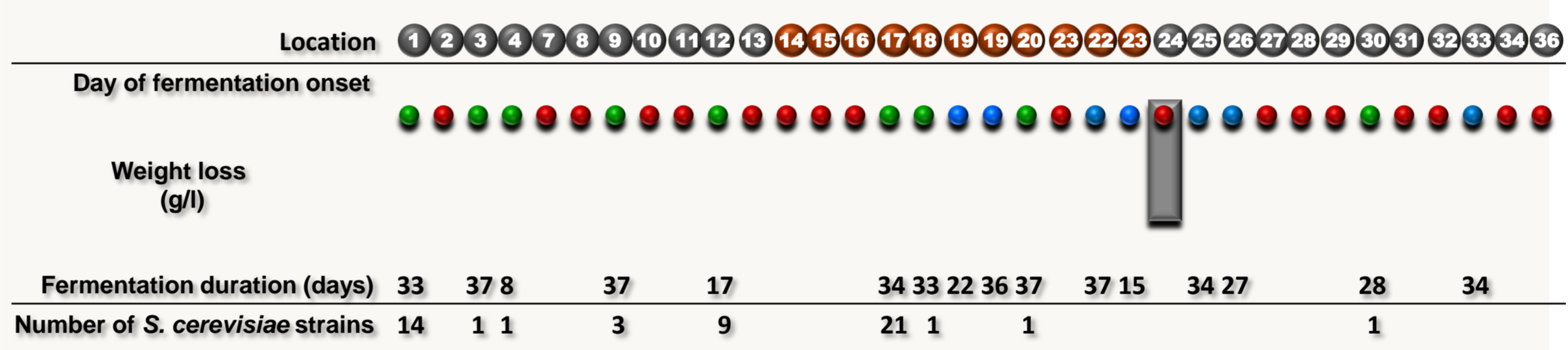
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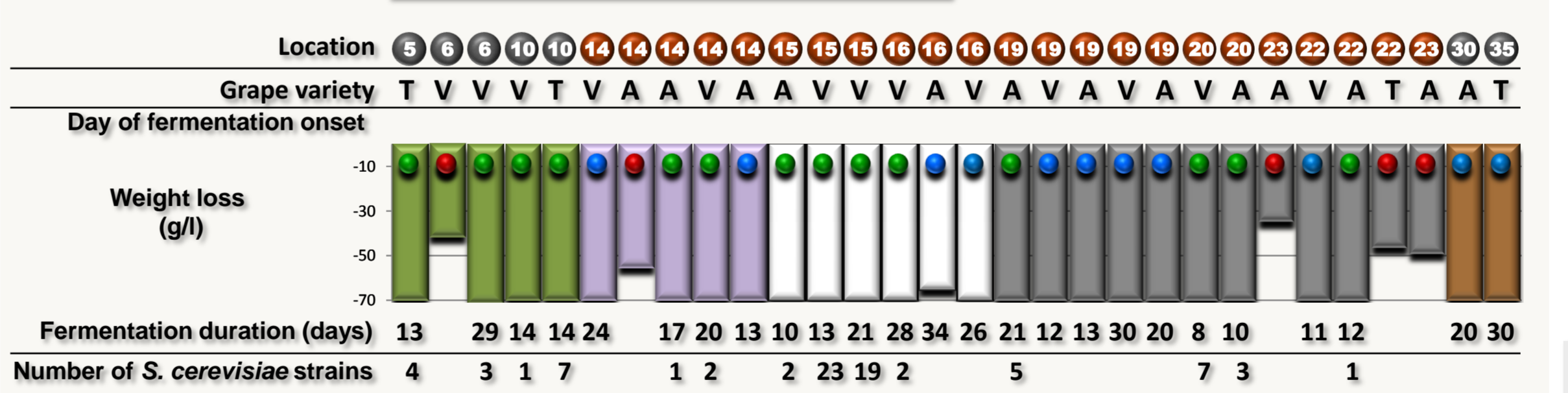
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