INHERITANCE OF WINEMAKING STRESS FACTORS TOLERANCE IN *Saccharomyces uvarum*/*S. eubayanus* x *S. cerevisiae* ARTIFICIAL HYBRIDS

Origone Andrea Cecilia[1,2], González Flores Melisa[1,3], Rodríguez María Eugenia[1,3], Querol Amparo[4] and Lopes Christian Ariel[1,2]

[1]Instituto de Investigación y Desarrollo en Ingeniería de Procesos, Biotecnología y Energías Alternativas (PROBIEN, Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina – Universidad Nacional del Comahue). Buenos Aires 1400, (8300) Neuquén, Argentina.

[2]Facultad de Ciencias Agrarias, Universidad Nacional del Comahue, Argentina.

[3]Facultad de Ciencias Médicas, Universidad Nacional del Comahue, Argentina.

[4]Instituto de Agroquímica y Tecnología de los Alimentos, IATA-CSIC. Agustín Escardino Benlloch, 7, 46980 Paterna, Spain.

Keywords: cryotolerance, wine, sulphite, ethanol, climate change

*Correspondence: clopes@conicet.gov.ar

ABSTRACT

Stress has been defined as any environmental factor that impairs the growth of a living organism. High concentrations of ethanol, sugars and SO_2 as well as temperature variations occurring during winemaking processes are some recognized stress factors that yeasts must overcome in order to avoid stuck or sluggish fermentations. At least two of these factors -sugar and ethanol concentrations- are strongly influenced by the global warming, which become them a worry for the future years in the winemaking industry. One of the most interesting strategies to face this complex situation is the generation of hybrids possessing, in a single yeast strain, a broader range of stress factors tolerance than their parents. In the present study, we evaluated four artificial hybrids generated with *S. cerevisiae*, *S. uvarum* and *S. eubayanus* using a non-GMO-generating method, in their tolerance to a set of winemaking stress factors. Their capacity to overcome specific artificial winemaking situations associated with global warming was also analyzed. All four hybrids were able to grow in a wider temperature range (8-37ºC) than their parents. Hybrids showed intermediate tolerance to higher ethanol, sugar and sulphite concentrations than their parents. Additionally, the hybrids showed an excellent fermentative behaviour in musts containing high fructose concentrations at low temperature as well as under a condition mimicking a stuck fermentation.

1. INTRODUCTION

Yeasts play a central role in winemaking processes determining through their metabolism the final quality of wines. During the whole fermentation process, yeasts should face constant physicochemical changes, such as osmotic pressure, variations in temperature, ethanol, SO_2 and nutrient concentrations (Cardona et al., 2007). All these situations notoriously affect the viability of the microorganisms and hence, several studies have been focused on understanding the global effect of winemaking stress

48 factors over certain strains, especially those belonging to *Saccharomyces cerevisiae*
49 species (Lacerda Ramos et al., 2012; Morard et al., 2019). *S. cerevisiae* industrial strains
50 have developed different cellular mechanisms to deal with these stress factors during
51 winemaking, most of them acquired during the domestication process (Diezmann and
52 Dietrich, 2009).
53 Today, a number of wine commercial yeast starters bearing different biotechnological
54 properties and selected to overcome specific winemaking situations are available in the
55 market. However, the permanent changes that wine industry makes to adapt their
56 technologies to both market requirements and environmental conditions make it
57 necessary to develop new yeast strains adapted to these conditions. In fact, even *S.*
58 *cerevisiae* strains have limitations to fulfill some problematic wine fermentation
59 resulting in sluggish or stuck fermentations (Bisson 1999; Novo et al., 2003).
60 One of the main problems that the winemaking industry has been facing during the last
61 years is related to global warming (Bock et al, 2013; Jones et al., 2005; Tate 2001). This
62 phenomenon gradually affects grapevine yield and wine quality as consequence of the
63 accelerated maturation of grapes that produces musts with high sugar and consequently
64 wines with high ethanol contents (Orduña 2010; White et al., 2006). Moreover,
65 increased concentrations of fructose in relation to glucose in the must have also been
66 hypothesized as a consequence of the climate change (Jones et al., 2005). In this
67 context, the well-known glucophilic character of the regular *S. cerevisiae* strains (Bauer
68 and Pretorius 2000; Berthels et al., 2004; Marsit and Dequin 2015) has become a
69 disadvantage that could lead to stuck fermentation with high concentrations of residual
70 fructose (Bisson 1999).
71 Another problem that winemaking industry must overcome is the need of strains better
72 adapted to extreme fermentation temperatures. Although conducted fermentations are

well controlled processes, temperature is known to be a factor that could also produce sluggish and even stuck fermentations (Moreno-Arribas and Polo, 2005; Pretorius and Høj 2005; Torija et al., 2003). Increasing temperature accelerates the yeast growth rate and subsequently the complete kinetic of the alcoholic fermentation; however, extremely high temperature could affect the yeast cell membrane and produce protein denaturation (Belloch et al., 2008; Serra et al., 2005). On the other hand, low temperature also affects the plasmatic membrane fluidity due to the increase of unsaturation of fatty acids (Torija et al., 2003). However, low fermentation temperature is nowadays a common strategy in winemaking to produce more aromatic wines generated because of the minimization of volatile compounds loss (Beltran et al., 2008; Torija et al., 2003). For those reasons, either to know the temperature growth range of the starter yeasts employed in the wine industry or to develop new strains able to grow in a bigger temperature range became a relevant feature to be evaluated in order to guarantee and optimize the fermentation conditions. Although *S. cerevisiae* showed excellent performance at high temperatures, it is not generally efficient in processes carried out at low temperature (Belloch et al., 2008; Novo et al., 2003). In this new scenario, wine starters based on the cryotolerant species *Saccharomyces uvarum, Saccharomyces eubayanus* or *S. kudriavzevii* have been proposed for low temperature fermentation (López-Malo et al., 2013; Masneuf-Pomarede et al., 2010; Origone et al., 2018). However, these species are more sensitive than *S. cerevisiae* to high temperatures and high ethanol concentrations (Arroyo-López et al., 2010; Belloch et al., 2008; Salvadó et al., 2011).

One of the most interesting strategies to face this complex situation, that allows having a single yeast strain able to tolerate a broader range of stress factors has been the development of artificial hybrids generated among different *Saccharomyces* species. In

fact, the well-known yeast species *S. pastorianus* widely studied because of its economic relevance in lager beer fermentations is a hybrid composed by *S. cerevisiae* and *S. eubayanus* genome portions that inherited the good fermentation performance from *S. cerevisiae* and the cold tolerance from *S. eubayanus* (Baker et al., 2015; Bing et al., 2014; Gibson and Liti 2015; Nakao et al., 2009; Peris et al., 2014, 2016; Su et al., 2019). During the last years, many reports have arisen about the generation of artificial hybrids between these two or other yeast species for beer (Hebly et al., 2015; Mertens et al., 2015), wine (Belloch et al., 2008; García-Ríos et al., 2019; Magalhães et al., 2017a; Su et al., 2019) and cider (Magalhães et al., 2017b) elaboration. In a recent study carried out in our laboratory, two *S. uvarum* strains selected for their differential oenological characteristics were hybridized with a commercial *S. cerevisiae* strain, resulting in hybrids with interesting features to be used in the elaboration of Sauvignon Blanc wines in Patagonia (Origone et al., 2018). Most of these works demonstrated that the hybrids showed a broader temperature range than the parental strains; however, the response of the hybrids to other stress factors has been poorly studied (Arroyo-López et al., 2009; Belloch et al, 2008; Serra et al., 2005).

The performance of the hybrids relative to their parents is the most important factor to be considered in hybridization protocols for winemaking. This performance is directly associated with the genetic events occurring as a consequence of hybridization such as heterosis or hybrid vigour, epistasis, dominance, among others (Bernardes et al., 2016; Plech et al., 2014; Shapira et al., 2014; Zörgo et al., 2012).

In this work, we compared for the first time, different artificial hybrids and their parental strains belonging to the species *S. cerevisiae*, *S. uvarum* and *S. eubayanus*, in both their tolerance to a set of typical winemaking stress factors and their capacity to overcome specific artificial winemaking situations.

2. MATERIALS AND METHODS

2.1. Yeast strains

In the present study two cryotolerant yeast strains isolated from *Araucaria araucana*, *S. eubayanus* NPCC 1292 (*Se*) and *S. uvarum* NPCC 1290 (*Su*^{a}) were selected to generate interspecific hybrids by crossing with two *S. cerevisiae*, a commercial strain NPCC 167 (*Sc*^{c}) and a wine strain NPCC 1178 isolated from Patagonian wines (*Sc*^{w}), and selected according to the biotechnological properties (Lopes et al., 2007). For the generation of homoploid cultures, parental strains were sporulated on acetate medium (% w/v: 1 CH3COONa, 0.1 glucose, 0.125 yeast extract and 2 agar) for 5–7 days at 28 °C. Following preliminary digestion of the asci walls with 2 mg/mL glucuronidase (Sigma), individual spores were seeded in GPY agar plates using a MSM Manual micromanipulator (Singer, UK). Monosporic cultures were able to sporulate in new acetate medium indicating their selfdiploidization, typical from homothallic strains. Natural auxotrophic (*lys*$^{-}$) strains of the two *S. cerevisiae* strains were obtained according to Zaret and Sherman (1985) methodology.

Additionally, two evolved hybrids (H13 or *Sc*^{c} x *Su*^{a} and H17 or *Sc*^{c} x *Su*^{ch}) previously obtained in our laboratory (Origone et al. 2018) were also used. Both parental and hybrid strains are deposited in the North Patagonian Culture Collection (NPCC) (Table 1).

2.2. Interspecific hybrids generation

Two hybrid yeasts were generated by mass-mating of a natural auxotrophic (*lys*$^{-}$) *S. cerevisiae* strain (either *S. cerevisiae* NPCC 167 or *S. cerevisiae* NPCC 1178) and a prototrophic cryotolerant yeast (either *S. eubayanus* NPCC 1292 or *S. uvarum* NPCC 1290): *Sc*^{c} x *Se* and *Sc*^{w} x *Su*^{a} following the methodology described by Origone et al. (2018). The parental were grown in the same tube containing 2 mL of GPY medium (% v/v: 0.5 peptone, 0.5 yeast extract, 2 glucose) and incubated in a static position for 5-10

149 days at 26°C. Hybrid colonies were selected on Minimum Medium (MM) plates (% p/v:
150 0.17 Yeast Nitrogen Base without aminoacids, 2 glucose, 2 agar-agar) and incubated
151 during 4-5 days at 37°C (only hybrids should be able to grow under these conditions).
152 The colonies were repiched in the same conditions and immediately conserved at 20%
153 v/v glycerol at -80°C for later molecular analysis. Hybrid nature was confirmed by PCR
154 amplification of *CBT2* and *GSY1* nuclear genes and subsequent RFLP analysis with
155 endonucleases *Hae* II and *EcoR* I, respectively, following the methodology described by
156 Origone et al. (2018).
157 Adaptive evolution of confirmed hybrid colonies was carried out by five successive
158 fermentations using Sauvignon blanc grape must at 20°C. Adaptive evolution of the
159 hybrids was monitored by RAPD-PCR as suggested by Pérez-Través et al. (2012) and
160 Origone et al. (2018). One evolved hybrid of each cross was additionally characterized
161 by PCR-RFLP of 33 nuclear genes, mtDNA-RFLP, *COX2* mitochondrial gene
162 sequencing and DNA content (ploidy) analyses as proposed by Pérez-Través et al.
163 (2012) and Origone et al. (2018).

164 **2.3. Molecular analysis**

165 ***2.3.1. RAPD-PCR analysis***

166 RAPD-PCR analysis using primers *p24* and *p28* was carried out according to the
167 methodology described in Baleiras Couto et al. (1996).

168 ***2.3.2. PCR-RFLP analysis of nuclear genes***

169 Total genomic DNA was obtained from the new hybrids according to Querol et al.
170 (1992). A total of 33 nuclear coding genes distributed along all of the 16 chromosomes
171 were amplified and digested with restriction enzymes as described previously (Pérez-
172 Través et al., 2014).

173 ***2.3.3. Sequencing analysis of the mitochondrial gene COX2***

Mitochondrial gene *COX2* from evolved hybrids was amplified and sequenced as described by Belloch et al. (2000). PCR products were purified with the AccuPrep PCR kit (Bioneer, Inc, USA) and submitted to an international sequencing service (Macrogen, Korea).

2.3.4. Flow cytometry analysis

The total DNA content was assessed by flow cytometry in a FACScan cytometer (Becton Dickinson Inmunocytometry System) according to the SYTOX Green methodology described by Haase and Reed (2002). DNA content values were determined on the basis of fluorescence intensity compared with the haploid (S288c) and diploid (FY16799) reference *S. cerevisiae* strains.

2.4. Inoculum preparation

For characterization of yeast strains in wine conditions assays, an inoculum of each strain was prepared from young cultures (24 h) previously grown in GPY-agar plates and inoculated in individual tubes containing 5 mL of GPY broth (% w/v: 0.5 peptone, 0.5 yeast extract, 2 glucose). The tubes were incubated for 24 h in agitation conditions at 27°C until the culture reached $2x10^8$ cells/mL. For the assays of simulated winemaking conditions, the inoculum was prepared in the same way but using modified synthetic must MS 300.

2.5. Tolerance to winemaking stress conditions

2.5.1. Temperature and SO_2 tolerance

Temperature and SO_2 stress tolerance were analyzed in YEPD-agar plates (Belloch et al., 2008; Park et al., 1999). YEPD-agar (w/v: 2 % dextrose, 2 % peptone, 1 % yeast extract, 2 % agar) plates were inoculated with six drops of five serial dilutions (1:5) from an initial concentration of $2x10^6$ cell/mL of each yeast strain. Temperature assays were performed by incubating the YEPD-agar plates at different temperature conditions (4, 8, 13, 20, 25, 30 and 37°C). SO_2 media culture was supplemented with tartaric acid

(pH 3.5) 75 mM and increasing concentrations of $Na_2S_2O_5$ to final concentrations of 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4 mM of SO_3^{2-}. Inoculated agar plates were incubated at 25°C. Both stress assays were incubated until colony development was observed at all dilutions under control conditions, 25°C and 0 mM of SO_3^{2-}, respectively.

2.5.2. Ethanol and sugar tolerance

Growth character was performed in 96 well microtiter plates containing 200 µL of YNB (Yeast Nitrogen Base) modified according to the stress condition in the study, adjusted to pH 3.5 and inoculated with an initial concentration of $1x10^6$ cell/mL of each yeast strain culture, reaching an initial OD of 0.2, approximately. At the beginning, the growth medium was modified with increasing concentrations of ethanol to obtain the following final concentrations: 0, 2, 3, 5, 7 and 8 % (v/v). On the other hand, sugars tolerance was achieved by supplementing the medium with 2.5, 5, 20, 60, 100, 120, 180, 240 and 300 g/L of glucose. All assays were performed by triplicate, considering a random distribution across plates and possitions and incubated at 25°C. Growth was monitored by OD at 630 nm (OD_{630}) using a manual microplate reader (MindrayMR-96A, Nanshan, Shenzhen, China). Measurements were taken every hour after a preshaking of 20 seconds. Experimental data were modelled using the reparametrized Gompertz function (Zwietering et al., 1990) for obtaining and comparing growth parameters belonging to each strain.

2.6. Evaluation of hybrids under specific simulated winemaking situations

2.6.1. Fermentations

Assays were carried out in 50 mL flasks containing 35 mL of synthetic must MS 300 modified to generate three simulated conditions: condition 1 (80 g/L glucose, 160 g/L fructose, fermented at 20°C); condition 2 (80 g/L glucose, 160 g/L fructose, fermented at 13°C) and condition 3 (20 g/L glucose, 50 g/L fructose, 8 % (v/v) ethanol, fermented at 13°C). Fermentations (by triplicate) were inoculated with a density of 2 x 10^6 cell/mL

and incubated at the corresponding temperatures. Fermentations evolution was monitored by weight loss of the system until at least two different strains produced a weight loss lower than 0.05 g for two consecutive days. Fermentative products were centrifuged (5 min, 4000 g) and clear supernatants were stored at 4°C until chemical evaluation.

2.6.2. Chemical analysis of fermentation products

Chemical analysis (glucose, fructose, ethanol, glycerol, and acetic acid) of fermented musts were carried out by HPLC using a Thermo Fisher Scientific chromatograph (Waltham, MA). A refraction index detector and the HyperREZTM XP carbohydrate H + 8 µm (Thermo Fisher Scientific) column, protected by a HyperREZTM XP Carbohydrate Guard (Thermo Fisher Scientific) were used. The conditions for the analysis were the following: eluent, 1.5 mM H_2SO_4; flux, 0.6 mL/min and 50°C oven temperature. Samples were diluted 5-fold, filtered through a 0.22 µm nylon filter (Symta, Madrid, Spain) and injected in duplicate.

2.7. Statistical analysis

Kinetic parameters of maximum specific growth rate (μ_{max}) and lag phase (λ) were individually obtained for each particular growth curve. $OD_{630\ nm}$ values and the amount of CO_2 lost daily, from microtiter plate and microfermentations (50 mL) assays respectively, were directly fitted to the reparametrized Gompertz equation (Zwietering et al., 1990):

$$y = A * \exp\left(-exp\left(\left(\frac{\mu_{max} * 2.718282}{A}\right) * (\lambda - t) + 1\right)\right)$$

Where y = ln (Nt/N0), being N_0 the initial OD and N_t the OD measured at time t; A = ln (N_∞/N_0) is the maximum population reached with N_∞ as the asymptotic maximum; μ_{max} is the maximum specific growth rate (h^{-1}) and λ is the length of the lag phase (h) by minimizing the sum of squares of the differences between the experimental data and the

fitted model (observed – predicted)^2. In the case of microfermentations y = ln (N_t/N_0) corresponds to the initial weight of the system (g), A = ln (N_∞/N_0) is the maximum CO_2 production, V_{max} is the maximum fermentation rate (h^{-1}) and λ the period of time to start the vigorous fermentation (h). The analysis was run using the non-linear module of the Statistica 8.0 software package and its Quasi-Newton option.

Physicochemical compounds and kinetic parameters were analyzed by mean comparison using ANOVA and Tukey honest significant differences test (HSD) with an α = 0.05, using the STATISTICA 8.0 Stat Soft Inc.3 software package. Model performance was checked by the lack of feat test and the determination coefficient R^2. Heatmap plots of kinetic parameters were generated employing the MeV Multi Experiment Viewer with Euclidean distance metrics and group clustering was based on group averages (average linkage).

2.8. Heterosis measurement

Heterosis or hybrid vigour was expressed as the percentage of the increase or decrease in the behavior of each diploid hybrid compared to parental strains, including best-parent heterosis (BPH), midparent heterosis (MPH) and worst parent heterosis (WPH) (Dan et al., 2014) according to the following equations:

$$BPH = \frac{F_1 - P_b}{P_b} \times 100$$

$$MPH = \frac{F_1 - \bar{P}}{\bar{P}} \times 100$$

$$WPH = \frac{F_1 - Pw}{Pw} \times 100$$

Where F_1 corresponds to the hybrid, P_b to the best parent, $\bar{P}$ to the mean-parent and Pw to the worst parent phenotypic values. The triploid hybrid H20 was not included in this analysis due to the potential effect of the ploidy on the evaluated traits.

3. RESULTS

3.1. Generation of artificial interspecific hybrids

Cryotolerant yeast strains *S. eubayanus* NPCC 1292 and S. *uvarum* NPCC 1290, previously selected for their interesting oenological features (González Flores et al., 2017; Origone et al., 2017) were employed as parental strains to generate artificial interspecific hybrids with *S. cerevisiae* wine strains. *S. eubayanus* NPCC 1292 was crossed with a natural *lys*$^{-}$ auxotrophic mutant strain of a commercial *S. cerevisiae* wine strain (Origone et al. 2018) and *S. uvarum* NPCC 1290 was crossed with a natural *lys*$^{-}$ auxotrophic mutant of a Patagonian *S. cerevisiae* wine strain selected in a previous work (Lopes et al., 2007). Putative hybrid colonies (named H19 and H20, respectively), selected from minimum medium agar plates at 37°C, were confirmed by PCR-RFLP of the nuclear genes *CBT1* and *GSY1*. Recently formed hybrids were evolved by five successive fermentation steps in Sauvignon blanc must at 20°C and their evolution was evaluated by invariability of RAPD-PCR profiles (Suppl. Figure 1). Flow cytometry analysis evidenced a DNA content of 2.0 ± 0.01 n for the *Sc*^{c} x *Se* (H19) and 2.9 ± 0.05 n for the *Sc*^{w} x *Su*^{a} (H20) hybrids. Moreover, PCR-RFLP analysis of 33 coding nuclear genes distributed along the 16 chromosomes evidenced that both interspecific hybrids conserved the complete subgenome of the two parental strains (data not shown). Both mtDNA-RFLP analysis and *COX2* mitochondrial gene sequencing evidenced the monoparental inheritance of *S. cerevisiae* mtDNA in the two hybrids.

3.2. Tolerance to different winemaking stress conditions

Both H19 (*Sc*^{c} x *Se*) and H20 (*Sc*^{w} x *Su*^{a}), as well as other two hybrids generated in a previous work named H13 (*Sc*^{c} x *Su*^{a}) and H17 (*Sc*^{c} x *Su*^{ch}), were compared with their respective parental strains in their tolerance to different winemaking stress factors. The effect of temperature was tested by the drop test in YEPD-agar plates incubated at different temperatures (4, 8, 13, 20, 25, 30 and 37°C). Both hybrids and parent yeasts were able to grow between 13ºC and 30 °C, and no growth was observed at 4ºC (Figure

297 1 and Suppl. Table 1). The cryotolerant *S. eubayanus* and *S. uvarum* strains were not
298 able to grow at 37ºC while the two *S. cerevisiae* strains were not able to grow at 8ºC. In
299 contrast, all four hybrids were able to grow at both 8ºC and 37ºC extreme conditions,
300 until dilutions 3 and 5 (Figure 1 and Suppl. Table 1).
301 Sulphite tolerance was also evaluated using the same methodology. In this case, YEPD-
302 agar plates supplemented with sodium metabisulphite to final concentrations of 0, 0.5,
303 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 mM. Results evidenced that the cryotolerant parental
304 strains were only able to grow in plates containing up to 1 mM (equivalent to 43,67
305 mg/L of free SO_2) of the antimicrobial compound, while *S. cerevisiae* parents developed
306 colonies at all evaluated concentrations (Figure 1 and Suppl. Table 1). Interestingly,
307 only the hybrid H20 (Sc^w x Su^a) evidenced a similar sulphite tolerance to that in *S.*
308 *cerevisiae* (4 mM or 170 mg/L sulphite). All remaining hybrids showed an intermediate
309 behaviour with regards to their parents, growing until sulphite concentrations of 1,5 mM
310 (Figure 1 and Suppl. Table 1).
311 In order to analyze the effect of the remaining stress factors (both ethanol and sugar
312 concentrations) over yeasts growth, microlite plates assays were carried out using YNB
313 broth supplemented with the respective stress factor. The OD data obtained in each
314 condition tested were fitted individually for each parent and hybrid yeast to Gompertz
315 equation in order to obtain their respective growth parameters (μ_{max} and λ). For these
316 quantitative traits, and only for the diploid hybrids, heterosis analysis was additionally
317 performed in order to support the results obtained from the comparison between hybrids
318 and parental strains.
319 Figure 2 shows the heatmaps generated from the kinetic parameters μ_{max} and λ obtained
320 for all strains in culture media containing 0 to 8 % (v/v) ethanol (Figure 2A) and 2.5 to
321 300 g/L of glucose (Figure 2B). In all cases, kinetic parameters were normalized for the

corresponding stress factors concentration using the average value calculated among the hybrid and their respective parental strains. The original values associated with these heatmaps are shown in Suppl. Table 2. As a general rule, hybrids evidenced lower μ_{max} and intermediate λ values than their respective parents at all analyzed ethanol concentrations (Figure 2A and Suppl. Table 2A). These observations were in accordance with the heterosis analysis (Suppl. Table 3). In this analysis, the diploid hybrids showed a better performance than the worst parental strains at a concentration of 8% v/v of ethanol (negative WPH values, Suppl. Table 3).

Similar behaviour was observed after evaluation of the μ_{max} and λ parameters at increasing sugars concentrations. Except for the hybrid H20 (*Sc*^{w} x *Su*^{a}), all remaining hybrids showed lower μ_{max} values than their parents at all different sugar concentrations (Figure 2B and Suppl. Table 2B). The hybrids showed, in general, the highest λ values. Again, the hybrid H20 (*Sc*^{w} x *Su*^{a}) evidenced a differential behaviour, with the lowest λ values at high glucose/fructose concentrations (240 to 300 g/L), values that are normally associated with most grape musts.

3.3. Evaluation of hybrids under specific simulated winemaking situations

3.3.1. Glucose/fructose unbalance related to climate change conditions

A first study was performed in microfermentations in 30 mL of synthetic must MS300 modified with a higher proportion of fructose than glucose (240 g/L sugars: 80 g/L of glucose and 160 g/L of fructose). Fermentations were conducted at two different temperatures, 20°C (Condition 1) and 13°C (Conditions 2). Table 2 shows both the kinetic parameters and the main chemical compounds obtained after 20 and 28 days of fermentation under conditions 1 and 2, respectively. Both V_{max} and λ parameters were significantly affected by the fermentation temperature, with the lowest V_{max} and the highest λ values found at 13ºC. Hybrid strains H17, H20 and the parental strain *Sc*^{c} showed the highest V_{max} values at 20ºC, while the complete set of hybrids showed the

highest V_{max} values at 13ºC (Table 2). However, the heterosis analysis evidenced an intermediate behavior in this parameter for most diploid hybrids at both 20ºC and 13ºC with regards to their respective parents (Suppl. Table 3). Regarding λ values, most hybrids (except for hybrid H19) showed intermediate values with respect to the parental strains at the two analysed temperatures. In particular, the hybrid H13 showed hybrid vigor in this parameter at 20ºC, with lower λ values than the best parent (BPH of -45,4%) (Suppl. Table 3).

Most strains were able to complete the sugar consumption (less than 2 g/L of residual sugars in the final wine) at 20ºC (condition 1), with the exception of the two cryotolerant strains isolated from natural habitats (*Su*[a] and *Se*), which left 4.53 and 45.16 g/L residual fructose, respectively (Table 2). In addition, all four hybrids produced significantly lower (approximately 59 % less) amounts of acetic acid than the parents (Table 2).

When the same medium was fermented at 13ºC by the same strains (condition 2), a clear effect of the temperature was observed in all fermentations. In this case, the fermentative processes were stopped after 28 days of fermentation and only the hybrids -particularly those generated with *S. uvarum* parental strains- were able to complete the sugar consumption. Not all parental strains were able to consume the sugars at the same time. Under this condition, the hybrids produced higher glycerol amounts and similar (low) acetic acid concentrations to the same at 20ºC (condition 1).

The heterosis analysis applied for acetic acid production, evidenced high BPH values for all hybrids (Suppl. Table 3). With respect to residual fructose concentrations, diploid hybrids also showed high BPH percentages at 13ºC (Suppl. Table 3).

3.3.2. Simulation of a stuck fermentation

A stuck fermentation condition was simulated using the same synthetic must MS300 containing 20 g/L of glucose, 50 g/L of fructose and 8 % (v/v) of ethanol (maximum exogenous concentration tolerated by both cryotolerant parents and hybrids according to the results exposed above) and the experiments were carried out at 13°C (Condition 3). Under this condition, no significant differences in both V_{max} and λ were observed among strains, with the only exception of hybrid H19 that showed a significantly higher λ value (Table 2). *S. eubayanus* was the only strain unable to complete the fermentations, leaving high (13.76 g/L) concentrations of residual fructose. The hybrids stood out again for the production of the lowest acetic acid concentrations (Table 2), which was also evidenced in the high BPH percentages (68.2 to 78.0%) (Suppl. Table 3).

4. DISCUSSION

Four different hybrids, with particular and differential phenotypic traits were obtained in this work, using the methodology known as mass-mating. Several works have observed the instability of the alloploid genomes in recently formed hybrids. This unstability can cause the lost of chromosomes from one or another parental strain, structural rearrangement in the genomes and changes in the genome size (for a review, see Sipiczki, 2018). For that reason, the propagation of the hybrid for long periods of time under "enriching" or selective conditions have been applied to obtain strains with specific properties (Lopandic, 2018; Sipiczki, 2018; Gorter de Vries et al., 2019). In our work, hybrid generation was followed by adaptive evolution on natural grape must in order to obtain hybrids adapted to the fermentation of this substrate.

The generation of interspecific hybrids has been recognized as an evolutionary method to overcome severe conditions of alcoholic fermentation (Lopandic 2018). Diverse studies have demonstrated that, in general, hybrids can better adapt to changing

conditions of the fermentation process (Masneuf et al., 1998; Morales and Dujon 2012; Sipiczki et al., 2001; Su et al., 2019). In particular, artificial hybridization has been proposed for improving phenotypic characteristics dependent on numerous loci distributed throughout the yeast genome like the ability to grow at a different temperature or in different ethanol concentrations (García-Ríos, et al., 2019; Giudici et al., 2005; Marullo et al., 2004). Bibliographic reports about natural hybrids evidenced that hybrids possessing subgenomes of both *S. cerevisiae* and a cryotolerant *Saccharomyces* species like *S. uvarum*, *S. eubayanus* or *S. kudriavzevii*, are able to grow in a broader temperature range than natural species (Arroyo-López et al., 2009; Belloch et al., 2008). Additionally, other works have demonstrated this inheritance in artificially made hybrids, in comparison with the specific parental strains involved in the hybridization (for a review, see Sipiczki, 2019). All four hybrids used in this work and obtained from two cryotolerant species (two different strains of *S. uvarum* and one strain of *S. eubayanus*) and two strains of *S. cerevisiae*, showed similar temperature growth profile (broader than their respective parents). As mentioned previously, the retention in hybrids of the ability to grow at low temperature is an interesting feature for its putative use in white wines elaboration, usually carried out at lower temperature than red wine.

Information about the behaviour of hybrids under other winemaking typical stresses different from temperature and compared with the parental strains is scarce. As the adaption to different growth temperatures, a large number of genes are involved in the ethanol tolerance in yeasts (Alexandre et al., 2001; Fujita et al., 2006; Teixeira et al., 2009; van Voorst et al., 2006). These genes, more than 200 according to the previously mentioned authors, are broadly distributed throughout the genome (Giudici et al., 2005) which might suggest that genetic improvement of yeasts based on hybridization could

422 be an interesting tool for the generation of more ethanol tolerant yeasts. Arroyo-López
423 et al. (2010) evaluated the ethanol tolerance of a set of yeast strains belonging to the
424 species *S. cerevisiae*, *S. uvarum* (*Saccharomyces bayanus* in that work), *S. kudriavzevii*
425 and *S. paradoxus*, and showed that *S. cerevisiae* was significantly the most resistant
426 species. They showed a maximum ethanol tolerance of 117 g/L for *S. cerevisiae* while
427 the same for *S. kudriavzevii* and *S. uvarum* was around 80 g/L (approximately 8 % v/v).
428 Additionally, these authors observed no differences in ethanol tolerance among strains
429 isolated from natural habitats or fermentative environments of both *S. cerevisiae* and *S.*
430 *uvarum* species suggesting that this physiological feature is not modified throughout the
431 adaptation to human-manipulated fermentative environments. Origone et al. (2017),
432 also observed that both *S. eubayanus* and *S. uvarum* species were not able to grow at
433 ethanol concentrations higher than 8% v/v. Additionally, the authors evidenced a lower
434 ethanol tolerance in the *S. uvarum* strains isolated from natural habitats with regards to
435 those from fermented beverages. In our work, no differences were observed in ethanol
436 tolerance among the two *S. uvarum* parental strains. The two strains showed worse
437 performance (lower μ_{max} and higher λ values) than *S. cerevisiae* at the maximum
438 ethanol concentration evaluated (8% v/v). Independently from the parental strains
439 involved, all hybrids showed intermediate or worse behaviour than their respective
440 parental strains at increasing ethanol concentrations. These results were validated by the
441 heterosis analysis, in which we compared the best parental heterosis, midparental
442 heterosis and worst parental heterosis (BPH, MPH and WPH, respectively) for all
443 diploid hybrids. This analysis was not carried out for the triploid hybrid H20, because
444 many traits are strongly affected by the ploidy (Zörgo et al., 2013). In a recent work
445 about comparative genomics of *S. cerevisiae* strains with different ethanol tolerances,
446 Morard et al. (2019) demonstrated that polysomy in chromosome III was associated

with the high tolerance to this compound. Interestingly, the hybrid H20 (*Sc*^{w} x *Su*^{a}) generated in this work -the only triploid hybrid- evidenced the best performance at the highest ethanol concentration evaluated (8% v/v), although it was not better than the performance of the best parental strain (*S. cerevisiae* NPCC 1278). Our results suggest that artificial hybridization did not improve ethanol tolerance in yeasts. Other experimental approaches, as adaptive evolution experiments based on the exposition of yeasts to increasing concentrations of ethanol, could be carried out in order to improve ethanol tolerance. This approach has already been employed by other authors using *S. cerevisiae* (Gorter de Vries et al., 2017; Voordeckers et al., 2015) and even newly generated hybrids (Piotrowski et al., 2012).

Another significant winemaking stress factor is sugar concentration, ranging in most grape musts between 120 and 250 g/L (Fleet and Heard, 1993). Under this condition, yeasts are subjected to strong osmotic pressure. Sugar concentrations from 200 g/L to 300 g/L have been reported to decrease significantly the growth rate of *S. cerevisiae* (Charoenchai et al., 1998; D'Amato et al., 2006). Different cellular mechanisms have been proposed to overcome the hyperosmotic stress, including the synthesis and accumulation of specific osmotically active compounds (i.e. glycerol or threalose), temporary arrest of cell cycle, modifications of both transcription and translation patterns (Babazadeh et al., 2017; Sipiczki, 2019; Scanes et al., 1998). Our results evidenced that both commercial *S. cerevisiae* and *S. eubayanus* parental strains showed the highest μ_{max} values. Numerous reports have evidenced the extraordinary ability of *S. cerevisiae* to grow under high sugar concentrations (Berthels et al., 2004) but little has been shown about *S. eubayanus*. Origone et al. (2017) has recently evidenced this ability in the cryotolerant species *S. eubayanus*. Contrarily to what could be expected

471 from the cross of these two species, the hybrids obtained from their cross evidenced
472 significantly lower μ_{max} values than their parents.
473 The ability to grow at high sugar concentrations was already evaluated in many yeasts
474 possessing chimeric genomes between *S. cerevisiae* and a cryotolerant species of the
475 genus generated by both natural and artificial processes. In some of these works,
476 hybrids displayed an even better sugar tolerance than *S. cerevisiae* (Belloch et al., 2008;
477 Bellon et al., 2015; Gibson and Liti 2015). Contrarily, a recent study that evaluated the
478 fermentative profiles of artificial *S. cerevisiae* x *S. eubayanus* hybrids evidenced an
479 intermediate osmotolerance of the hybrids in relation to their parents (Magalhães et al.,
480 2017a). Most of these previously mentioned studies employed the drop test to evaluate
481 the ability of the yeasts to grow at different sugar concentrations. This methodology is
482 useful but it has limitations related to its semiquantitative nature. The methodology used
483 in our work allows having a more complete analysis of yeast growth parameters,
484 including both μ_{max} and λ. Interestingly, in our work, the specific analysis of the kinetic
485 parameters evidenced that the triploid hybrid Sc^{w} x Su^{a} showed the lowest λ and higher
486 μ_{max} values at the highest sugar concentrations (240-300 g/L). Krogerus et al. (2016)
487 also observed that both allotriploid and allotetraploid hybrids between *S. cerevisiae* and
488 *S. eubayanus*, but not the allodiploid hybrids, showed a better performance in high-
489 gravity wort fermentations than their parents.
490 The ability to grow is not necessarily related to the ability to complete the fermentation
491 in grape must with high sugar concentration, leaving low levels of residual sugars in the
492 wine. Moreover, it is well known that different yeast strains have a differential affinity
493 to glucose or fructose, the two main sugars present in the grape must (Leandro et al.,
494 2009). In fact, the species *S. uvarum* has been associated with a more fructophilic
495 character than *S. cerevisiae* (Tronchoni et al., 2009). Taking these aspects into

consideration, the complete set of both parental and hybrid strains were evaluated in fermentation conditions using synthetic must with 240 g/L total sugars but containing unbalanced glucose/fructose concentrations (80 g/L/ 160 g/L). Under this condition, all strains completed the fermentations carried out at 20ºC but only hybrids were able to consume the total reducing sugars at 13ºC, with the additional advantage of producing very low amounts of acetic acid. Considering both, the hybrid vigour as an improvement of the performance of the parental strains, and a low acetic acid production as a good fermentative trait of the yeasts in winemaking, our data suggest the existance of hybrid vigour in this particular conditions for all the analyzed hybrids. The disturbance in the glucose/fructose proportion (grape must have equimolar amounts of the two monosaccharides) has recently been hypothesized to be associated with climate change (Jones et al., 2005), which would turn hybrids into interesting tools in the future winemaking industry.

Additionally, during the last stages of fermentation, yeasts must efficiently consume the residual sugars under the additional effect of ethanol. These residual sugars are mostly composed by fructose due to the glucophilic character of most yeasts (Leandro et al., 2009). Interestingly, the hybrid *Sc*^{w} x *Su*^{a} also showed the lowest λ values at the lowest sugar concentration evaluated (2,5-5 g/L), even lower than the one in the two *S. cerevisiae* parental strains. Incomplete alcoholic fermentations constitute another typical problem in winemaking and hybrids, because of their complex genomic constitution, could be an interesting biotechnological tool to overcome this situation. Considering the maximum ethanol tolerance observed in this work for most strains (both hybrids and parents) as well as the excellent behaviour of hybrids in glucose/fructose unbalance conditions at low temperature, all strains were subsequently compared in their fermentation ability using a synthetic must simulating a stuck fermentation (20 g/L

glucose, 50 g/L fructose, 8% v/v ethanol). In order to avoid the effect of nitrogen limitation as a putative cause of stuck fermentation (Beltran et al., 2005), a total of 300 mg/L YAN was used in this work for the elaboration of the synthetic must. Numerous studies have been carried out with the aim of identifying special yeasts able to restart stuck fermentations, as well as the cellular mechanisms involved (Beltran et al. 2005; Cavazza et al., 2004; Llauradó et al., 2005; Santos et al., 2008). Our results evidenced that both hybrids and parental strains were able to restart the stuck fermentations at 13°C, with the exception of *S. eubayanus*. This species was able to grow at both ethanol (8% v/v) and sugars concentrations (70 g/L total sugar) when these factors were evaluated independently; however, this yeast cannot complete a fermentation in which these two factors are together. Again, the four hybrids generated fermentation products with a reduced acetic acid content, which represents an interesting advantage of the hybrid over the parental strains. Some authors have described this differential ability to produce acetic acid by yeasts as a strain dependent feature (Antonelli et al., 1999; Castellari et al., 1994; Rainieri et al., 1999). The low acetic acid production was observed as a common feature of the four hybrids in every different situation evaluated in this work, which could be associated with common interaction strategies between the subgenomes of the parental strains involved in hybrid generation. In a previous work carried out in our laboratory, all 18 *S. cerevisiae* x *S. uvarum* hybrid strains (including the two hybrids used in this work) also showed significantly lower acetic acid production than their parents in wines obtained from Sauvignon blanc grape must (Origone et al., 2018). González et al. (2007) and Gamero et al. (2013) also evidenced a lower acetic acid production in both *S. cerevisiae* x *S. uvarum* and *S. cerevisiae* x *S. kudriavzevii* hybrids compared to *S. cerevisiae* and *S. kudriavzevii* strains used as references (in this case, the authors did not have the real parental strains because they

546 used natural hybrids). By means of quantitative trait loci (QTL) mapping, Marullo et al.
547 (2007) demonstrated that acetic acid production in wine yeasts is due to a non-
548 synonymous single-nucleotide polymorphism in the asparaginase *ASP1*. However, the
549 explanation for the low acetic acid by the hybrids is still a matter of exploration.
550 Finally, the tolerance to the antimicrobial compound SO_2 was also suggested as a key
551 characteristic to be present in a starter for winemaking. This compound is a very
552 reactive molecule that binds to a high number of cell metabolites and enzymes; its
553 impact on wine yeasts, particularly *S. cerevisiae*, has been widely studied (Divol et al.,
554 2012). Sulphite tolerance in yeast has been associated to one specific gene named *SSU1*,
555 codifying for a plasma membrane protein responsible for the efficient sulphite efflux, as
556 well as to its transcription factor *FZF1* (Avram and Bakalinsky, 1997; Avram et al.,
557 1999). *S. cerevisiae* also face the high SO_2 levels in wine by means of the production of
558 high levels of acetaldehyde (highly reactive molecule that binds sulphite) as well as the
559 upregulation of sulphite reduction systems or whole sulphur metabolism (Casalone et
560 al., 1992). Contrarily to what happens with the tolerance to the other stresses, sulphite
561 tolerance has been proved to be a domestication sign present in yeasts associated with
562 industrial fermentations and absent in wild strains (Barrio et al., 2006; Pérez-Ortín et al.,
563 2002). *S. cerevisiae* wine strains present a reciprocal translocation between
564 chromosomes VIII and XVI, generating a recombinant *SSU1* promoter involved in the
565 higher tolerance to sulphite (Pérez-Ortín et al., 2002). *Saccharomyces* non *cerevisiae*,
566 generally associated to less anthropic environments, are more sensitive to sulphite
567 concentrations than *S. cerevisiae*. In *S. uvarum*, the presence of a *S. eubayanus*
568 sequence integrated into the gene *FZF1* confers a higher tolerance to sulfite (Zhang et
569 al., 2015). Many strains of this species from Holarctic origin have demonstrated to
570 possess this integrated region, absent in *S. uvarum* strains from other origins including

571 the South American strains from natural habitats (Albertin et al., 2018; Almeida et al.,
572 2014). In accordance with this phenomenon, Origone et al. (2017) observed that the
573 Patagonian strains of *S. eubayanus* and *S. uvarum* species, including those used in this
574 work, were extremely sensitive to this antimicrobial compound, indicating that sulphite
575 tolerance should be improved to propose the use of these strains in winemaking. For
576 that reason, the inheritance of sulphite tolerance from the *S. cerevisiae* parental strain is
577 a valuable tool in hybrids generated for its use in winemaking industry. Two out of four
578 hybrids analyzed in the present study evidenced high sulphite tolerance, similar to that
579 observed for the two *S. cerevisiae* parental strains. Two hybrids generated in this work
580 (both Sc^{c} x *Se* and Sc^{w} x Su^{a}) were able to grow in media containing high (4 mM)
581 sulphite concentrations. This differential behaviour among the obtained hybrids could
582 be related to a different inheritance of specific genes alleles associated with the
583 tolerant/sensitive phenotypes in the hybrids. Interestingly, the hybrid Sc^{w} x Su^{a} was the
584 most tolerant; this particular feature might be associated with the presence of a higher
585 copy number (this hybrid exhibited higher DNA content than the remaining hybrids,
586 approximately 3n) of the *S. cerevisiae* genes responsible for the sulphite tolerance.
587 Hybridization was also used recently as a method to improve sulphite tolerance in *S.*
588 *uvarum*, (Liu et al., 2017).
589 The authors obtained hybrids between sensitive and tolerant parental strains of this
590 species, that were able to ferment Sauvignon blanc grape juice containing 2 mM (Liu et
591 al., 2017). In our work, the tolerant hybrids were able to grow in media containing 4
592 mM of this compound. According to these results, hybridization is a useful strategy to
593 rapidly generate strains with characteristics typically acquired by the yeasts during long
594 term domestication processes.

595 **5. CONCLUSIONS**

This study contributes to know more about interspecific laboratory created hybrids and their possible response under winemaking stress conditions. The capability of hybrids to develop within a wider range of temperatures than parent yeasts (8-37°C) and to adapt to fermentative conditions in musts with unequal contents of sugar at different temperatures with low acetic acid production turn hybrid yeasts in an interesting oenological tool. Furthermore, a good adaptation to musts in the presence of the antimicrobial compound SO_2 suggests its potential for winemaking industry. Our data also support that the hybridization method of mass-mating, allows the generation of strains with different ploidy levels, that could be associated with differential adaptive features.

ACKNOWLEDGEMENTS

This work was supported by grants PICT 2015-1198 from ANPCyT, PIP 2015-555 from CONICET and PI04-A128 from Universidad Nacional del Comahue (Argentina) to CAL, as well as Grant RTI2018-093744-B-C31 from the Spanish Government and FEDER to AQ. ACO and MGF thanks CONICET for their fellowships.

REFERENCES

Albertin, W., Chernova, M., Durrens, P., Guichoux, E., Sherman, D.J., Masneuf-Pomarede, I., Marullo, P., 2018. Many interspecific chromosomal introgressions are highly prevalent in Holarctic *Saccharomyces uvarum* strains found in human-related fermentations. YEAST 35, 141-156.

Alexandre, H., Heintz, D., Chassagne, D., Guilloux-Benatier,M., Charpentier, C., Feuillat, M., 2001. Pretease A activity and nitrogen fractions released during alcoholic fermentation and autolysis in enological conditions. J. Ind. Microbiol. Biot. 26, 235-240.

620 Almeida, P., Goncalves, C., Teixeira, S., Libkind, D., Bontrager, M., Masenuf-
621 Pomarede, I., Albertin, W., Durrens, P., Sherman, D.J., Marullo, P., Hittinger,
622 C.T., Sampaio, J.P. 2014. A Gondwanan imprint on global diversity and
623 domestication of wine and cider yeast *Saccharomyces uvarum*. Nat. Commun. 5,
624 4044.
625 Antonelli, A., Castellari, L., Zambonelli, C., Carnacini, A., 1999. Yeast influence on
626 volatile composition of wines. J. Agric. Food Chem. 47, 1139-1144.
627 Arroyo-López, F.N., Orlić, S., Querol, A., Barrio, E., 2009. Effects of temperature, pH
628 and sugar concentration on the growth parameters of *Saccharomyces cerevisiae*,
629 *S. kudriavzevii* and their interspecific hybrid. Int. J. Food Microbiol. 131, 120-
630 127.
631 Arroyo-López, F.N., Salvadó, Z., Tronchoni, J., Guillamón, J.M., Barrio, E., Querol, A.,
632 2010. Susceptibility and resistance to ethanol in *Saccharomyces* strains isolated
633 from wild and fermentative environments. Yeast 27, 1005-1015.
634 Avram, D., Bakalinsky, A.T., 1997. SSU1 encodes a plasma membrane protein with a
635 central role in a network of proteins conferring sulfite tolerance in *S. cerevisiae*. J.
636 Bacteriol. 179, 5971-5974.
637 Avram, D., Leid, M., Bakalinsky, A.T., 1999. Fzf1p of *S. cerevisiae* is a positive
638 regulator of *SSU1* transcription and its first zinc-finger region is required for DNA
639 binding. Yeast 15, 473-480.
640 Babazadeh, R., Lahtvee, P.J., Adiels, C.B., Goksör, M., Nielsen, J.B., Hohmann, S.,
641 2017. The yeast osmostress response is carbon source dependent. Sci. Rep. 7, 990.
642 Baker, E.C., Wang, B., Bellora, N., Peris, D., Hulfachor, A.B., Koshalek, J.A., Adams,
643 M., Libkind, D., Hittinger, C.T., 2015. The genome sequence of *Saccharomyces*

644 *eubayanus* and the domestication of lager brewing yeasts. Mol. Biol. Evol. 32,
645 2818-2831.
646 Baleiras Couto, M.M., Eijsma, B., Hofstra, H., Huis, J.H.J., Van der Vossen, J.M.B.M.,
647 1996. Evaluation of molecular typing techniques to assign genetic diversity
648 among *Saccharomyces cerevisiae* strains. Appl. Env. Microbiol. 62, 41‑46.
649 Barrio, E., González, S.S., Arias, A., Belloch, C., Querol, A., 2006. Molecular
650 mechanisms involved in the adaptive evolution of industrial yeasts. In: Querol A.
651 y Fleet G.H., eds. The Yeast Handbook Vol. 2: Yeasts in Food and Beverages.
652 Chapter 6. Springer Verlag, Berlin, Alemania.
653 Bauer, E.F., Pretorius, L.S., 2000. Yeast stress response and fermentation efficiency:
654 how to survive the making of wine - A review. South African J. Enol. Vitic. 21,
655 27-51.
656 Belloch, C., Querol, A., García, M.D., Barrio, E., 2000. Phylogeny of the genus
657 *Kluyveromyces* inferred from the mitochondrial cytochrome-c oxidase II gene. Int.
658 J. Syst. Evol. Micr. 50, 405-416.
659 Belloch, C., Orlic, S., Barrio, E., Querol, A., 2008. Fermentative stress adaptation of
660 hybrids within the *Saccharomyces* sensu stricto complex. Int. J. Food Microbiol.
661 122, 188–195.
662 Bellon, J.R., Yang, F., Day, M.P., Inglis, D.L., Chambers, P.J., 2015. Designing and
663 creating *Saccharomyces* interspecific hybrids for improved, industry relevant,
664 phenotypes. Appl. Microbiol. Biotechnol. 99, 8597-8609.
665 Beltran, G., Esteve-Zarzoso, B., Rozès, N., Mas, A., Guillamón, J.M., 2005. Influence
666 of the timing of nitrogen additions during synthetic grape must fermentations on
667 fermentation kinetics and nitrogen consumption. J. Agric. Food Chem. 53, 996-
668 1002

669 Beltran, G., Novo, M., Guillamón, J.M., Mas, A., Rozès, N., 2008. Effect of
670 fermentation temperature and culture media on the yeast lipid composition and
671 wine volatile compounds. Int. J. Food Microbiol. 121, 169-177.
672 Bernardes, J.P., Stelkens, R.B., Greig, D., 2016. Heterosis in hybrids within and
673 between yeast species. J. Evol. Biol. 30, 538-548.
674 Berthels, N.J., Cordero Otero, R.R., Bauer, F.F., Thevelein, J.M., Pretorius, I.S., 2004.
675 Discrepancy in glucose and fructose utilisation during fermentation by
676 *Saccharomyces cerevisiae* wine yeast strains. FEMS Yeast Res. 4, 683-689.
677 Bing, J., Han, P.J., Liu, W.Q., Wang, Q.M., Bai, F.Y., 2014. Evidence for a far east
678 asian origin of lager beer yeast. Curr. Biol. 24, 1-12.
679 Bisson, L., 1999. Stuck and sluggish fermentations. Am. J. Enol. Viticult. 50, 107–109.
680 Bock, A., Sparks, T.H., Estrella, N., Menzel, A., 2013. Climate-induced changes in
681 grapevine yield and must sugar content in Franconia (Germany) between 1805
682 and 2010. PLoS One 8 (7): e69015.
683 Cardona, F., Carrasco, P., Pérez-Ortín, J.E., del Olmo, M. Lí, Aranda, A., 2007. A novel
684 approach for the improvement of stress resistance in wine yeasts. Int. J. Food.
685 Microbiol. 114, 83-91.
686 Casalone, E., Colella, C.M., Daly, S., Gallori, E., Moriani, L., Polsinelli, M., 1992.
687 Mechanism of resistance to sulfite in *Saccharomyces cerevisiae*. Curr. Genet. 22,
688 435–440.
689 Castellari, L., Ferruzzi, M., Magrini, A., Giudici, P., Passarelli, P., Zambonelli, C.,
690 1994. Unbalanced wine fermentation by cryotolerant vs. non-cryotolerant
691 *Saccharomyces* strains. Vitis 33, 49-52.
692 Cavazza, A., Poznanski, E., Trioli, G. (2004) Restart of fermentation of simulated stuck
693 wines by direct inoculation of active dry yeasts. Am. J. Enol. Vitic. 55, 160-167.

694 Charoenchai, C., Fleet, G., Henschke, P.A., 1998. Effects of temperature, pH and sugar
695 concentration on the growth rates and cell biomass of wine yeasts. Am. J. Enol.
696 Viticult. 49, 283–288.
697 Coloretti, F., Zambonelli, C., Tini, V., 2006. Characterization of flocculent
698 *Saccharomyces* interspecific hybrids for the production of sparkling wines. Food
699 Microbiol. 23, 672-676.
700 D'Amato, D., Corbo, M.R., Del Nobile, M.A., Sinigaglia, M., 2006. Effects of
701 temperature, ammonium and glucose concentrations on yeast growth in a model
702 wine system. Int. J. Food Sci. Tech. 41, 1152–1157.
703 Dan, Z., Liu, P., Huang, W., Zhou, W., Yao, G., Hu, J., Zhu, R., Lu, B., Zhu, Y. 2014.
704 Balance between a higher degree of heterosis and increased reproductive
705 isolation: strategic design for breeding inter-subspecific hybrid rice. Plos One. 9,
706 e93122.
707 Diezmann, S., Dietrich, F.S., 2009. *Saccharomyces cerevisiae*: Population divergence
708 and resistance to oxidative stress in clinical, domesticated and wild isolates. PLoS
709 One 4 (4): e5317.
710 Divol, B., Du Toit, M., Duckitt, E., 2012. Surviving in the presence of sulphur dioxide:
711 Strategies developed by wine yeasts. Appl. Microbiol. Biotechnol. 95, 601-613.
712 Fujita, K., Matsuyama, A., Kobayashi, Y., Iwahashi, H., 2006. The genome-wide
713 screening of yeast deletion mutants to identify the genes required for tolerance to
714 ethanol and other alcohols. FEMS Yeast Res. 6 (5), 744-50.
715 Gamero, A., Tronchoni, J., Querol, A., Belloch, C., 2013. Production of aroma
716 compounds by cryotolerant *Saccharomyces* species and hybrids at low and
717 moderate fermentation temperatures. J. Appl. Microbiol. 114, 1405-1414.

718 Gangl, H., Batusic, M., Tscheik, G., Tiefenbrunner, W., Hack, C., Lopandic, K., 2009.
719 Exceptional fermentation characteristics of natural hybrids from *Saccharomyces*
720 *cerevisiae* and *S. kudriavzevii*. New Biotechnol. 25 (4), 244-251.
721 García-Ríos, E., Guillén, A., de la Cerda, R., Pérez-Través, L., Querol, A., Guillamón,
722 J.M., 2019. Improving the cryotolerance of wine yeast by interspecific
723 hybridization in the genus *Saccharomyces.* Front. Microbiol. 9, 3232.
724 Gibson, B., Liti, G., 2015. *Saccharomyces pastorianus*: genomic insights inspiring
725 innovation for industry. Yeast 32, 17-27.
726 Giudici, P., Solieri, L., Pulvirenti, A.M. y Cassanelli, S. (2005) Strategies and
727 perspectives for genetic improvement of wine yeasts. Appl. Microbiol.
728 Biotechnol. 66, 622-628.
729 González, S.S., Gallo, L., Climent, M.D., Barrio, E., Querol, A., 2007. Enological
730 characterization of natural hybrids from *Saccharomyces cerevisiae* and *S.*
731 *kudriavzevii.* Int. J. Food Microbiol. 116, 11-18.
732 González Flores, M., Rodríguez, M.E., Oteiza, J.M., Barbagelata, R.J., Lopes, C.A.,
733 2017. Physiological characterization of *Saccharomyces uvarum* and
734 *Saccharomyces eubayanus* from Patagonia and their potential for cidermaking.
735 Int. J Food. Microbiol. 249, 9-17.
736 Gorter de Vries, A.R., Pronk, J.T., Daran, J.-M., 2017. Industrial relevance of
737 chromosomal copy number variation in *Saccharomyces* yeasts. Appl. Environ.
738 Microbiol. 83 (11) e 3206-16.
739 Haase, S.B., Reed, S.I., 2002. Improved flow cytometric analysis of the budding yeast
740 cell cycle. Cell Cycle 1, 117-121.
741 Hebly, M., Brickwedde, A., Bolat, I., Driessen, M.R.M., De Hulster, E.A.F., Van Den
742 Broek, M., Pronk, J.T., Geertman, J-M, Daran J-M, Daran-Lapujade, P., 2015. *S.*

cerevisiae x *S. eubayanus* interspecific hybrid, the best of both worlds and beyond. FEMS Yeast Res. 15 (3).

Jones, G. V., White, M.A., Cooper, O.R., Storchmann, K., 2005. Climate change and global wine quality. Climate Change 73 (3), 319–343.

Krogerus, K., Arvas, M., De Chiara, M., Magalhaes, F., Mattinen, L., Oja, M., Vidgren, V., Yue, J.X., Liti, G., Gibson, B., 2016. Ploidy influences the functional attributes of de novo lager yeast hybrids. Appl. Microbiol. Biotechnol. 100, 7203-7222.

Lacerda Ramos, R., Ferreira Duarte,W., Freire A. L., Ribeiro Dias, D., Araújo Eleutherio, E.C., Freitas Schwan, R., 2013. Evaluation of stress tolerance and fermentative behavior of indigenous *Saccharomyces cerevisiae*. Braz. J. Microbiol. 44 (3), 935–944.

Leandro, M.J., Fonseca, C., Gonçalves, P., 2009. Hexose and pentose transport inascomycetousyeasts: an overview. FEMS Yeast Res. 9, 511-525.

Liu, X.Z., Sang, M., Zhang, X.A., Zhang, T.K., Zhang, H.Y., He, X., Li, S.X., Sun, X.D., Zhang, Z.M., 2017. Enhancing expression of SSU1 genes in *Saccharomyces uvarum* leads to an increase in sulfite tolerance and transcriptome profiles change.

Llauradó, J.M., Rozeés, N., Constantí, M., Mas, A., 2005. Study of some *Saccharomyces cerevisiae* strains for winemaking after preadaptation at low temperatures. J. Agric. Food Chem. 53, 1003-1011.

Lopandic, K., 2018. *Saccharomyces* interspecies hybrids as model organisms for studying yeast adaptation to stressful environments. Yeast 35, 21-38.

Lopes, C.A., Rodríguez, M.E., Sangorrín, M., Querol, A., Caballero, A.C., 2007. Patagonian wines: Implantation of an indigenous strain of *Saccharomyces*

767 *cerevisiae* in fermentations conducted in traditional and modern cellars. J. Ind.
768 Microbiol. Biotechnol. 34, 139-149.
769 López-Malo, M., Querol, A., Guillamon, J.M., 2013. Metabolomic comparison of
770 *Saccharomyces cerevisiae* and the cryotolerant species *S. bayanus* var. *uvarum*
771 and *S. kudriavzevii* during wine fermentation at low temperature. PLoS One 8 (3)
772 e60135.
773 Magalhães, F., Krogerus, K., Castillo, S., Ortiz-Julien, A., Dequin, S., Gibson, B.,
774 2017a. Exploring the potential of *Saccharomyces eubayanus* as a parent for new
775 interspecies hybrid strains in winemaking. FEMS Yeast Res. 17.
776 Magalhães, F., Krogerus, K., Vidgren, V., Sandell, M., Gibson, B., 2017b. Improved
777 cider fermentation performance and quality with newly generated *Saccharomyces*
778 *cerevisiae* × *Saccharomyces eubayanus* hybrids. J Ind. Microbiol. Biotechnol. 44
779 (8), 1203-1213.
780 Marullo, P., Bely, M., Masneuf-Pomarede, I., Aigle, M., Dubourdieu, D., 2004.
781 Inheritable nature of enological quantitative traits is demonstrated by meiotic
782 segregation of industrial wine yeast strains. FEMS Yeast Res. 4, 711-719.
783 Marullo, P., Yvert, G., Bely, M., Aigle, M., Dubourdie, D., 2007. Efficient use of DNA
784 molecular markers to construct industrial yeast strains. FEMS Yeast Res.7, 1295-
785 1306.
786 Marsit, S., Dequin, S., 2015. Diversity and adaptive evolution of *Saccharomyces* wine
787 yeast: a review. FEMS Yeast Res. 15, 1-12.
788 Masneuf, I., Hansen, J., Groth, C., Piskur, J., Dubourdieu, D., 1998. New hybrids
789 between *Saccharomyces* sensu stricto yeast species found among wine and cider
790 production strains. Appl. Environ. Microbiol. 64, 3887-3892.

Masneuf-Pomarède, I., Bely, M., Marullo, P., Lonvaud-Funel, A., Dubourdieu, D., 2010. Reassessment of phenotypic traits for *Saccharomyces bayanus* var. *uvarum* wine yeast strains. Int. J. Food Microbiol. 139, 79-86.

Mertens, S., Steensels, J., Saels, V., De Rouck, G., Aerts, G., Verstrepen, K.J., 2015. A large set of newly created interspecific *Saccharomyces* hybrids increases aromatic diversity in lager beers. Appl. Environ. Microbiol. 81, 8202-8214.

Morales, L., Dujon, B., 2012. Evolutionary role of interspecies hybridization and genetic exchanges in yeasts. Microbiol. Mol. Biol. Rev. 76, 721-739.

Morard, M., Macías, L. G., Adam, A. C., Lairón-Peris, M., Pérez-Torrado, R., Toft, C., Barrio, E., 2019. Aneuploidy and Ethanol Tolerance in *Saccharomyces cerevisiae*. Front. Genet. 10 (82).

Moreno-Arribas, M.V., Polo, M.C., 2005. Winemaking biochemistry and microbiology: current knowledge and future trends. Crit. Rev. Food Sci. Nutr. 45, 265-286.

Nakao, Y., Kanamori, T., Itoh, T., Kodama, Y., Rainieri, S., Nakamura, N., Shimonaga, T., Hattori, M., Ashikari, T., 2009. Genome sequence of the lager brewing yeast, an interspecies hybrid. DNA Res. 16, 115-129.

Novo, M.T., Beltran, G., Torija, M.J., Poblet, M., Rozés, N., Guillamón, J.M., Mas, A., 2003. Changes in wine yeast storage carbohydrate levels during preadaptation, rehydration and low temperature fermentations. Int. J. Food Microbiol. 86, 153-161.

Orduña, R.M., 2010. Climate change associated effects on grape and wine quality and production. Food Res. Int. 43, 1844-1855.

Origone, A.C., del Mónaco, S.M., Ávila, J.R., González Flores, M., Rodríguez, M.E., Lopes, C.A., 2017. Tolerance to winemaking stress conditions of Patagonian

strains of *Saccharomyces eubayanus* and *Saccharomyces uvarum.* J. Appl. Microbiol. 123, 450-463.

Origone, A.C., Rodríguez, M.E., Oteiza, J.M., Querol, A., Lopes, C.A., 2018. *Saccharomyces cerevisiae* x *Saccharomyces uvarum* hybrids generated under different conditions share similar winemaking factors. Yeast 35, 157-171.

Park, H., Lopez, N.I., Bakalinsky, A.T., 1999. Use of sulfite resistance in *Saccharomyces cerevisiae* as a dominant selectable marker. Curr. Genet. 36, 339-344.

Pérez-Ortín, J., Querol, A., Puig, S., Barrio, E., 2002. Molecular characterization of a chromosomal rearrangement involved in the adaptive evolution of yeast strains. Genome Res. 12, 1533-1539.

Pérez-Través, L., Lopes, C.A., Barrio, E., Querol, A., 2012. Evaluation of different genetic procedures for the generation of artificial hybrids in *Saccharomyces* genus for winemaking. Int. J. Food Microbiol. 156, 102-111.

Pérez-Través, L., Lopes, C.A., Querol, A., Barrio, E., 2014. On the complexity of the *Saccharomyces bayanus* taxon: hybridization and potential hybrid speciation. PLoS One 9 (4) e93729.

Peris, D., Sylvester, K., Libkind, D., Gonçalves, P., Sampaio, J.P., Alexander, W.G., Hittinger, C.T., 2014. Population structure and reticulate evolution of *Saccharomyces eubayanus* and its lager-brewing hybrids. Mol. Ecol. 23, 2031-2045.

Peris, D., Langdon, Q.K., Moriarty, R. V., Sylvester, K., Bontrager, M., Charron, G., Leducq, J-B, Landry, C.R., Libkind, D., Hittinger, C.T., 2016. Complex ancestries of lager-brewing hybrids were shaped by standing variation in the wild yeast *Saccharomyces eubayanus.* PLoS Genet. 12 (7): e1006155.

840 Piotrowski, J.S., Nagarajan, S., Kroll, E., Stanbery, A., Chiotti, K.E., Kruckeberg, A.L.,
841 Dunn, B., Sherlock, G., Rosenzweig, F., 2012. Different selective pressures lead
842 to different genomic outcomes as newly-formed hybrid yeasts evolve. BMC Evol.
843 Biol. 12, 46.
844 Plech, M., de Visser, J.A.G.M., Korona, R., 2014. Heterosis is prevalent among
845 domesticated but not wild strains of *Saccharomyces cerevisiae*. G3. 4, 315-323.
846 Pretorius, I.S., Høj, P.B., 2005. Grape and wine biotechnology: challenges,
847 opportunities and potential benefits. Aust. J. Grape Wine Res. 11, 83-108.
848 Querol, A., Barrio, E., Ramón, D., 1992. A comparative study of different methods of
849 yeast strain characterization. System. Appl. Microbiol. 15, 439-446.
850 Rainieri, S., Zambonelli, C., Hallsworth, J.E., Pulvirenti, A., Giudici, P., 1999.
851 *Saccharomyces uvarum*, a distinct group within *Saccharomyces* sensu stricto.
852 FEMS Microbiol. Lett. 177, 177-185.
853 Salvadó, Z., Arroyo-López, F.N., Guillamón, J.M., Salazar, G., Quero, A., Barrio, E.,
854 2011.Temperature adaptation markedly determines evolution within the genus
855 *Saccharomyces.* Appl. Environ. Microbiol. 77, 2292-2302.
856 Santos, J., Sousa, M.J., Cardoso, H., Inácio, J., Silva, S., Spencer-Martins, I., Leao, C.,
857 2008. Ethanol tolerance of sugar transport, and the rectification of stuck wine
858 fermentations. Microbiology 154, 422-430.
859 Scanes, K.T., Hohmann, S., Prior, B.A., 1998. Glycerol production by the yeast
860 *Saccharomyces cerevisiae* and its relevance to wine: a review. S. Afr. J. Enol.
861 Vitic. 19, 17-24.
862 Serra, A., Strehaiano, P., Taillandier, P., 2005. Influence of temperature and pH on
863 *Saccharomyces bayanus* var. *uvarum* growth; impact of a wine yeast interspecific
864 hybridization on these parameters. Int. J. Food Microbiol. 104, 257-265.

865 Shapira, R., Levy, T., Shaked, S., Fridman, E., David, L., 2014. Extensive heterosis in
866 growth of yeast hybrids is explained by a combination of genetic models.
867 Heredity. 113, 316-326.
868 Sipiczki, M., 2018. Interspecies hybridisation and genome chimerisation in
869 *Saccharomyces*: combining of gene pools of species and its biotechnological
870 perspectives. Front. Microbiol. 9, 3071.
871 Sipiczki, M., 2019. Yeast two- and three-species hybrids and high-sugar fermentation.
872 Microb. Biotechnol. 12, 1101-1108.
873 Sipiczki, M., Romano, P., Lipani, G., Miklos, I., Antunovics, Z., 2001. Analysis of
874 yeasts derived from natural fermentation in a Tokaj winery. Anton. Leeuw. Int. J.
875 G. 79, 97-105.
876 Slaninova, I., Sestak, S., Svoboda, A., Farkas,V., 2000. Cell Wall and cytoskeleton
877 reorganization as the response to hypermostic shock in *Saccharomyces cerevisiae.*
878 Arch. Microbiol. 173, 245-252.
879 Su, Y., Gamero, A., Rodríguez, M.E., Lopes, C.A., Querol, A., Guillamón, J.M., 2019.
880 Interspecific hybridisation among diverse Saccharomyces species: A combined
881 biotechnological solution for low-temperature and nitrogen limited wine
882 fermentations. Int J Food Microbiol 310, 108331.
883 Tate, A.B., 2001. Global Warming's Impact on Wine. J. Wine Res. 12 (2), 95-109.
884 Teixeira, M.C., Raposo, L.R., Mira, N.P., Lourenc ̧o, A.B., Sá-Correia, I., 2009.
885 Genome-wide identification of *Saccharomyces cerevisiae* genes required for
886 maximal tolerance to ethanol. Appl. Environ. Microbiol. 75 (18), 5761-5772.
887 Torija, M.J., Rozés, N., Poblet, M., Guillamónn, J.M., Mas, A., 2003. Effects of
888 fermentation temperature on the strain population of *Saccharomyces cerevisiae*.
889 Int. J. Food Microbiol. 80, 47-53.

Tronchoni, J., Gamero, A., Arroyo-López, F.N., Barrio, E., Querol, A. 2009. Differences in the glucose and fructose consumption profiles in diverse *Saccharomyces* wine species and their hybrids during grape juice fermentation. Int. J. Food Microbiol. 134, 237-243.

Van Voorst, F., Houghton-Larsen, J., JØnson, L., Kielland-Brandt, M.C., Brandt, A., 2006. Genome-wide identification of genes requiered for growth of *Saccharomyces cerevisiae* under ethanol stress. Yeast 23, 351-359.

Voordeckers, K., Kominek, J., Das, A., Espinosa-Cantú, A., De Maeyer, D., Arslan, A., Van Pee, M., van der Zande, E., Meert, W., Yang, Y., Zhu, B., Marchal, K., DeLuna, A., Van Noort,V., Jelier, R., Verstrepen1,K. , 2015. Adaptation to high ethanol reveals complex evolutionary pathways. PLOS Genet. 11 (11), 1-31.

White M.A., Diffenbaugh N.S., Jones G.V., Pal J.S., Giorgi, F., 2006. Extreme heat reduces and shifts United States premium wine production in the 21st century. PNAS 103 (30) 11217-11222.

Zaret, K.S., Sherman, F., 1985. α-Aminoadipate as a primary nitrogen source for *Saccharomyces cerevisiae* mutants. J. Bacteriol. 162, 579-583.

Zhang, H., Richards, K.D., Wilson, S., Lee, S.A., Sheehan, H., Roncoroni, M., Gardner, R.C., 2015. Genetic characterization of strains of *Saccharomyces uvarum* from New Zealand wineries. Food Microbiol. 46, 92–99.

Zörgo, E., Gjuvsland, A., Cubillos, F., Louis, E.J., Liti, G., Blomberg, A., Omholt, S., Warringer, J., 2012. Life history shapes trait heredity by accumulation of loss-of-function alleles in yeast. Mol. Biol. Evol. 29(7):1781–1789.

Zörgo, E., Chwialkowska, K., Gjuvsland, A.B., Garré, E., Sunnerhagen, P., Liti, G., Blomberg, A., Omholt, S., Warringer, J., 2012. Ancient evolutionary trade-offs between yeast ploidy states. Plos Genet. 9, e1003388.

915 Zwietering, M.H., Jongenburger, I., Rombouts, F.M., van't Riet, K., 1990. Modeling of
916 the bacterial growth curve. Appl. Environ. Microbiol. 56, 1875-81.

917 **FIGURE CAPTIONS**

918 **Figure 1:** Yeast growth evaluation by drop test at different temperatures and sulphur
919 dioxide concentration**.**

920 **Figure 2:** Heatmap representation of maximum growth rate (μ_{max}) and lag phase (λ)
921 values of artificial hybrids in comparison with the respective parent strains at increasing
922 **(A)** ethanol and **(B)** glucose concentrations. Lines correspond to each yeast strain and
923 columns to different concentrations of the compounds. The colour key bars at the top
924 indicate the growth parameter values relative to the average for each ethanol and
925 glucose concentration: values higher that the average are in red and values lower than
926 the average are in green. Hierarchical clustering is shown on the left. The statistical
927 significance is shown in Supplementary material Table 2.

Table 1

Table 1: Yeast strains used in this work

Species	Denomination	Origin	Source
S. uvarum (*Su*[a])	NPCC[b] 1290	*Araucaria araucana*	Rodríguez *et al.* (2014)
S. uvarum (*Su*[ch])	NPCC[b] 1314	Apple chicha	Rodríguez *et al.* (2017)
S. cerevisiae (*Sc*[c])	NPCC[b] 167 (K1M)	Commercial	
S. eubayanus (*Se*)	NPCC[b] 1292	*Araucaria araucana*	Rodríguez *et al.* (2014)
S. cerevisiae (*Sc*[w])	NPCC[b] 1178 (MMf9)	Wine	Lopes (2002)
Hybrid *Su*[c] x *Su*[a]	H13	Artificial hybrid (rare-mating)	Origone *et al.* (2018)
Hybrid *Su*[c] x *Su*[ch]	H17	Artificial hybrid (rare-mating)	Origone *et al.* (2018)
Hybrid *Su*[c] x *Se*	H19	Artificial hybrid (rare-mating)	Origone *et al.* (2018)
Hybrid *Su*[w] x *Su*[a]	H20	Artificial hybrid (rare-mating)	Origone *et al.* (2018)

Superscript letters "a" and "ch" indicate the isolation origin of *A. araucana* and *chicha* for *S. uvarum* strains, while "c" and "w" stand for commercial and winery isolation origins for *S. cerevisiae* strains, respectively.

[b]North Patagonian Culture Collection, Neuquén, Argentina.

Table 2

Table 2. Physicochemical characteristics and kinetic parameters of fermentation products generated under specific conditions.

Condition[a]		Species[b]	Kinetic parameters[c]		Chemic composition[d]				
			V_{max} (h^{-1})	λ (h)	Glucose (g/L)	Fructose (g/L)	Glycerol (g/L)	Ethanol (% v/v)	Acetic acid (g/L)
Glu/fru unbalance	**20°C**	***S. cerevisiae*[c] (*Sc*[c])**	0.045(0.004)^{bc}	19.69(0.50)^{d}	0(0.00)^{a}	0.28(0.00)^{a}	7.17(0.19)^{abc}	14.17(0.11)^{d}	1.62(0.06)^{d}
		***S. cerevisiae*[w] (*Sc*[w])**	0.032(0.008)^{ab}	22.82(1.32)^{e}	0(0.00)^{a}	0.49(0.28)^{a}	7.62(0.38)^{abc}	13.82(0.47)^{bcd}	1.04(0.01)^{c}
		***S. uvarum*[a] (*Su*[a])**	0.025(0.001)^{a}	14.81(1.30)^{c}	0(0.00)^{a}	4.53(2.11)^{a}	7.73(0.71)^{c}	13.24(0.30)^{c}	1.46(0.29)^{de}
		***S. uvarum*[ch] (*Su*[ch])**	0.031(0.008)^{ab}	17.13(0.79)^{cd}	0(0.00)^{a}	0.69(0.01)^{a}	8.16(0.01)^{c}	13.49(0.01)^{bc}	1.58(0.01)^{d}
		***S. eubayanus* (*Se*)**	0.034(0.003)^{ab}	10.98(1.06)^{b}	2.19(0.33)^{b}	45.16(4.54)^{b}	6.80(0.38)^{ab}	11.06(0.38)^{a}	1.25(0.10)^{c}
		***Sc*[c] x *Su*[a] (H13)**	0.038(0.004)^{ab}	8.09(0.85)^{a}	0(0.00)^{a}	0.73(0.10)^{a}	7.63(0.72)^{bc}	13.70(0.17)^{b}	0.33(0.05)^{a}
		***Sc*[c] x *Su*[ch] (H17)**	0.043(0.002)^{bc}	14.76(0.39)^{c}	0(0.00)^{a}	1.03(0.00)^{a}	7.54(0.63)^{abc}	13.82(0.37)^{cd}	0.55(0.17)^{ab}
		***Sc*[c] x *Se* (H19)**	0.032(0.006)^{ab}	28.59(1.50)^{f}	0(0.00)^{a}	0.94(0.01)^{a}	6.76(0.09)^{a}	13.68(0.16)^{bcd}	0.61(0.03)^{b}
		***Sc*[w] x *Su*[a] (H20)**	0.048(0.001)^{c}	15.78(0.51)^{c}	0(0.00)^{a}	0.62(0.29)^{a}	7.17(0.12)^{abc}	13.14(0.21)^{bc}	0.79(0.05)^{bc}
	13°C	***S. cerevisiae*[c] (*Sc*[c])**	0.013(0.002)^{abc}	37.23(6.08)^{a}	0(0.00)^{a}	5.06(2.79)^{a}	6.56(0.53)^{ab}	13.91(0.49)^{b}	1.37(0.07)^{de}
		***S. cerevisiae*[w] (*Sc*[w])**	0.009(0.001)^{ab}	130.48(11.12)^{d}	0.44(0.12)^{b}	19.72(3.54)^{c}	6.92(0.19)^{bc}	13.03(0.40)^{ab}	1.39(0.18)^{e}
		***S. uvarum*[a] (*Su*[a])**	0.008(0.0)^{a}	96.37(5.47)^{c}	2.36(0.10)^{d}	44.51(0.98)^{e}	8.25(0.31)^{ef}	11.79(0.33)^{a}	1.17(0.05)^{cd}
		***S. uvarum*[ch] (*Su*[ch])**	0.010(0.001)^{ab}	51.62(4.09)^{ab}	0.11(0.09)^{a}	12.55(6.12)^{b}	6.88(0.23)^{bc}	12.82(0.74)^{ab}	1.09(0.12)^{c}
		***S. eubayanus* (*Se*)**	0.015(0.005)^{bc}	55.24(13.43)^{ab}	0.74(0.10)^{c}	34.85(3.12)^{d}	8.65(0.20)^{f}	11.87(0.16)^{a}	1.73(0.16)^{f}
		***Sc*[c] x *Su*[a] (H13)**	0.014(0.001)^{abc}	51.89(6.56)^{ab}	0(0.00)^{a}	0.68(0.06)^{a}	8.04(0.25)^{e}	13.51(0.38)^{b}	0.39(0.01)^{a}
		***Sc*[c] x *Su*[ch] (H17)**	0.014(0.001)^{abc}	54.33(5.54)^{ab}	0(0.00)^{a}	0(0.00)^{a}	7.24(0.17)^{cd}	13.78(0.35)^{b}	0.39(0.01)^{a}
		***Sc*[c] x *Se* (H19)**	0.017(0.002)^{c}	56.61(1.58)^{ab}	0(0.00)^{a}	3.21(0.62)^{ab}	8.21(0.08)^{def}	13.08(0.26)^{ab}	0.72(0.04)^{ab}
		***Sc*[w] x *Su*[a] (H20)**	0.014(0.002)^{abc}	68.76(4.62)^{b}	0(0.00)^{a}	0.90(0.41)^{a}	6.05(0.28)^{a}	13.19(0.29)^{ab}	1.04(0.11)^{bc}
Stuck	**13°C**	***S. cerevisiae*[c] (*Sc*[c])**	0.006(0.001)^{c}	90.35(8.72)^{a}	0(0.00)^{a}	0(0.00)^{a}	2.14(0.16)^{a}	10.88(0.33)^{a}	1.4(0.15)^{d}
		***S. cerevisiae*[w] (*Sc*[w])**	0.003(0.001)^{ab}	154.76(3.59)^{cd}	0(0.00)^{a}	0(0.00)^{a}	2.46(0.28)^{ab}	11.11(0.67)^{ab}	0.82(0.09)^{bc}
		***S. uvarum*[a] (*Su*[a])**	0.003(0.0)^{ab}	138.87(16.27)^{bcd}	0(0.00)^{a}	0(0.00)^{a}	5.27(0.17)^{e}	11.38(0.08)^{ab}	1.09(0.26)^{cd}
		***S. uvarum*[ch] (*Su*[ch])**	0.004(0.0)^{abc}	103.38(18.19)^{ab}	0(0.00)^{a}	0(0.00)^{a}	4.03(0.83)^{d}	11.06(0.50)^{ab}	0.85(0.09)^{bc}
		***S. eubayanus* (*Se*)**	0.003(0.01)^{a}	114.92(13.86)^{ab}	0.22(0.06)^{b}	13.76(1.87)^{b}	3.70(0.29)^{cd}	11.78(0.02)^{bc}	0.78(0.12)^{bc}
		***Sc*[c] x *Su*[a] (H13)**	0.004(0.0)^{abc}	103.97(13.42)^{ab}	0(0.00)^{a}	0(0.00)^{a}	2.93(0.47)^{abc}	11.59(0.41)^{ab}	0.24(0.10)^{a}
		***Sc*[c] x *Su*[ch] (H17)**	0.004(0.01)^{abc}	123.09(7.21)^{abc}	0(0.00)^{a}	0(0.00)^{a}	3.06(0.41)^{bc}	11.47(0.24)^{ab}	0.27(0.12)^{a}
		***Sc*[c] x *Se* (H19)**	0.005(0.0)^{bc}	165.45(13.59)^{d}	0(0.00)^{a}	0(0.00)^{a}	3.19(0.36)^{bc}	12.57(0.21)^{c}	0.32(0.12)^{a}
		***Sc*[w] x *Su*[a] (H20)**	0.006(0.002)^{c}	112.53(5.68)^{ab}	0(0.00)^{a}	0(0.00)^{a}	2.46(0.18)^{ab}	10.78(0.36)^{a}	0.68(0.22)^{b}

a- Experimental conditions: **Glu/fru unbalance:** 80 g/L of glucose, 160 g/L of fructose at 20 °C and 13 °C; **Stuck:** 8 % (v/v) of ethanol; 20 g/L of glucose, 50 g/L of fructose at 13 °C.

b- Superscript letters "a" and "ch" indicate the isolation origin of *A. araucana* and *chicha* for *S. uvarum* strains, while "c" and "w" stand for commercial and wine origins of *S. cerevisiae* strains, respectively.

c- V_{max}: maximum fermentation rate; λ: time required to start the tumultuous fermentation.

d- Standard deviation calculated for the experiments in triplicate into parenthesis. Different superscript letters indicate significant differences between values in the same column for an experimental condition (p-value≤0.005). In all cases, R^2 values were higher than 0.98.

Supplementary Table 1: Growth profiles of hybrid and parental strains at different temperatures.

	Strains[a]	Growth temperatures (°C)[b]							Sulphur dioxide concentration (mM)								
		4	**8**	**13**	**20**	**25**	**30**	**37**	**0**	**0.5**	**1.0**	**1.5**	**2.0**	**2.5**	**3.0**	**3.5**	**4.0**
Parental	***S. cerevisiae*[c] (*Sc*[c])**	0	0	5	6	6	6	6	6	6	6	6	6	6	6	6	6
	***S. cerevisiae*[w] (*Sc*[w])**	0	0	4	6	6	6	5	6	6	6	6	6	6	6	6	6
	***S. uvarum*[a] (*Su*[a])**	0	5	6	5	6	5	0	6	6	5	0	0	0	0	0	0
	***S. uvarum*[ch] (*Su*[ch])**	0	5	6	6	6	5	0	6	6	6	0	0	0	0	0	0
	***S. eubayanus* (*Se*)**	0	5	6	6	6	5	0	6	6	5	0	0	0	0	0	0
Hybrid	***Sc*[c] x *Su*[a] (H13)**	0	2	4	6	6	6	5	6	6	6	3	0	0	0	0	0
	***Sc*[c] x *Su*[ch] (H17)**	0	3	5	6	6	6	5	6	6	6	6	6	5	6	3	1
	***Sc*[c] x *Se* (H19)**	0	2	6	6	6	5	5	6	6	6	2	0	0	0	0	0
	***Sc*[w] x *Su*[a] (H20)**	0	2	6	6	6	5	5	6	6	6	6	6	5	6	6	5

a-Superscript letters “a” and “ch” indicate the isolation origin of *A. araucana* and *chicha* for *S. uvarum* strains, while “c” and “w” are commercial and winery origins for *S. cerevisiae* strains, respectively.

b-Numbers 1 to 6 indicate the dilutions where colony development was observed (0 indicates no growth and 6 indicates growth until the last dilution)

Supplementary Table 2A: Maximum growth rate ($\mu_{máx}$) and lag phase (λ) of parental and hybrid strains under increasing ethanol concentrations.

Species[a]	Ethanol concentration (% v/v)[b]											
	0		2		3		5		7		8	
	μ (h^{-1})	λ (h)	μ (h^{-1})	λ (h)	μ (h^{-1})	λ (h)	μ (h^{-1})	λ (h)	μ (h^{-1})	λ (h)	μ (h^{-1})	λ (h)
***S. cerevisiae* (*Sc*^{c})**	0.22 (0.03)^{b}	1.15 (0.22)^{a}	0.20 (0.03)^{b}	1.03 (0.10)^{a}	0.19 (0.01)^{b}	1.45 (0.1)^{a}	0.18 (0.00)^{c}	1.75 (0.04)^{a}	0.15 (0.01)^{c}	1.98 (0.67)^{a}	0.16 (0.01)^{c}	2.85 (0.67)^{a}
***S. uvarum*^{a} (*Su*^{a})**	0.21 (0.03)^{ab}	6.53 (0.11)^{b}	0.17 (0.01)^{b}	6.44 (0.97)^{c}	0.20 (0.05)^{b}	6.72 (0.17)^{b}	0.12 (0.00)^{b}	6.27(1.02)^{b}	0.10(0.01)^{b}	11.3 (0.94)^{c}	0.10(0.00)^{b}	13.15 (0.66)^{c}
***Sc*^{c} x *Su*^{a} (H13)**	0.15 (0.01)^{a}	4.05 (1.07)^{c}	0.10 (0.01)^{a}	3.77 (0.75)^{b}	0.10 (0.00)^{a}	2.74 (0.81)^{a}	0.09 (0.00)^{a}	2.23 (0.31)^{a}	0.07 (0.0)^{a}	3.84(0.27)^{b}	0.07 (0.0)^{a}	8.00 (1.22)^{b}
***S. cerevisiae* (*Sc*^{c})**	0.22 (0.03)^{b}	1.15 (0.22)^{a}	0.20 (0.03)^{b}	1.03 (0.10)^{a}	0.19 (0.01)^{b}	1.45 (0.1)^{a}	0.18 (0.00)^{b}	1.75 (0.04)^{a}	0.15 (0.01)^{c}	1.98 (0.67)^{a}	0.16(0.01)^{b}	2.85 (0.67)^{a}
***S. uvarum*^{ch} (*Su*^{ch})**	0.13 (0.01)^{a}	5.25 (0.66)^{c}	0.11 (0.0)^{a}	6.44 (0.30)^{b}	0.09 (0.0)^{a}	7.87 (0.17)^{b}	0.06 (0.04)^{a}	8.39(0.95)^{b}	0.04 (0.00)^{a}	8.96(0.98)^{b}	0.08 (0.01)^{a}	22.12 (3.83)^{c}
***Sc*^{c} x *Su*^{ch} (H17)**	0.13 (0.00)^{a}	3.07 (0.59)^{b}	0.10 (0.01)^{a}	2.23 (0.85)^{a}	0.09 (0.0)^{a}	3.73 (0.01)^{a}	0.08 (0.0)^{a}	7.2 (1.59)^{b}	0.07 (0.0)^{b}	7.41(1.44)^{b}	0.09 (0.0)^{a}	12.86 (2.84)^{b}
***S. cerevisiae* (*Sc*^{c})**	0.22 (0.03)^{a}	1.15 (0.22)^{a}	0.20 (0.03)^{b}	1.03 (0.1)^{a}	0.19 (0.01)^{a}	1.45 (0.1)^{a}	0.18 (0.00)^{b}	1.75 (0.04)^{a}	0.15(0.01)^{b}	1.98 (0.67)^{a}	0.16(0.01)^{ab}	2.85 (0.67)^{a}
***S. eubayanus* (*Se*)**	0.19 (0.08)^{a}	4.75 (0.71)^{b}	0.16 (0.02)^{a}	4.37 (1.21)^{b}	0.17 (0.05)^{a}	6.54 (1.55)^{b}	0.17(0.05)^{ab}	6.46 (0.64)^{a}	0.2 (0.00)^{c}	9.12 (0.03)^{c}	0.21(0.05)^{b}	8.7 (0.27)^{b}
***Sc*^{c} x *Se* (H19)**	0.16 (0.00)^{a}	4.25 (0.71)^{b}	0.12 (0.02)^{ab}	3.37 (0.91)^{b}	0.12 (0.01)^{a}	3.19 (1.10)^{ab}	0.09 (0.01)^{a}	5.08 (1.99)^{a}	0.08 (0.0)^{a}	5.28(1.73)^{b}	0.07 (0.01)^{a}	5.3 (2.14)^{ab}
***S. cerevisiae* (*Sc*^{w})**	0.19 (0.00)^{a}	1.65 (0.28)^{a}	0.18(0.02)^{a}	1.41 (0.50)^{a}	0.16 (0.00)^{a}	1.81 (0.19)^{a}	0.15 (0.01)^{b}	2.14 (0.08)^{a}	0.13(0.00)^{b}	5.03 (0.28)^{a}	0.11 (0.00)^{b}	4.98 (0.3)^{a}
***S. uvarum*^{a} (*Su*^{a})**	0.21 (0.03)^{a}	6.53 (0.11)^{c}	0.17 (0.01)^{a}	6.44 (0.97)^{b}	0.20 (0.05)^{a}	6.72 (0.17)^{c}	0.12 (0.00)^{a}	6.27(1.02)^{b}	0.10(0.01)^{ab}	11.3(0.94)^{b}	0.09(0.00)^{a}	13.15 (0.66)^{c}
***Sc*^{w} x *Su*^{a} (H20)**	0.18 (0.01)^{a}	3.45 (0.11)^{b}	0.15 (0.01)^{a}	2.01 (0.01)^{a}	0.14 (0.0)^{a}	2.82 (0.48)^{b}	0.15 (0.00)^{b}	4.46(0.57)^{b}	0.09 (0.02)^{a}	8.08(2.06)^{ab}	0.10(0.00)^{ab}	7.18 0.04)^{b}

a- Superscipt letters "a", "ch", "c" and "w" indicate the origin of the *S.uvarum* and *S. cerevisiae* strains, being *A. araucana*, chicha, commercial and wine, respectively.

b- Standard deviations, in parenthesis, were calculated from the experiments by triplicate. Different superscript letters indicate significant differences between values in the same column and between the hybrid and the corresponding parental strains (ANOVA and Tukey Test; p-value ≤ 0.005). In all cases, R^2 values were higher than 0.98.

Supplementary Table 2B: Maximum growth rate ($\mu_{máx}$) and lag phase (λ) of parental and hybrid strains under increasing glucose concentrations.

Species[a]	Glucose concentration (g/L)[b]									
	2.5		5		20		60		100	
	μ (h^{-1})	λ (h)	μ (h^{-1})	λ (h)	μ (h^{-1})	λ (h)	μ (h^{-1})	λ (h)	μ (h^{-1})	λ (h)
***S. cerevisiae* (*Sc*^{c})**	0.21(0.03)^{b}	3.03(0.4)^{a}	0.19(0.02)^{b}	3.11(0.59)^{a}	0.19(0.0)^{b}	4.42(2.06)^{a}	0.18(0.03)^{b}	4.22(2.29)^{a}	0.21(0.01)^{b}	3.21(0.41)^{a}
***S. uvarum*^{a} (*Su*^{a})**	0.14(0.02)^{ab}	3.9(1.39)^{a}	0.14(0.01)^{a}	3.01(0.57)^{a}	0.12(0.0)^{a}	2.26(0.0)^{a}	0.13(0.02)^{ab}	2.47(1.14)^{a}	0.11(0.01)^{a}	2.10(0.96)^{a}
***Sc*^{c} x *Su*^{a} (H13)**	0.11(0.06)^{a}	7.13(0.11)^{b}	0.11(0.01)^{a}	10.01(0.41)^{b}	0.1(0.03)^{a}	7.06(0.3)^{a}	0.12(0.02)^{a}	7.49(1.92)^{a}	0.09(0.03)^{a}	8.58(1.05)^{b}
***S. cerevisiae* (*Sc*^{c})**	0.21(0.03)^{b}	3.03(0.4)^{a}	0.19(0.01)^{b}	3.11(0.59)^{a}	0.19(0.0)^{b}	4.42(2.06)^{a}	0.18(0.03)^{b}	4.22(2.29)^{a}	0.21(0.01)^{c}	3.21(0.41)^{a}
***S. uvarum*^{ch} (*Su*^{ch})**	0.15(0.02)^{ab}	5.58(1.0)^{b}	0.11(0.01)^{a}	3.32(0.49)^{a}	0.14(0.03)^{a}	4.06(1.69)^{a}	0.12(0.0)^{ab}	3.76(1.02)^{a}	0.12(0.01)^{b}	3.58(0.53)^{a}
***Sc*^{c} x *Su*^{ch} (H17)**	0.11(0.04)^{a}	2.1(0.13)^{a}	0.08(0.01)^{a}	2.09(0.94)^{a}	0.11(0.01)^{a}	4.31(1.4)^{a}	0.08(0.03)^{a}	5.4(1.27)^{a}	0.09(0.01)^{a}	3.68(1.94)^{a}
***S. cerevisiae* (*Sc*^{c})**	0.21(0.03)^{b}	3.03(0.4)^{a}	0.19(0.01)^{b}	3.11(0.59)^{a}	0.19(0.0)^{ab}	4.42(2.06)^{a}	0.18(0.03)^{b}	4.22(2.29)^{a}	0.21(0.01)^{b}	3.21(0.41)^{a}
***S. eubayanus* (*Se*)**	0.16(0.01)^{ab}	4.96(0.13)^{ab}	0.19(0.0)^{b}	6.61(0.0)^{ab}	0.20(0.0)^{b}	6.31(0.97)^{a}	0.20(0.01)^{b}	4.61(0.38)^{a}	0.20(0.01)^{b}	5.18(0.23)^{a}
***Sc*^{c} x *Se* (H19)**	0.12(0.03)^{a}	6.81(2.02)^{b}	0.14(0.01)^{a}	10.37(2.10)^{b}	0.13(0.01)^{a}	11.2(0.68)^{b}	0.13(0.01)^{a}	7.88(2.42)^{a}	0.12(0.0)^{a}	8.69(1.25)^{b}
***S. cerevisiae* (*Sc*^{w})**	0.13(0.03)^{a}	1.81(0.5)^{a}	0.14(0.01)^{a}	4.31(0.52)^{b}	0.13(0.0)^{a}	1.72(0.5)^{a}	0.12(0.03)^{a}	2.84(0.4)^{a}	0.11(0.03)^{a}	3.9(1.39)^{ab}
***S. uvarum*^{a} (*Su*^{a})**	0.14(0.02)^{a}	3.9(1.39)^{b}	0.14(0.02)^{a}	3.9(1.39)^{ab}	0.12(0.0)^{a}	2.26(0.0)^{a}	0.13(0.02)^{a}	2.47(1.14)^{a}	0.11(0.01)^{a}	2.10(0.96)^{a}
***Sc*^{w} x *Su*^{a} (H20)**	0.15(0.02)^{a}	2.19(0.0)^{a}	0.14(0.07)^{a}	2.18(0.23)^{a}	0.15(0.01)^{b}	3.28(1.2)^{a}	0.13(0.0)^{a}	12.72(1.6)^{b}	0.10(0.06)^{a}	6.73(1.94)^{b}

a - Superscript letters "a", "ch", "c" and "w" indicate the origin of the *S. uvarum* and *S. cerevisiae* strains, being *A. araucana*, chicha, commercial and wine, respectively.

b - Standard deviations, in parenthesis, were calculated from the experiments by triplicate. Different superscript letters indicate significant differences between values in the same column and between the hybrid and the corresponding parental strains (ANOVA and Tukey Test; p-value ≤ 0.005). In all cases, R^2 values were higher than 0.98.

Supplementary Table 2B (cont.): Maximum growth rate ($\mu_{máx}$) and lag phase (λ) of parental and hybrid strains under increasing glucose concentrations.

Species[a]	Glucose concentration (g/L)[b]							
	120		180		240		300	
	μ (h^{-1})	λ (h)	μ (h^{-1})	λ (h)	μ (h^{-1})	λ (h)	μ (h^{-1})	λ (h)
***S. cerevisiae* (*Sc*^{c})**	0.16(0.03)^{b}	2.34(0.58)^{a}	0.17(0.01)^{b}	3.37(0.32)^{a}	0.13(0.01)^{b}	7.42(0.55)^{a}	0.13(0.01)^{b}	7.42(0.55)^{a}
***S. uvarum*^{a} (*Su*^{a})**	0.12(0.02)^{ab}	1.53(0.09)^{a}	0.1(0.02)^{a}	3.51(0.55)^{a}	0.09(0.02)^{a}	6.03(1.07)^{a}	0.06(0.01)^{a}	5.09(2.39)^{a}
***Sc*^{c} x *Su*^{a} (H13)**	0.08(0.0)^{a}	5.13(0.03)^{b}	0.08(0.02)^{a}	11.46(2.75)^{b}	0.07(0.01)^{a}	14.63(0.29)^{b}	0	0
***S. cerevisiae* (*Sc*^{c})**	0.16(0.03)^{b}	2.34(0.58)^{a}	0.17(0.01)^{b}	3.37(0.32)^{a}	0.13(0.01)^{b}	7.42(0.55)^{a}	0.13(0.01)^{b}	7.42(0.55)^{b}
***S. uvarum*^{ch} (*Su*^{ch})**	0.10(0.01)^{a}	2.93(1.17)^{a}	0.10(0.0)^{a}	5.05(0.53)^{a}	0.08(0.01)^{a}	5.24(1.63)^{a}	0.04(0.0)^{a}	5.43(0.0)^{a}
***Sc*^{c} x *Su*^{ch} (H17)**	0.10(0.01)^{a}	2.81(0.65)^{a}	0.07(0.03)^{a}	6.68(0.65)^{b}	0.06(0.01)^{a}	6.27(0.86)^{a}	0.04(0.0)^{a}	4.79(0.21)^{a}
***S. cerevisiae* (*Sc*^{c})**	0.16(0.03)^{c}	2.34(0.58)^{a}	0.17(0.01)^{b}	3.37(0.32)^{a}	0.13(0.01)^{b}	7.42(0.55)^{a}	0.13(0.01)^{b}	7.42(0.55)^{a}
***S. eubayanus* (*Se*)**	0.19(0.01)^{b}	4.95(0.28)^{b}	0.17(0.01)^{b}	6.56(0.5)^{b}	0.15(0.01)^{b}	9.26(0.67)^{b}	0.11(0.01)^{b}	10.53(0.4)^{a}
***Sc*^{c} x *Se* (H19)**	0.11(0.0)^{a}	8.86(0.98)^{c}	0.09(0.02)^{a}	14.04(3.04)^{c}	0.09(0.02)^{a}	6.32(0.63)^{a}	0.07(0.01)^{a}	16.33(1.98)^{b}
***S. cerevisiae* (*Sc*^{w})**	0.12(0.0)^{a}	4.42(0.5)^{b}	0.12(0.0)^{a}	4.97(0.06)^{b}	0.10(0.01)^{a}	6.26(0.88)^{b}	0.07(0.01)^{a}	4.69(0.93)^{a}
***S. uvarum*^{a} (*Su*^{a})**	0.12(0.02)^{a}	1.53(0.09)^{a}	0.10(0.02)^{a}	3.51(0.55)^{a}	0.09(0.02)^{a}	6.03(1.07)^{b}	0.06(0.01)^{a}	5.09(2.39)^{a}
***Sc*^{w} x *Su*^{a} (H20)**	0.13(0.01)^{a}	5.13(0.03)^{b}	0.12(0.02)^{a}	4.53(0.20)^{b}	0.09(0.03)^{a}	1.74(0.11)^{a}	0.11(0.0)^{b}	2.29(1.66)^{a}

a- Superscript letters "a", "ch", "c" and "w" indicate the origin of the *S. uvarum* and *S. cerevisiae* strains, being *A. araucana*, chicha, commercial and wine, respectively.

b- Standard deviations, in parenthesis, were calculated from the experiments by triplicate. Different superscript letters indicate significant differences between values in the same column and between the hybrid and the corresponding parental strains (ANOVA and Tukey Test; p-value ≤ 0.005). In all cases, R^2 values were higher than 0.98.

Supplementary Table 3: Heterosis percentages calculated for kinetic and physicochemical parameters obtained for hybrid strains in different conditions.

Condition[a]		Parameter[b]	*Sc*[c] x *Su*[a] (H13)			*Sc*[c] x *Su*[ch] (H17)			*Sc*[c] x *Se* (H19)		
			BPH (%)	MPH (%)	WPH (%)	BPH (%)	MPH (%)	WPH (%)	BPH (%)	MPH (%)	WPH (%)
Ethanol	**8 % v/v**	**μ_{max} (h^{-1})**	-55.7 S.c.	-44.0	-23.7 S.u.	-45.0 S.c.	-27.2	7.7 S.u.	-66.0 Se..	-61.1	-54.4 S.c.
		λ (h)	180.0 S.c.	0.03	-39.1 S.u.	351.0 S.c.	3.0	-41.9 S.u.	85.2 S.c.	-8.6	-39.3 S.e.
Glucose	**240 g/L**	**μ_{max} (h^{-1})**	-46.2 S.c.	-35.4	-19.2 S.u.	-53.8 S.c.	-41.5	-20.0 S.u.	-35.6 S.e.	-32.1	-28.2 S.c.
		λ (h)	142.7 S.u.	117.6	97.2 S.c.	19.8 S.u.	-0.9	-15.5 S.c.	-14.8 S.c.	-24.2	-31.8 S.e.
	300 g/L	**μ_{max} (h^{-1})**	---	---	---	-69.2 S.c.	-52.9	0.02 S.u	-48.7 S.c.	-44.4	-39.4 S.e.
		λ (h)	---	---	---	-11.8 S.c.	-25.4	-35.4 Su..	120.2 S.c.	82.0	55.1 S.e.
Glu/fru unbalance	**20°C**	**V_{max} (h^{-1})**	-15.6 S.c.	8.6	52.0 S.u	-4.4 S.c.	13.2	38.7 S.u	-28.9 S.c.	-19.0	-5.9 Se.
		λ (h)	**-45.4** S.u	-53.1	-58.9 S.c.	-13.8 S.u	-19.8	-25.0 S.c.	160.4 S.e.	86.4	45.2 Sc.
		Fructose (g/L)	160.7 S.c.	-69.7	-83.9 S.u	267.9 S.c.	112.4	49.3 S.u	235.7 S.c.	-95.9	97.9 Se.
		Ethanol (% v/v)	3.47 S.u	-0.04	-3.3 S.c.	2.45 S.u	-0.07	-2.5 S.c.	23.69 S.e.	8.44	-3.5 S.c.
		Glycerol (g/L)	6.4 S.c.	2.4	-1.3 S.u	-7.6 S.u	-1.6	5.2 S.c.	-5.7 S.c.	-3.2	-0.6 S.e.
		Acetic acid (g/L)	**-77.4 S.u**	-78.6	-79.6 S.c.	**-65.2 S.u**	-65.6	-66.1 S.c.	**-51.2 S.e.**	-57.5	-62.3 S.c.
	13°C	**V_{max} (h^{-1})**	7.7 S.c.	33.3	75.0 S.u	7.7 S.c.	21.7	40.0 S.u	13.3 S.e.	21.4	30.8 S.c.
		λ (h)	39.4 S.c.	-22.3	-46.2 S.u	45.9 S.c.	22.3	5.3 S.u	52.1 S.c.	22.4	2.5 S.e.
		Fructose (g/L)	**-86.6 S.c.**	-97.3	-98.5 S.u	**-100 S.c.**	-100	-100 S.u	**-36.6 S.c.**	-83.9	-90.8 S.e.
		Ethanol (% v/v)	14.59 S.u	5.14	-2.9 S.c.	7.49 S.u	3.11	-0.9 S.c.	10.19 S.e.	1.47	-6.0 S.c.
		Glycerol (g/L)	-2.6 S.u	8.	22.6 S.c.	5.2 S.u	7.7	10.4 S.c.	-5.1 S.e.	8.0	25.2 S.c.
		Acetic acid (g/L)	**-66.7 S.u**	-69.3	-71.5 S.c.	**-64.2 S.u**	-68.3	-71.4 S.c.	**-58.38 S.e.**	-53.6	-47.45 S.c.
Stuck	**13°C**	**V_{max} (h^{-1})**	-33.3 S.c.	-11.1	33.0 S.u	-33.3 S.c.	-20.0	0 S.u	-16.7 S.c.	11.1	66.7 S.e.
		λ (h)	15.1 S.c.	-9.3	-25.1 S.u	36.2 S.c.	27.1	19.1 S.u	83.1 S.c.	61.2	44.0 S.e.
		Fructose (g/L)	---	---	---	---	---	---	---	---	---
		Ethanol (% v/v)	6.53 S.c.	4.13	1.9 S.u	5.42 S.c.	4.56	3.7 S.u	15.53 S.c.	10.94	6.7 S.e.
		Glycerol (g/L)	-44.4 S.u	-20.1	36.9 S.c.	-24.1 S.u	-0.8	43.0 S.c.	-13.8 S.e.	9.3	49.1 S.c.
		Acetic acid (g/L)	**-78.0 S.u**	-80.7	-82.9 S.c.	**-68.2 S.u**	-76.0	-80.7 S.c.	**-59.0 S.e.**	-70.6	-77.1 S.c.

a-Experimental extreme conditions of hybrid strains growth (8 % v/v of ethanol, 240 and 300 g/L of glucose) and fermentation traits obtained under glu/fru unbalance and stuck fermentations at 13 and 20°C.

b-Growth (μ_{max}, λ), kinetic (V_{max}, λ) and physicochemical parameters evaluated for heterosis indexes. BPH: best parent heterosis, MPH: mid-parent heterosis, WPH: worst parent heterosis, expressed in percentages.

Values indicating hybrid vigour are showed in bold letters. Superscript letters in heterosis percentages, indicate the parental strain used for comparison in each specific case.

Figure 1

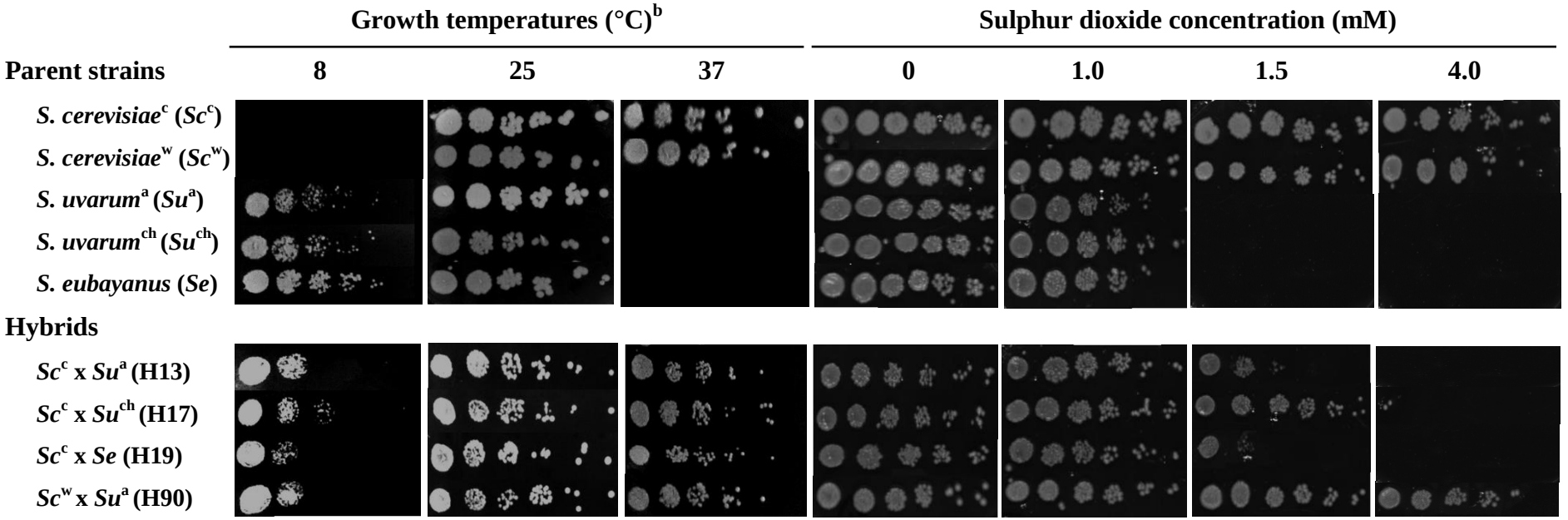

Growth temperatures (°C)[b]
Sulphur dioxide concentration (mM)
Parent strains
8
25
37
0
1.0
1.5
4.0
S. cerevisiae[c] (Sc[c])
S. cerevisiae[w] (Sc[w])
S. uvarum[a] (Su[a])
S. uvarum[ch] (Su[ch])
S. eubayanus (Se)
Hybrids
Sc[c] x Su[a] (H13)
Sc[c] x Su[ch] (H17)
Sc[c] x Se (H19)
Sc[w] x Su[a] (H90)

Figure 2

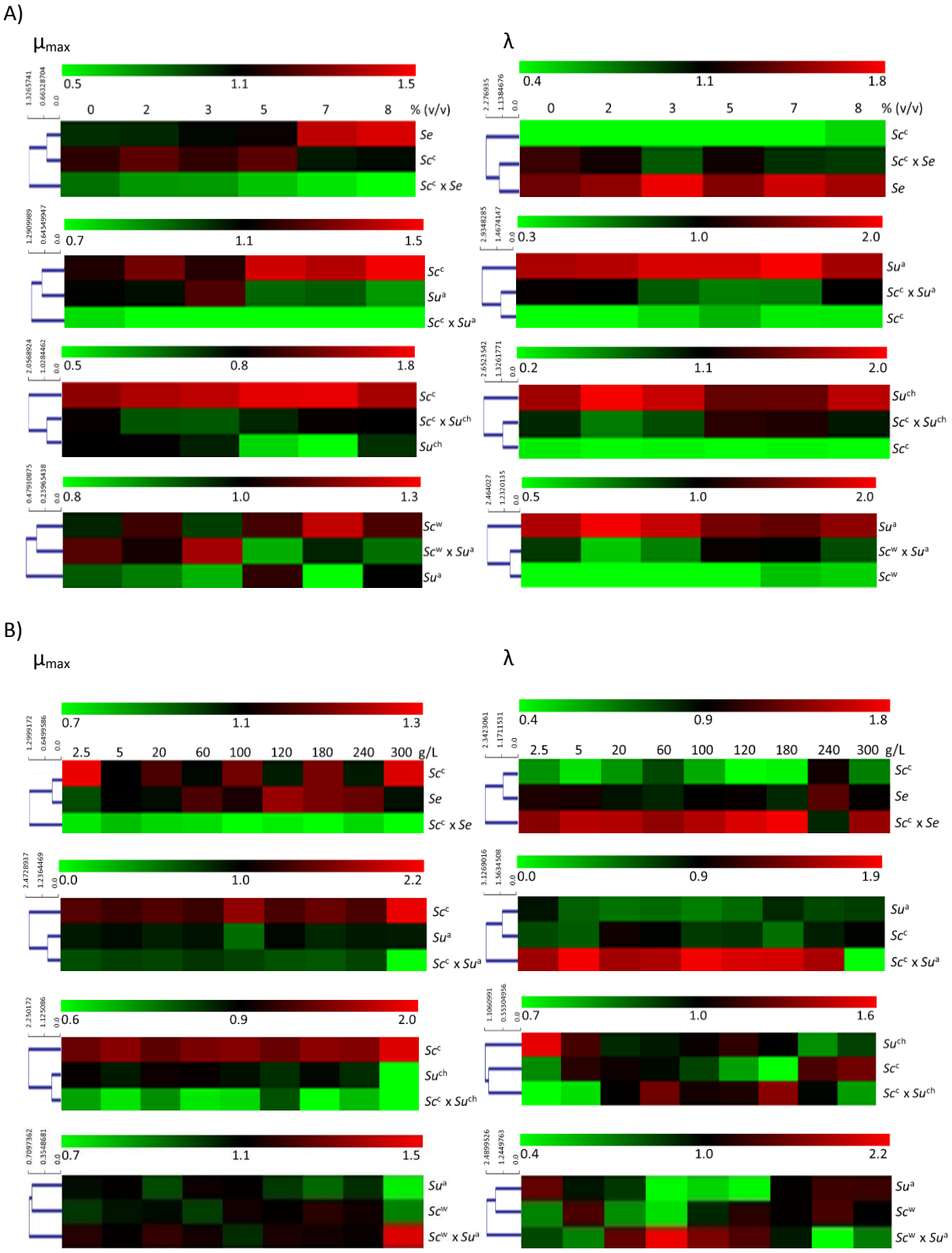
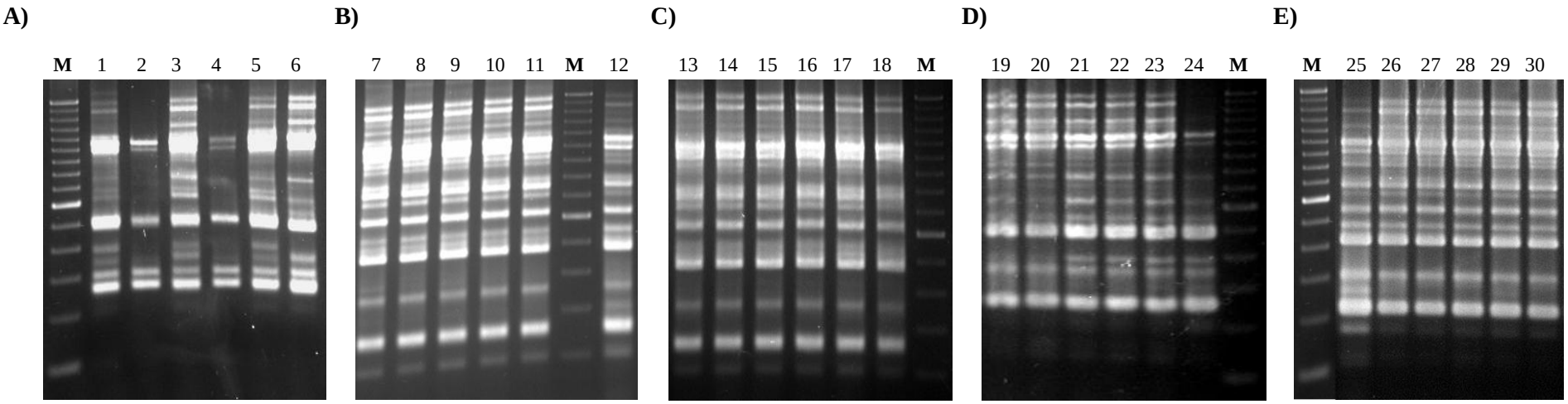


Figure 2.

Suppl Figure 1



Supplementary Figure 1: Evolution of the hybrid *Sc* x *Se* by means of RAPD-PCR analysis. Letters A to E indicate each particular evolution step during hybrid evolution. Numbers 1-30 in the top of the images indicate different yeast colonies, randomly selected from each particular evolution step. **M:** 100 bp DNA ladder marker.