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Reduced gamete viability associated to somatic genome rearrangements increases fruit set sensitivity to the environment in Tempranillo Blanco grapevine cultivar

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ABSTRACT

Sensitivity of fruit set to the environment is a genetic feature of uncertain origin that limits production in some grapevine clones and cultivars. Here we studied the developmental causes and environmental conditions leading to decreased fruit yield associated to somatic loss of fruit colour in Tempranillo Blanco (TB) white-berried derivative cultivar. We first compared fruit set-related production traits between TB and its black-berried clonal ancestor, Tempranillo Tinto (TT) cultivar, both grown under the same field conditions. We identified that lower cluster weight in TB correlated with a genetic reduction in pollen viability and lower fruit and seed setting. Then, a combination of correlation and two-way-ANOVA (Analysis of Variance) procedures, along with a series of multivariate linear regression models were developed to examine the effect of genotype and pre-flowering environmental factors on the reproductive performance of TB, using data recorded in nine field plots. These analyses identified prolonged pre-flowering cold periods and abundant rainfalls at flowering time as the main conditions increasing the ratio of seedless to seeded berries in the cluster, whereas the low pollen viability and low number of seeds characteristic of TB barely varied across plots. Collectively, these findings indicate that decreased gamete viability caused by complex genome rearrangements is in the origin of increased susceptibility to fruit set disorders in grapevine. The case of TB shows that such dysfunctions can be selected in clonally propagated crops as a trade-off of novel interesting traits emerged after genome reshuffling.

Abbreviations: *Eto_WeekF*, mean daily evapotranspiration of the week of flowering (mm/day); *Eto_Week1PreF*, mean daily evapotranspiration of the first week before flowering (mm/day); *Eto_Week2PreF*, mean daily evapotranspiration of the second week before flowering (mm/day); *Eto_Week3PreF*, mean daily evapotranspiration of the third week before flowering (mm/day); *Eto_Week4PreF*, mean daily evapotranspiration of the fourth week before flowering (mm/day); *Ndays10_WeekF*, number of days of the week of flowering with a minimum temperature below 10°C; *Ndays10_Week1PreF*, number of days of the first week before flowering with a minimum temperature below 10°C; *Ndays10_Week2PreF*, number of days of the second week before flowering with a minimum temperature below 10°C; *Ndays10_Week3PreF*, number of days of the third week before flowering with a minimum temperature below 10°C; *Ndays10_Week4PreF*, number of days of the fourth week before flowering with a minimum temperature below 10°C; *Ndays30_WeekF*, number of days of the week of flowering with a maximum temperature over 30°C; *Ndays30_Week1PreF*, number of days of the first week before flowering with a maximum temperature over 30°C; *Ndays30_Week2PreF*, number of days of the second week before flowering with a maximum temperature over 30°C; *Ndays30_Week3PreF*, number of days of the third week before flowering with a maximum temperature over 30°C; *Ndays30_Week4PreF*, number of days of the fourth week before flowering with a maximum temperature over 30°C; *P_WeekF*, mean daily precipitation of the week of flowering (l/m^2); *P_Week1PreF*, mean daily precipitation of the first week before flowering (l/m^2); *P_Week2PreF*, mean daily precipitation of the second week before flowering (l/m^2); *P_Week3PreF*, mean daily precipitation of the third week before flowering (l/m^2); *P_Week4PreF*, mean daily precipitation of the fourth week before flowering (l/m^2); *R_WeekF*, mean global radiation of the week of flowering (w/m^2); *R_Week1PreF*, mean global radiation of the first week before flowering (w/m^2); *R_Week2PreF*, MEAN global radiation of the second week before flowering (w/m^2); *R_Week3PreF*, mean global radiation of the third week before flowering (w/m^2); *R_Week4PreF*, mean global radiation of the fourth week before flowering (w/m^2); *SV*, Genome structural variation; *T_WeekF*, mean temperature registered during the week of flowering (°C); *T_Week1PreF*, mean temperature registered during the first week before flowering (°C); *T_Week2PreF*, mean temperature registered during the second week before flowering (°C); *T_Week3PreF*, mean temperature registered during the third week before flowering (°C); *T_Week4PreF*, mean temperature registered during the fourth week before flowering (°C); *TB*, Tempranillo Blanco; *TT*, Tempranillo Tinto.

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1. Introduction

Fruit set defines the transition from an ovary to a growing young fruit. This process is critical to ensure crop production in cultivated plant species where seeds and fruits are key components of yield (de Jong et al., 2009). Fruit set and also seed set are established during fertilisation, or early after it, committing the ovary and the ovule to become a fruit and a seed, respectively. This commitment determines the number of fruits and the number of seeds per fruit that the plant will develop, and to some extent their size and weight (Yong-Ling et al., 2012). Nevertheless, fruit set success is not only genetically determined, since the whole process of plant sexual reproduction behaves itself as highly susceptible to diverse environmental cues (Barnabás et al., 2008; De Storme and Geelen, 2014; Hedhly et al., 2008; Thakur et al., 2010; Zinn et al., 2010).

Balanced yield and high-quality fruit production is essential for viticulturists, winemakers and the grape processing industry, although wine and table grape production pursue different fruit quality aims (Ibáñez et al., 2020). Bud fertility (number of inflorescences that will develop per winter bud) is the main component of grape yield (up to 60%), whereas flower number and fruit set, along with fruit size and number of seeded fruits contribute to the remaining 40% (Carmona et al., 2008, Dry et al., 2010, Li-Mallet et al., 2016). In grapevine, fruit set is determined by the successful completion of pollination, whereas subsequent fruit growth is affected by seed development, which is in turn triggered by fertilisation (Dauelsberg et al., 2011). Unfertilised ovaries are dropped in a complex energy and hormone-driven process that finishes one or two weeks after anthesis, when the final fruit set rate can be definitely determined from the organs remaining in the cluster compared to the initial number of flowers (Bessis and Fournioux, 1992; Kühn et al., 2014). Fruit set rates vary among grapevine cultivars (Dry et al., 2010). While low fruit set rate is a trait that apparently was selected in table grape cultivars (Ibáñez et al., 2020), there is also variation among genotypes intended for wine production and even among clones of the same cultivar (Bowed and Kliewer, 1990; Grimplet et al., 2019). Poor fruit set in grapevine often relates with increased number of seedless berries (or berries with seed traces) and live green ovaries (LGOs) in the clusters of seeded cultivars (Dry et al., 2010). The phenomenon of a high incidence of seedless berries and LGOs in seeded cultivars is known as *millerandage*, a reproductive disorder that limits vine yield in highly susceptible cultivars (Friend and Trought, 2007). *Coulure* (also termed shatter) is another grapevine reproductive disorder contributing to poor fruit set. It refers to an excessive drop of pre-fruit set ovaries, yielding ragged clusters with few regular berries (Collins and Dry, 2009). Few studies in different varieties have related fruit set rate variation or development of parthenocarpic fruits with defective pollen viability and germination (Baby et al., 2016b; Costantini et al., 2021; Royo et al., 2016) or abnormal flower development (Costantini et al., 2021; Longbottom et al., 2004).

Fruit set and production success in grapevine rely on favourable environmental conditions around flowering time (Dry et al., 2010; Keller, 2010a; Li-Mallet et al., 2016; May, 2004; Vasconcelos et al., 2009). Fruit set is highly influenced by the weather during pollination, with solar radiation, temperature and rainfall usually being the most critical factors (see May (2004) and Keller (2010a) for comprehensive reviews in this topic). Particularly in grapevine, extreme temperatures (heat and cold) and rainy conditions are suggested to affect gamete development and viability (Ebadi et al., 1995a, 1995b; Ewart and Kliewer, 1977; Pereira et al., 2014). Likewise, low pre-flowering temperatures delay pollen grain germination and limit pollen tube growth (Staudt, 1982). Fruit set is also affected by vineyard soil and nutritional status, and nutrients like boron and zinc have been suggested to be essential for proper fertilisation processes (Alva et al., 2015; Baby et al., 2016a; Duchene et al., 2001). Other factors such as vineyard management practices and rootstock selection also affect fruit set success (Carrillo et al., 2020; Diago et al., 2010; Esteban et al., 2001; Marín et al., 2019). Remarkably, the effects of environmental factors on fruit set success are not even across grapevine genotypes. Wine cultivars like Merlot, Gewürztraminer, Riesling, Cabernet Franc, Semillon and Garnacha have been indicated as especially sensitive to unfavourable environmental conditions, whereas others like Pinot, Chardonnay and Sylvaner are much less susceptible to fruit set disorders (Keller, 2010a; May, 2004). *Coulure* occurs naturally in susceptible grapevine cultivars, when affected by environmental factors such as low temperature and limited sun radiation, and it is intensified under water and nutritional deficits (Lebon et al., 2008). Under unfavourable circumstances, susceptible cultivars can end up with clusters of limited commercial value due to their relatively low number of well-developed berries (Carrillo et al., 2020; Dry et al., 2010). Regardless, the genetic origin of susceptibility to environment-dependent fruit set disorders in grapevine remains largely unknown.

Given the highly heterozygosity of grapevine cultivars, they are vegetatively propagated to keep the varietal attributes and shorten production lapses (This et al., 2006). Considering that functional sexual reproduction is not used for cultivar multiplication, purifying selection limiting the transmission of mutations affecting gametogenesis, fertilisation or embryo development does not take place and those mutations can accumulate in cultivars as far as they do not compromise berry production (Carbonell-Bejerano et al., 2019). In some cases, these deleterious mutations haven been even artificially selected because of the singular phenotype they produce (Costantini et al., 2021; Royo et al., 2016, 2018). Recent genome sequencing studies are unveiling that genome structural variation (SV) events including deletions, inversions and translocations are frequent among and within grapevine cultivars (Carbonell-Bejerano et al., 2017; Vondras et al., 2019; Zhou et al., 2019). While such chromosomal rearrangements can impair gamete viability (Tan et al., 2015), their effects on fruit set have not been extensively analysed in grapevine.

Complex chromosomal rearrangements are in the origin of Tempranillo Blanco (TB) (Carbonell-Bejerano et al., 2017), a somatic variant of Tempranillo Tinto (TT) cultivar that appeared in 1988 in an old vineyard of Murillo de Rio Leza (La Rioja, Spain). From that bud sport, TB cultivar was developed and it became in a short time one of the most relevant white grape varieties of the D.O.Ca. Rioja, grown in more than 750 ha in 2019 and with an average production of 8,500-9,000 kg of grapes per ha (https://www.riojawine.com/). TB cultivar is highly valued to elaborate fresh and fruity wines, with citrus and tropical fruits characteristics, whereas its ampelographic features are similar to those of its somatic ancestor TT (Balda and Martínez de Toda, 2017; Martinez and García-Escudero, 2017). A genetic analysis showed reduced sexual transmission of the rearranged chromosome segments in TB, which was associated to reduced pollen viability with respect to TT (Carbonell-Bejerano et al., 2017). TB also displays reduced fruit set rate and fruit yield (Carbonell-Bejerano et al., 2017), indicating that complex SV reducing gamete viability might cause fruit set disorders.

To understand the effects of SV on grapevine fruit set and production, we have compared cluster yield components between TB and TT. To identify environmental factors involved in fruit set anomalies that decrease grapevine yield we also analysed these components in a network of TB vineyards. The results point out the effects of gamete viability on seed and fruit production disorders, as well as the environmental conditions that increase TB fruit set sensitivity. As a whole, these results clarify the possible effects of SV in relevant cluster and production traits in grapevine.

2. Material and methods

2.1. Plant material

For a paired comparison between the derivative Tempranillo Blanco (TB) and the ancestral Tempranillo Tinto (TT) somatic variants (Fig. 1), we used an experimental plot at the ICVV Grapevine Collection (FAO

code ESP-217), located at "Finca La Grajera" (LOG; Logroño, Spain). Three interspersed vineyard rows of each genotype were used in this plot. We also collected data from the same TB clone in eight additional locations corresponding to a network of commercial vineyards distributed along the Ebro river valley in La Rioja region (Spain): Albelda de Iregua (ALB), Alfaro (ALF), Azofra (AZO), Cenicero (CEN), Corera (COR), Fonzaleche (FON), Nalda (NAL) and Rincón de Soto (RIN). The R packages *ggplot2, ggmap* and *ggrepel* were used to plot the location and altitude of these vineyards in La Rioja region (Supplementary File 1). TT clone RJ51 was used as reference as it is one of the most widely grown ones, whilst for TB we used the only clone available. All vineyards and plots were cultivated under similar agricultural practices, with plants grafted onto the same rootstock and maintained under the same training and pruning systems (Table 1). For each location and genotype, three replicates were considered, each representing a different vineyard row. On each replicate, four randomly distributed plants were followed for most traits. To avoid fruit set alteration due to pollen collection, another 9-12 plants in total from the same three rows were used as replicates for pollen viability analysis.

2.2. Phenology observations

Phenological stages were evaluated according to the modified E-L system (Coombe, 1995). Dates of budburst (modified E-L stage 4), full flowering (modified E-L stage 23), veraison (modified E-L stage 35) and harvest (modified E-L stage 38) were recorded for TB in the nine locations under study and for TT in LOG. The observed date of full-flowering per location and genotype was used to reference periods at which environmental conditions were compared.

2.3. Meteorological data

Meteorological data were recorded by weather stations located nearby the nine locations analysed in this work. These weather stations are part of the Agroclimatic Information Network of La Rioja, and data (validated according to the normative UNE 500540:2004) can be accessed at: http://www.larioja.org/siar. Within the information available, we retrieved data on mean daily temperature (T, °C), maximum and minimum daily temperatures (°C), mean daily precipitation (P, l/m^2), mean daily global radiation (R, w/m^2) and mean daily reference evapotranspiration (Eto, mm/day). Reference evapotranspiration was estimated from data collected in the weather stations using the FAO Penman-Monteith method (Allen et al., 1998). For T, P, R and Eto we considered the mean value of seven consecutive days, which correspond to five weekly periods: the week of flowering and four weeks before flowering. Thus, meteorological data on the week of flowering (_WeekF) correspond to the mean value of the three days before the date of flowering, the day of the date of flowering and the three days after that date. Weeks before flowering (_Week1PreF, _Week2PreF, _Week3PreF, and _Week4PreF,) were set as 7-day periods before the week of flowering (Supplementary File 2).

Following the work of Jones and Davis (2000), maximum and minimum daily temperatures were used to calculate two additional variables to evaluate the negative effect that recurrent low and high temperatures around flowering time may have on grapevine reproductive performance. Thus, we calculated the number of days per week with a maximum daily temperature over 30°C (Ndays30), and the number of days per week with a minimum daily temperature below 10°C (Ndays10). These values were estimated for five week periods, which were determined as described before. These two temperature thresholds (<10 and >30°C) were set in our analysis after adaptation to La Rioja specific climate conditions of the suggested optimum range of temperatures for successful flowering and fruit set in grapevine (Keller, 2010b).

2.4. Phenotypic descriptions

TB and TT plants were analysed for 12 cluster yield-related traits in two consecutive seasons (2014 and 2015) (Table 2). For each location (and genotype), one well-developed inflorescence on each of the four plants per replicate was selected. Following the procedure detailed in Ibáñez et al. (2020), these inflorescences were tagged and bagged with a fine nylon mesh bag at approximately two weeks before flowering (modified E-L stage 17-18 (Coombe, 1995)). Pollen viability was estimated at full flowering (modified stage E-L 23 (Coombe, 1995)) from additional inflorescences in plants of the same plot, considering that this trait does not vary within the inflorescences of a certain genotype grown

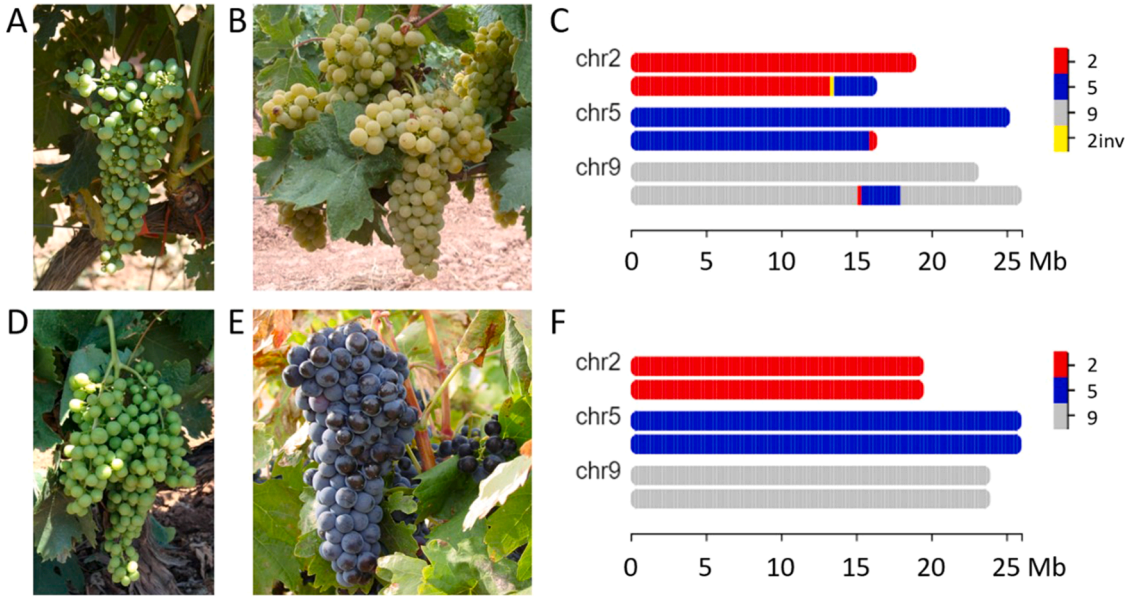

Fig. 1. Features of Tempranillo Blanco grapevine somatic variant. Cluster at pea-size green stage (A, D) and clusters at maturity (B, E) and conformation of differential chromosomes (C, F) are shown, respectively for Tempranillo Blanco derivative white-berried cultivar (A, B and C) as well as for the ancestor Tempranillo Tinto black-berried cultivar (D, E and F). For each somatic variant, genome rearrangements involving chromosomes 2, 5 and 9 (chr2, chr5, and chr9), causing loss of berry colour in TB and an affection of grape production performance are schematically shown. Chromosome maps (C and F) were built using the ChromoMap R package (https://cran.r-project.org/web/packages/chromoMap/vignettes/chromoMap.html) according to previous data (Carbonell-Bejerano et al., 2017).

Table 1
Location and main characteristics of the vineyard plots analysed in this work.

Vineyard	Location	Cultivar	Rootstock	Coordinates	Altitude (m.a.s.l.)	Training/Pruning systems	Year of plantation
ALB	Albelda de Iregua	Tempranillo Blanco	R-110	N 42° 21′ 44.43′′ W 2° 27′ 7.889′′	740	Double cordon/spur	2011
ALF	Alfaro	Tempranillo Blanco	R-110	N 42° 9′ 30.701′′ W 1° 50′ 3.824′′	340	Double cordon/spur	2009
AZO	Azofra	Tempranillo Blanco	R-110	N 42° 25′ 45.04′′ W 2° 48′ 22.43′′	555	Double cordon/spur	2009
CEN	Cenicero	Tempranillo Blanco	R-110	N 42° 28′ 41.28′′ W 2° 38′ 51.419′′	490	Double cordon/spur	2009
COR	Corera	Tempranillo Blanco	R-110	N 42° 20′ 52.47′′ W 2° 13′ 7.079′′	490	Double cordon/spur	2009
FON	Fonzaleche	Tempranillo Blanco	R-110	N 42° 36′ 34.44′′ W 2° 58′ 50.639′′	550	Double cordon/spur	2010
LOG	Logroño	Tempranillo Blanco	R-110	N 42° 26′ 22.08′′ W 2° 30′ 51.129′′	410	Double cordon/spur	2002
LOG	Logroño	Tempranillo Tinto	R-110	N 42° 26′ 22.08′′ W 2° 30′ 51.129′′	410	Double cordon/spur	2002
NAL	Nalda	Tempranillo Blanco	R-110	N 42° 21′ 44.2′′ W 2° 30′ 6.75′′	725	Double cordon/spur	2010
RIN	Rincón de Soto	Tempranillo Blanco	R-110	N 42° 12′ 36.89′′ W 1° 52′ 36.549′′	350	Double cordon/spur	2009

Table 2
Phenotypic variation (mean ± standard deviation) observed for 12 cluster yield-related traits and two-way ANOVA results obtained for Tempranillo Blanco and Tempranillo Tinto somatic variants in Logroño experimental plot (LOG) in two consecutive seasons (2014 and 2015).

Trait	Tempranillo Blanco (2014)	Tempranillo Tinto (2014)	Tempranillo Blanco (2015)	Tempranillo Tinto (2015)	G[1]	E[1]	GxE[1]
Berry weight (g)	2.20 ± 0.09	2.50 ± 0.18	1.56 ± 0.11	1.70 ± 0.12	*	***	ns
Cluster weight (g)	206.43 ± 14.5	283.78 ± 53.02	197.67 ± 15.7	379.33 ± 49.51	***	ns	*
Coulure	1.65 ± 1.00	1.19 ± 0.41	4.49 ± 0.33	3.06 ± 0.15	*	***	ns
Dropped ovaries	64.33 ± 35.47	39.83 ± 21.24	216.58 ± 41.27	173.92 ± 50.16	ns	***	ns
Flowers	353.33 ± 23.71	273.75 ± 42.01	425.92 ± 50.53	494.42 ± 107.04	ns	**	ns
Fruit set (%)	32.16 ± 3.26	61.43 ± 5.82	36.37 ± 3.73	54.95 ± 2.36	***	ns	*
LGOs	180.14 ± 51.86	74.58 ± 16.48	68.42 ± 3.79	66.75 ± 18.83	*	**	*
Millerandage	7.34 ± 0.21	3.11 ± 0.32	3.64 ± 0.38	2.21 ± 0.33	***	***	***
Pollen viability (%)	42.14 ± 19.41	94.5 ± 3.34	17.52 ± 4.68	90.14 ± 9.39	***	ns	ns
Seeded berries	81.83 ± 20.89	157.67 ± 23.53	133.42 ± 15.83	251.92 ± 54.02	***	**	ns
Seedless berries	30.28 ± 16.21	1.70 ± 0.95	7.50 ± 2.38	1.80 ± 1.90	**	*	*
Seeds per berry	1.13 ± 0.08	2.17 ± 0.49	1.20 ± 0.10	1.92 ± 0.15	***	ns	ns

[1] Two-way ANOVA. G: Genotype; E: Environment; GxE: Genotype x Environment; n.s.: p-value > 0.05; *: p-value < 0.05; **: p-value < 0.01; ***: p-value < 0.001.

under the same conditions (Tello et al., 2018). To this aim, 9-12 inflorescences, each from a different plant, were collected from randomly selected plants equally distributed in number across the three vineyard rows used for each location and genotype and transported to the laboratory. Pollen samples were either sieved and allowed to dry over-night in 2014 or directly submitted to Alexander's modified staining method in 2015 (Peterson et al., 2010), which can differentiate between non-viable and viable pollen grains. One slide per inflorescence was prepared, photographed and analysed as detailed in Royo et al. (2016). Ten pictures per slide were taken and >1,000 pollen grains per picture were classified as viable or inviable according to their staining pattern using Fiji (Schneider et al., 2012).

After the completion of fruit set, at grape pea-size stage (modified E-L stage 31 (Coombe, 1995)) in 2014 or at cluster closure (modified E-L stage 32) in 2015, bags were removed from the inflorescences, sealed, transported to the laboratory, and stored at room temperature to analyse their content. After air-drying at room temperature, the flower debris of each bag (which contains flower caps, abscised flowers, dropped ovaries and other senescent floral organs) were spread and scanned using an EPSON Perfection V370 Photo scanner. Images were then manually analysed with Fiji (Schindelin et al., 2012) to determine the number of flower caps + abscised flowers (which corresponds to the total number of flowers initially present in the inflorescence), and the number of ovaries dropped after flowering.

At harvest time (modified E-L stage 38 (Coombe, 1995)), mature clusters developed from tagged inflorescences were collected and transported to the laboratory. Cluster weight was determined in a scale (Blauscal AC-5000, Gram Precision, Barcelona, Spain), and the three post-flowering organs indicated in Friend and Trought (2007) were manually counted in each cluster: the number of ripe seeded berries, the number of ripe seedless berries, and the number of live green ovaries (LGOs). These numbers were added to the number of flowers and dropped ovaries found in the nylon mesh bag. The detailed analysis of these variables allowed us to calculate the fruit set rate, and the *millerandage* and *coulure* indices, according to Collins and Dry (2009). Lastly, five random seeded berries per cluster were weighted to determine the mean berry weight, and their seed number was counted to determine the number of seeds per seeded berry for the corresponding cluster. For all these traits, values for each replicate per year were obtained as the mean of the four corresponding clusters.

2.5. Statistical analysis

2.5.1. Comparative analysis of Tempranillo Blanco vs Tempranillo Tinto

Pearson's partial correlation coefficients (r) with season as correcting factor were calculated between all traits, considering data from TB and TT (both from the same vineyard, Table 1). Correlations were considered significant if $p \leq 0.05$. Two-way analyses of variance (ANOVAs) were calculated to determine the effect of year, genotype, and their interaction on the variation of the twelve traits considered in this work. Factors were evaluated to have a significant effect on trait variation at three different levels ($p \leq 0.001$, $p \leq 0.01$ and $p \leq 0.05$). Lastly, a PCA under Varimax rotation was calculated to identify the underlying relationships between all traits and the factor "season" and "genotype". All

analyses were performed by means of SPSS v.26.0.

2.5.2. Comparative analysis of Tempranillo Blanco in nine locations

Pearson's partial correlation coefficients (*r*) with season as correction factor were calculated between all traits and all environmental factors by SPSS, considering the data collected for TB at the nine experimental plots (Table 1). Two-way ANOVAs were calculated as detailed before to determine the effect of year, location, and their interaction on the 12 phenotypic traits. Effects were considered significant at $p \leq 0.001$, $p \leq 0.01$ and $p \leq 0.05$ levels. A PCA was calculated as defined before to identify the underlying relationships between all traits and the factors "season", "location", "altitude", "latitude" and "longitude". Multiple regression analyses were performed for all traits, considering as independent variables those environmental factors with significant ($p \leq 0.05$) Pearson's partial correlation coefficients with the modelled trait. The optimum set of regressors in each model was determined through the forward stepwise method implemented in SPSS, which starts with a null model and ends in a model with the highest possible explained variance (R^2) from the minimum number of regressors that explain non-redundant variance. Regressors were included in the model considering both a probability-of-F-to-enter (PIN) ≥ 0.05 and a probability-of-F-to-remove (POUT) ≤ 0.1. Similarly, a critical factor of variance-inflation-factor (VIF) ≤ 5.0 was set to avoid multicollinearity between regressors. Lastly, the R *relaimpo* package (Grömping, 2006) was used to determine the relative contribution of the selected regressors to the total variance of each model. To ease interpretation, the individual contribution of the regressors were forced to sum 100% (option rela=TRUE).

3. Results

3.1. Effect of somatic genome rearrangements on Tempranillo Blanco cluster yield traits

To evaluate for side effects on fruit production of loss of fruit colour-associated somatic genome variation in TB (Fig. 1), cluster yield components were compared between the white-berried derivative TB and the ancestral black-berried TT (Table 2). Cluster weight was significantly lower in TB than in TT in 2014 (-27.3%) and 2015 (-47.9%), as it was the number of seeded berries in TB when compared to TT (-48.1% in 2014 and -47.0% in 2015). In addition to the lower number of seeded berries, the white-berried variant showed a lower number of seeds per berry in seeded berries, and a higher number of seedless berries and LGOs per cluster (Table 2). Fruit set rate was indeed lower in TB compared to TT (-47.6% and -33.8% for 2014 and 2015, respectively). These differences might ultimately derive from the significantly lower pollen viability registered in TB when compared to TT in 2014 and 2015 (-55.4% and -80.6%, respectively). Two-way ANOVA results revealed a major significant effect of the genotype on the pollen viability variation, with no significant effect of the environment or the GxE interaction (Table 2).

On the other hand, two-way ANOVA results indicated a non-significant effect of the genotype on the initial number of flowers per inflorescence, with a phenotypic variance significantly depending only on the effect of the environment. A similar result was obtained for the number of dropped ovaries after fruit set, which was significantly higher in 2015 than in 2014 for both genotypes (Table 2). *Coulure* and *millerandage* reproductive disorders (both measured as indices) were found to be significantly affected by both the genotype (in both cases, higher in TB than in TT) and the environment (Table 2). Lastly, both the genotype and the environment were found to have significant effects in weight variation of seeded berries, with TB berries being 12.0% and 8.2% lighter than those of TT (for 2014 and 2015, respectively). For both genotypes, seeded berries were significantly heavier in 2014 than in 2015 (Table 2).

The univariate relationship among the 12 cluster yield-related traits compared between TB and TT were evaluated by a partial correlations analysis, with season as correcting factor (Fig. 2). Results indicated a high and positive correlation between pollen viability and fruit set rate, which in turn correlated significantly and positively with the number of seeded berries in the cluster, the number of seeds per berry and the final cluster weight. In addition, fruit set rate was significantly and negatively correlated with the number of seedless berries and the number of LGOs in the cluster at harvest time, as well as with the *millerandage* index. The number of seeded berries in the cluster correlated significantly and negatively with the number of seedless berries, as well as with the *millerandage* index. A significant and positive correlation among the number of berries, cluster weight and the number of flowers was also identified.

The 12 traits analysed in both somatic variants were submitted to a principal component analysis (PCA) to globally detect the underlying relationship among them, including the factor season and genotype to evaluate how they relate to phenotypic data variation. Results revealed a three PC model explaining 92.7% of the total data variance, with the two first PCs explaining 46.5 and 37.0% of the total data variability, respectively. Interestingly, the factor season is related to PC-1, whilst the factor genotype is more related to PC-2. Regarding the phenotypic traits, PC-1 is mainly associated with berry weight, the number of dropped ovaries, the *coulure* index, and the number of flowers per inflorescence. The most influential variables for PC-2 are cluster weight, the number of seeded berries and pollen viability (Fig. 3). The analysis of the loadings of each variable in these two PCs revealed their clustering in five different groups, suggesting these are variables that share similar variance. For instance, the number of LGOs, the number of seedless berries and the *millerandage* rate clustered in the same group, as cluster weight did with the number of seeded berries (Fig. 3). The variables clustered in each of these five groups showed significant and positive coefficients in the partial correlation analyses (Fig. 2). The factor season clustered in a group that included three agronomic variables: *coulure*, the number of dropped ovaries in the cluster, and the number of flowers in the inflorescence. According to ANOVA results, these three variables were found to be significantly affected by the different environmental conditions recorded in 2014 and 2015 (Table 2). Likewise, the factor genotype clustered with pollen viability, the fruit set rate and the number of seeds per seeded berry (Fig. 3). These three variables were significantly

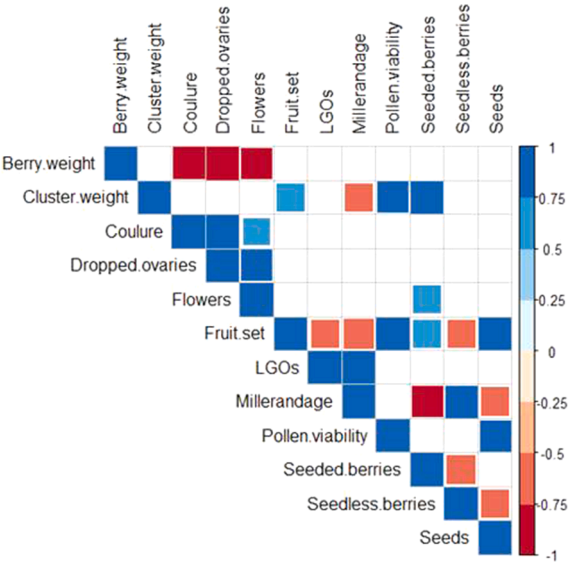

Fig. 2. Pearson's correlation coefficients obtained for 12 cluster yield-related traits analysed in Tempranillo Blanco and Tempranillo Tinto somatic variants in Logroño (LOG) in two consecutive seasons (2014 and 2015). Squares size and colour vary according to correlation coefficients (see blue-to-red scale). Only significant values are shown (p-value <0.05).

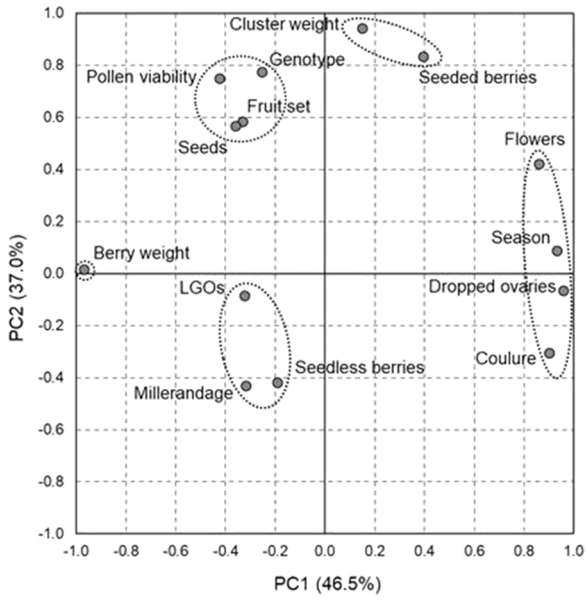

Fig. 3. Principal components analysis obtained for 12 cluster yield-related traits analysed in Tempranillo Blanco and Tempranillo Tinto somatic variants in Logroño (LOG) in two consecutive seasons (2014 and 2015).

different between TB and TT (Table 2).

3.2. Analysis of Tempranillo Blanco performance under different environmental conditions

To identify production traits subjected to environmental variation in TB, as well as the conditions limiting fruit yield success in this derivative cultivar, variation for 12 cluster yield-related traits in TB was recorded across nine locations (Supplementary File 1) and in two seasons. As a reference, results in the nine plots were compared with those obtained for TT in one location (Fig. 4). The meteorological information retrieved for the analysed period of time in 2014 and 2015 presented high variability within the nine locations explored (Supplementary File 2). As expected, temperature raised as flowering time approached, reaching the highest temperature in most locations at flowering time. In 2014, average temperature values at flowering time ranged from 21.0 ± 3.7°C (in LOG) to 15.0 ± 1.5°C (ALB). In 2015, these values ranged from 21.0 ± 1.7°C (NAL) to 13.9 ± 1.4°C (FON). Regarding the accumulated rainfalls registered during the analysed period, relatively low variation took place in 2014, with maximum values registered in COR (69.5 l/m^2), ALB (52.6 l/m^2) and FON (51.0 l/m^2), and the minimum values were recorded in ALF (24.4 l/m^2), LOG (34.4 l/m^2) and CEN (34.7 l/m^2). Regarding 2015, maximum precipitations were registered in FON (73.8 l/m^2), ALB (61.3 l/m^2) and AZO (47.9 l/m^2). Minimum values were recorded in RIN, LOG and COR, with extremely low precipitations (1.6, 2.7 and 3.1 l/m^2, respectively).

We observed a relevant variability in many of the analysed traits across the nine locations, such as the number of seedless berries, which varied by a 62.5-fold factor in 2015 (ranging from 2.1 ± 2.2 in ALF to 130.1 ± 51.2 in ALB), the number of seeded berries, which varied by a 5.8-fold factor in 2015 (from 36.6 ± 11.3 in ALB to 212.0 ± 11.0 in RIN), or *coulure*, which varied by a 5.6-fold factor in 2014 (from 0.9 ± 0.2 in AZO to 4.8 ± 0.7 in ALF). Other traits were less affected by the location effect, including the number of seeds per berry, which varied by a 1.1-fold factor in 2014 (from 1.1 ± 0.1 in AZO to 1.2 ± 0.2 in COR) as well as in 2015 (from 1.1 ± 0.1 in AZO to 1.2 ± 0.1 in NAL). ANOVA results confirmed such trends, with location having a significant effect on the phenotypic variation of all traits but the number of seeds per seeded berry (Table 3 and Supplementary File 3).

Similarly, ANOVA results indicated a significant effect of season on 11 out of the 12 traits analyzed (Table 3). *Millerandage* indices in TB tended to be higher in 2014 than in 2015, with up to a 2.7-fold difference in RIN. The number of seeded berries per cluster also varied significantly between seasons. For example, TB clusters in ALF had 2.1-fold more seeded berries in 2015 (189.5 ± 45.5) than in 2014 (92.2 ± 21.1), and in RIN they had 2.1-fold more in 2015 (212.0 ± 11.0) than in 2014 (99.2 ± 10.4). Significant seasonal differences were also obtained in TB cluster weight: in ALF it was 1.8-fold higher in 2015 than in 2014 (293.2 ± 65.3g and 164.5 ± 11.8g, respectively), whereas in RIN it was 2.1-fold higher in 2015 than in 2014 (343.1 ± 22.5g and 165.3 ± 12.5g, respectively). TB pollen viability was higher in 2014 than in 2015 in all analyzed locations, and marked significant differences were also found for *coulure*. Interestingly, a PCA computed to identify the relationship between season, location and TB production traits indicate that location coordinates (altitude, latitude and longitude) and season load in different dimensions (Supplementary File 4), suggesting that these two main factors explain different sections of the overall variability.

3.3. Relationship between environmental variables and Tempranillo Blanco production traits

Our results indicated a strong effect of vineyard location on TB production traits, with altitude, latitude, and longitude correlating significantly with seven, three and six phenotypic traits, respectively (Fig. 5). A significant correlation was also observed between latitude and longitude, as well as between altitude and longitude. These correlations derive from the singular geography and topography of La Rioja region, which include the presence of some mountainous systems in its western side and some plains with not much change in elevation in its eastern side (Supplementary File 1). As expected, altitude affects environmental conditions, as it correlated significantly with evapotranspiration, precipitation, mean solar radiation and the number of days below 10°C and over 30°C registered at different pre-flowering or flowering week periods (Fig. 5). On the contrary, latitude and longitude only correlated significantly with the variable Ndays10_Week3PreF. The number of significant correlations obtained between environmental variables and phenotypic traits was very different, from variables that do not correlate with any trait (like T_Mean_WeekF or P_Week2PreF) to others correlating with up to seven or six traits (P_WeekF and Ndays10_Week3PreF, respectively) (Fig. 5). Thus, P_WeekF correlated significantly with cluster weight, the number of dropped ovaries, fruit set ratio, *millerandage*, the number of seeded and seedless berries, and the number of seeds per seeded berry. Ndays10_Week3PreF correlated significantly with *coulure*, cluster weight, the number of dropped ovaries, seeded berries and seedless berries, and *millerandage*.

The number of significant partial correlations obtained for each trait was highly variable too, with *millerandage*, the number of seedless berries, and the number of seeded berries correlating significantly with more than ten environmental variables. On the contrary, pollen viability and the number of LGOs correlated significantly with only two variables (Ndays10_WeekF and Ndays10_Week1PreF for pollen viability, and Eto_Week2PreF and R_Mean_Week2PreF for LGOs), whilst the number of seeds per seeded berry correlated significantly with only three variables (P_WeekF, T_Mean_Week4PreF, and Ndays30_Week4PreF), and the number of flowers per inflorescence with five variables (Eto_WeekF, R_Mean_WeekF, T_Mean_Week3PreF, T_Mean_Week1PreF, and Ndays30_Week2PreF).

Stepwise regression modelling identified the environmental and topological variables with a major influence on each production trait (Fig. 6 and Supplementary File 5). No models were released for three traits: LGOs, pollen viability and the number of seeds per seeded berry, as the input variables did not accomplish model fitting criterion. For the modelled traits, the number of retained variables ranged from one to six (for berry weight and the number of seeded berries, respectively). Thus, for berry weight, only T_Mean_Week4PreF was selected, which explained 40.4% of trait variance. For the number of seeded berries, the

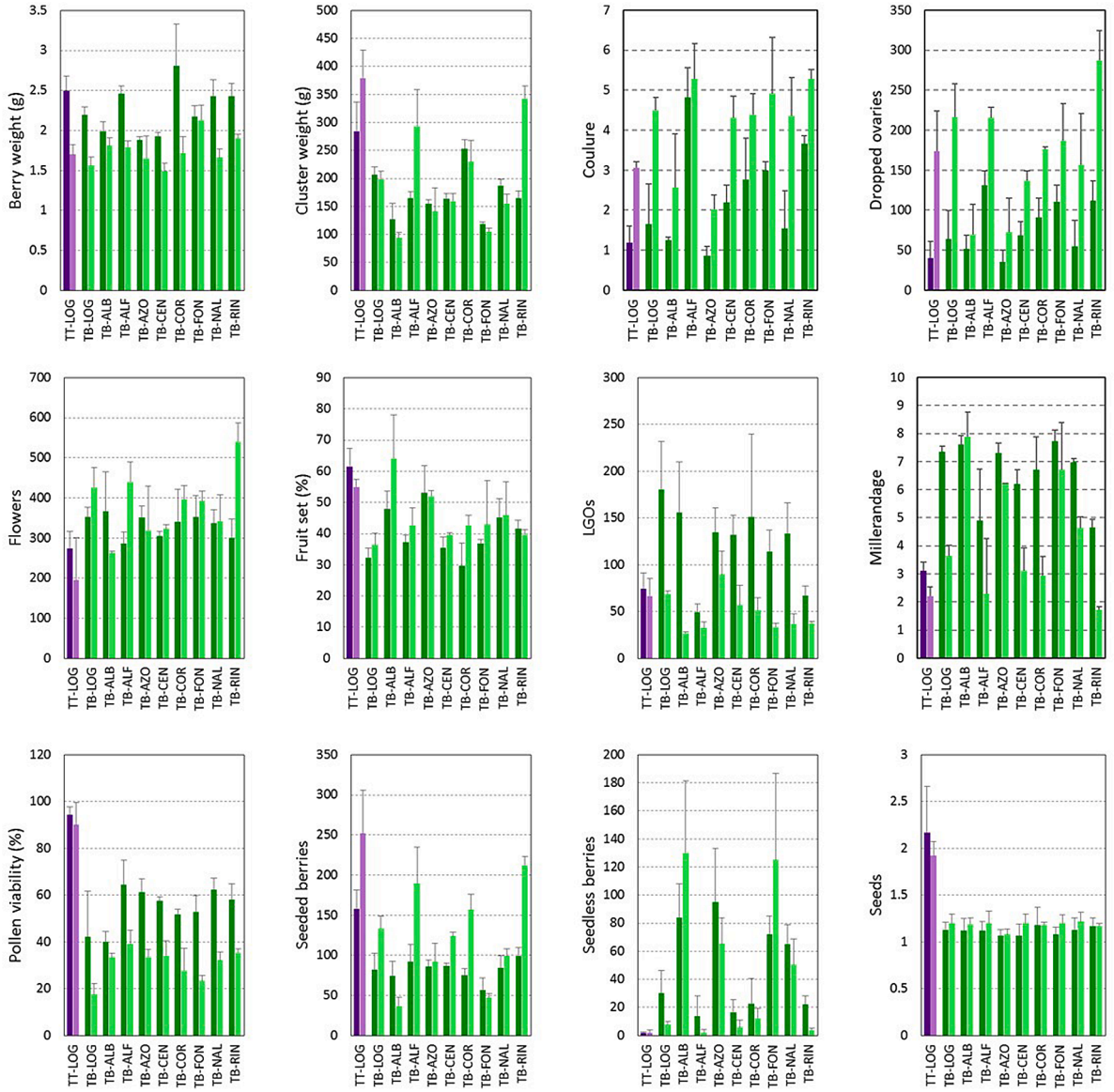

Fig. 4. Phenotypic variation (mean ± standard deviation) estimated for 12 cluster yield-related traits in grapevine Tempranillo Tinto (TT, purple) and Tempranillo Blanco (TB, green, in nine vineyards) somatic variants. Each bar represents data of one season (dark purple/dark green: 2014; light purple/light green: 2015). Vineyards are named according to Table 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
Two-way ANOVA results to evaluate the effects of location (Loc) and season (Sea) on the phenotypic variability observed for 12 cluster yield-related traits in Tempranillo.

Trait	Location	Season[1]	Loc x Sea[1]
Berry weight	***	***	***
Cluster weight	***	**	***
Coulure	***	***	ns
Dropped ovaries	***	***	**
Flowers	*	**	***
Fruit set	***	**	ns
LGOs	**	***	ns
Millerandage	***	***	**
Pollen viability	***	***	ns
Seeded berries	***	***	***
Seedless berries	***	ns	*
Seeds per berry	ns	*	ns

[1] n.s.: p-value > 0.05; *: p-value < 0.05; **: p-value < 0.01; ***: p-value < 0.001.

stepwise approach retained Ndays10_Week1PreF, Ndays10_Week3PreF, Ndays30_Week2PreF, P_WeekF, Eto_WeekF, and Eto_Week3PreF as predictive regressors, which jointly could explain up to 95.1% of trait variance. Modelling results indicated Eto_WeekF and Longitude as the two most determining variables for cluster weight variance (cR^2=63.2%). In the other modelled traits, recurrent low and high pre-flowering and full-flowering temperatures (below 10°C and above 30°C, respectively) were commonly retained as significantly predictive regressors (Fig. 6 and Supplementary File 5). Only two variables were retained for fruit set modelling (Ndays10_WeekF and Ndays30_Week1PreF), which explained 54.2% of trait variance. The same number of variables was selected to model *millerandage*, with Ndays10_Week3PreF and Ndays30_Week1PreF as regressors in a model that explained 86.6% of trait variance. These two environmental variables were retained for modelling the number of dropped ovaries, which, together with Ndays10_Week2PreF, explained 80.4% of trait variance. For *coulure*, four variables were selected (Ndays10_WeekF, Ndays10_Week2PreF, Ndays10_Week3PreF, and P_Week3PreF) in a model that explained 82.3% of trait variance. Lastly, two environmental

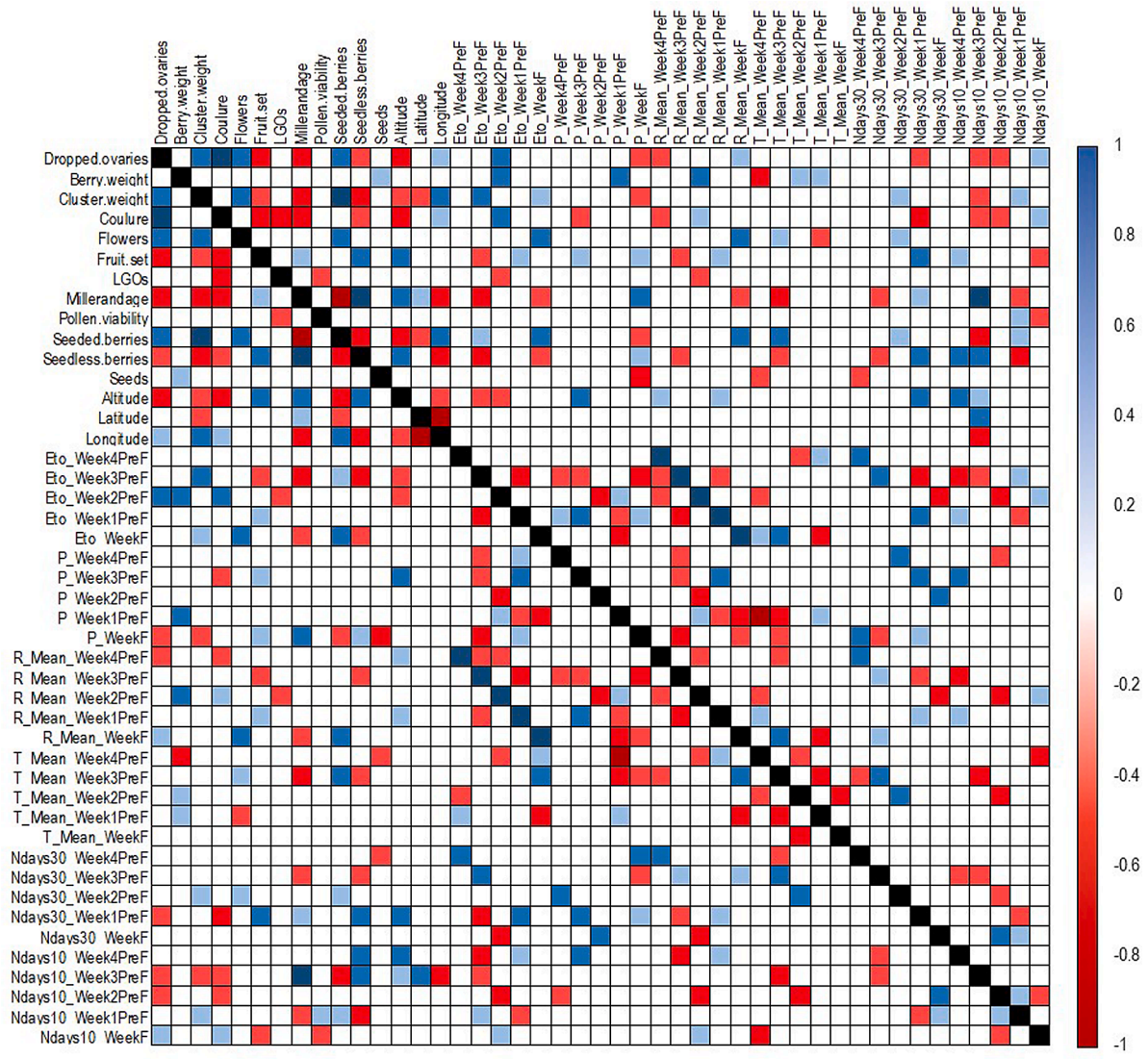

Fig. 5. Correlogram based on Pearson partial correlation coefficients between 12 cluster yield-related traits evaluated in grapevine cv. Tempranillo Blanco in nine vineyards and meteorological variables. Partial correlation coefficients were obtained using season as random variable. Only coefficients for significant correlations are shown (p-value <0.05), which are coloured according to the blue-to-red scale (see adjacent bar).

variables (Ndays30_Week2PreF and Eto_WeekF) were selected for modelling the number of flowers in the inflorescence (cR^2=55.1%), whereas three were selected for the number of seedless berries: Ndays10_Week1PreF, T_Mean_Week3PreF, and Altitude (cR^2=78.6%).

4. Discussion

4.1. Low gamete viability drives to a reduction of cluster yield in Tempranillo Blanco by limiting seed and fruit set success

The extreme chromosomal restructuring event that generated the loss of berry colour in TB is associated with alterations of its reproductive performance (Fig. 1). In particular, decreased pollen viability and reduced yield were reported in TB compared to its black-berried somatic ancestor (Carbonell-Bejerano et al., 2017). Confirming previous reports that describe TB as a less-yielding cultivar than TT (Balda and Martínez de Toda, 2017), the pairwise comparison between TB and TT carried out here points out a relevant reduction in TB cluster weight (Table 2). Correlation and PCA analyses revealed that the difference in cluster weight between TB and TT is mainly due to differences in the number of seeded berries between both genotypes, with an intrinsic significant negative effect of *millerandage* and a related inverse contribution of fruit set rate (Figs. 2 and 3). Interestingly, although the seeded berries from TB were found to be significantly lighter than those from TT (Table 2), no significant correlation between berry weight and cluster weight was obtained (Fig. 2).

The number of berries per cluster depends on the initial number of flowers in the grapevine inflorescence and the fruit set rate (Dry et al., 2010). Therefore, considering the absence of significant differences in flower number between TB and TT (Table 2), the lower number of seeded berries in TB derives from its markedly low fruit set rate and the increased development of seedless berries, which collectively contribute to an increased *millerandage* (Table 2). The decreased number of seeded berries in TB and the associated increased fraction of parthenocarpic berries and LGOs is also generally extended to other grapevine varieties with poor fruit set (Dry et al., 2010; May, 2004). TT has been described as a cultivar with low susceptibility to *millerandage*, with similar rates to those found in Pinot Noir, Chardonnay and Shiraz (Dry et al., 2010). Therefore, higher *millerandage* in TB than in TT (Table 2) is likely a side effect of the chromosomal restructuring event that generated this white-berried somatic variant (Carbonell-Bejerano et al., 2017). *Coulure* was also significantly higher in TB than in TT (Table 2), despite no

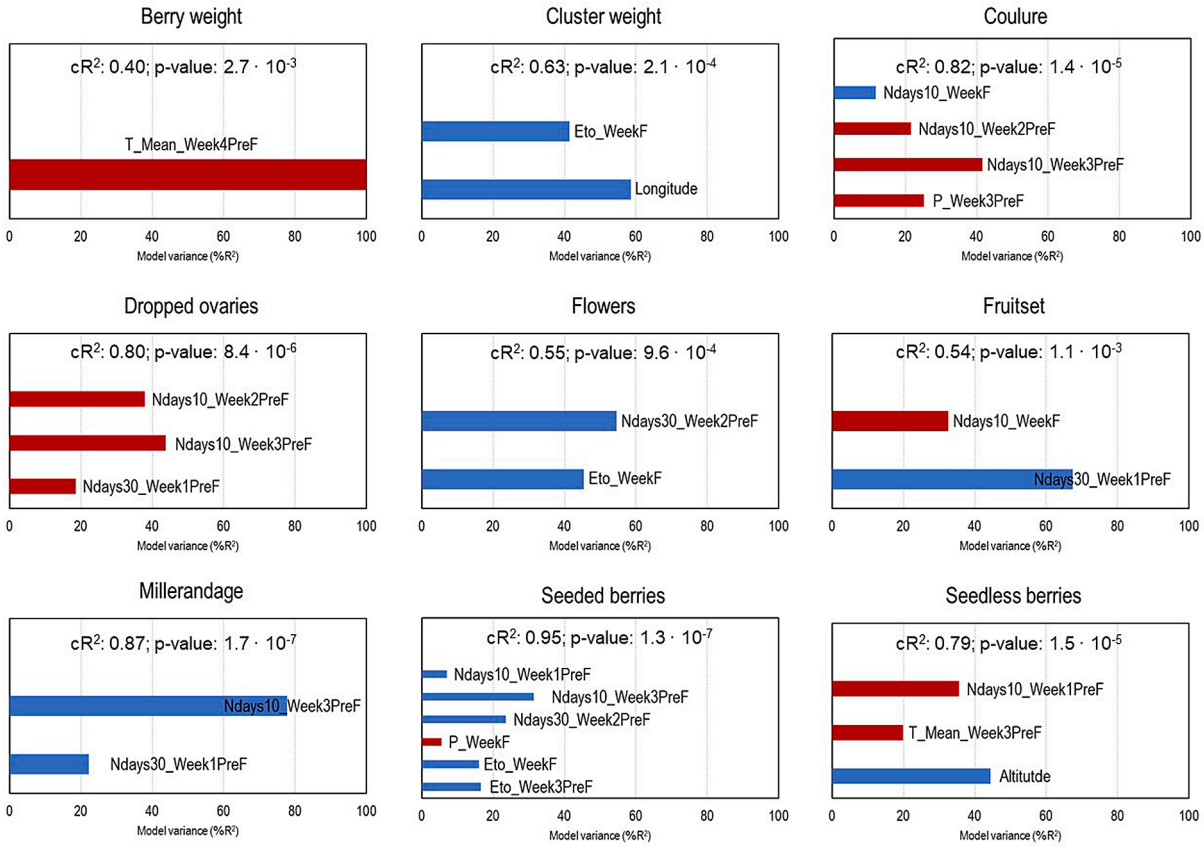

Fig. 6. Relative contribution of the meteorological variables selected by stepwise multiple regression modelling to explain the phenotypic variance observed for nine cluster yield-related traits in in nine plots of grapevine cv. Tempranillo Blanco. Bars colour indicates if the regressor has positive (blue) or negative (red) effect on trait variance. Metrics are normalized to sum 100%. Models corrected-R^2 (cR^2) and associated p-values are shown for each trait. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

significant difference between TB and TT was observed for the number of dropped ovaries (Table 2).

Obviously, the genetically-determined affection of TB cluster yield-related traits discussed above have a common origin. Confirming previous results (Carbonell-Bejerano et al., 2017; Tello et al., 2018), TB shows a marked genetic decrease in pollen viability of 45-65% compared to its black-berried clonal ancestor (Table 2), supporting that the genome rearrangements that generated this somatic variant affected gametogenesis processes. Highly rearranged chromosomes have been suggested to be deleterious for the resulting unbalanced haploid gametes (Pellestor, 2014), which in plants is translated into increased gamete sterility (Tan et al., 2015). Findings in *Capsicum annuum* L. indicate that the negative effects of the unbalanced migration of genetic material during male meiosis ends in pollen grain sterility (Qiu et al., 2017). Low pollen viability hinders pollen grain germination and pollen tube growth processes, which ultimately affects grapevine fruit set and reduces cluster yield (Baby et al., 2016b), as it happens for the increased *millerandage* in TB compared to TT. In a similar way, in the *millerandage*-susceptible table grape cultivar Rubi, reduced pollen germination rates related with erratic meiotic chromosome behaviour have been associated with a major tendency to form seedless berries (Da Silva et al., 2001). Likewise, in parthenocarpic grapevine cultivars such as Corinto Bianco and Corinto Nero, which can be considered as the most extreme cases of *millerandage*, practically full sterility of pollen grains is observed (Royo et al., 2016; Costantini et al., 2021). Nonetheless, pollen is usually produced in a large excess in grapevine hermaphroditic flowers (Kelen and Demirtas, 2003), which might ensure successful fruit set even at very low pollen viability rates. Although female gamete viability has not directly been measured in TB, the significant and environment-independent lower number of seeds observed in TB seeded berries suggests the existence of additional development anomalies decreasing TB ovule viability. Lack of segregation of rearranged chromosomes 2 and 5 after TB selfing also supports that the viability of both male and female gametes is in fact reduced in TB (Carbonell-Bejerano et al., 2017). Linkage between pollen sterility and ovule sterility has also been reported in Corinto Bianco and Corinto Nero parthenocarpic variants (Royo et al., 2016; Costantini et al., 2021), as well as after pharmacological treatments of grapevine inflorescences (Iyer and Randhawa, 1966). Therefore, the causes of gamete sterility in grapevine often act in a sex-inespecific fashion, as it can be expected for the consequences of the SV present in TB. Altogether, these findings suggest that reduced gamete viability due to unbalanced chromosomes generated after somatic genome reshuffling as in TB (Fig. 1 and Table 2), might also be related with the tendency to *millerandage* and poor fruit set that is often found in other grapevine cultivars. Genome variation causing this kind of reproductive dysfunction would be lost in natural populations due to purifying selection taking place during sexual cycles (Kim and Zhang, 2018). However, because grapevine cultivars are vegetatively propagated, such dysfunctions can be perpetuated on time and, sometimes, they are even submitted to positive artificial selection when associated to novel interesting agronomic traits (e.g.: somatic loss of fruit colour in TB).

4.2. Genome reshuffle in Tempranillo Blanco compromises grape production under environmental conditions that are unfavourable for grapevine gametogenesis and fertilisation

The high variability observed here for TB grape production

components across vineyards (Fig. 4) is mostly attributable to local environmental conditions since the same clone, rootstock and management practices were used. Cluster weight was indeed one of the traits involving larger variability in TB yield performance under different locations (Additional file 3 and Fig. 4). TB cluster weight variation across vineyard locations was not only related with the number of seeded berries in the cluster as expected, but it also had a strong dependence on the number of seedless berries (Additional file 3 and Fig. 5). Thus, decreased flower and seeded berry numbers, as well as increased proportion of seedless berries and *millerandage*, appear as the main features resulting in decreased TB cluster weight yield in some of the analysed locations (Fig. 5 and Supplementary File 4). Among these traits, variation in flower number would not be specifically related with somatic variation in TB as no significant effect of the genotype was identified when compared to TT (Table 2 and Fig. 3). Indeed, the variation in the number of flowers across locations and seasons was generally lower than that observed for the number of seedless berries and *millerandage* in TB (Fig. 4). In contrast, the pair-wise comparison between TB and TT showed a significant GxE effect on the last two traits, which was especially strong for *millerandage* with a specific season effect in TB (Table 2 and Fig. 3). In the same line, the number of seedless berries was not only one of the traits with larger variation across locations in TB but it also showed a reproducible pattern between the two analysed seasons (Fig. 4 and Table 3), suggesting for consistent effects of the local environment in TB performance. While both TB and TT showed some parthenocarpy capability (both genotypes can develop seedless fruits), the increased proportion of seedless berries per cluster showed as a marked genetic feature of TB (Table 2 and Fig. 3). Altogether, these results indicate that limited gamete viability in TB leads to increased sensitivity of seeded fruit setting to the environment.

When considering all nine locations, the variation of cluster weight in TB seems to be affected by the vineyard altitude (Supplementary File 4). Rather than affecting the number of flowers, gain in altitude was related with higher *millerandage* and seedless berries (Fig. 5). The lowest mean cluster weight observed in this work was obtained in 2015 in the vineyard located at the highest altitude (ALB, 740 m.a.s.l.). Here, TB clusters were characterized by an excessive number of seedless berries (130.11 ± 51.25) and LGOs (26.83 ± 1.61) relative to seeded berries (36.56 ± 11.34). Compared to other locations at lower altitudes, this plot had prolonged cold periods (high number of consecutive days below 10°C) and abundant rainy episodes (Supplementary File 2). Under this specific conditions, TB seedless berries represent more than 65% of all berries, an unsustainable value considering that seedless berries typically represent less than 10% of all berries, or 20-25% in susceptible cultivars (Dry et al., 2010). In contrast, pollen viability and the number of seeds in seeded berries were rather constant across vineyards on each season, and did not correlate with any topographic variable and only with few meteorological ones (Figs. 3 and 4), showing again that reduction of gamete viability in TB is a genetic trait associated to its SV that is barely affected by the environment. Altogether, these results suggest that the acquired reduced gamete viability of TB compromises its production yield under pre-flowering/flowering environmental conditions that are unfavourable for gamete development and fertilisation, such as low temperature and heavy rains (Ebadi et al., 1995b; González-Fernández et al., 2020; May, 2004)

Proper flowering and fruit set performance in grapevine require a combination of adequate environmental conditions (warm temperatures, medium values of humidity and adequate light intensity) (Li-Mallet et al., 2016; Vasconcelos et al., 2009). These conditions can be strongly interrelated, which hinders to interpret their singular effect. Here, the use of univariate and multivariate analyses allowed us to get some insights into the individual effect of some environmental factors on TB grape production. Correlation and modelling analyses indicate that long periods at low and high temperatures during pre-flowering and full-flowering impact TB grape production (Figs. 5 and 6). This finding agrees with the general assumption that both cool and hot temperatures (below 15°C and above 35°C, respectively) decrease grapevine fruit set and grape production (Keller, 2010b; Pagay and Collins, 2017). Data from the last decade indicate that pre-flowering/flowering temperatures above 35°C are unusual in La Rioja region, whilst it is very common to have minimum daily temperatures below 15°C (Ramos and Martínez de Toda, 2020). Therefore, we considered two slightly different values (10°C and 30°C) to explore the effect of low and high temperatures on TB production performance. Our results suggest a detrimental effect of temperatures below 10°C occurring 2-3 weeks before flowering, as they associate with the development of seedless berries and higher *millerandage* rates (Fig. 5). In fact, this stage is known to be critical for proper gamete development (Ebadi et al., 1995b). As previously shown for cvs. Shiraz and Chardonnay, flowers exposed to low temperatures (below 12°C) at this period show a high number of imperfect ovules with incompletely o missing embryo sac, as well as a reduced pollen activity (Ebadi et al., 1995b), which ultimately reduce fruit set and berry number (Ebadi et al., 1995a).

High temperatures recorded the week before full-flowering positively correlated with grape production in TB (Fig. 5). Indeed, modelling results indicated a beneficial effect of temperatures above 30°C on TB fruit set (Fig. 6). Findings in cvs. Pinot Noir and Carignan indicate that optimum pollen germination and ovule fertilisation occur at mild temperatures (25°C) (Kliewer, 1977). Our results suggest that even higher temperatures just before flowering could also favour the last stages of flower development in TB, such as stigma maturation and proper anthesis (flower and anthers opening mechanisms) (Fougère-Rifot et al., 1995). It might enhance subsequent pollination and fertilisation efficiency, and partially counteract the consequences of reduced pollen viability in TB.

On the other hand, we observed a detrimental effect of precipitations at flowering time on many TB grape production traits (Fig. 5), having a relevant impact on the number of seeded berries (Fig. 6). Atmospheric pollen concentration is reduced by rainfall events (González-Fernández et al., 2020), which ultimately hinders the fertilisation process and thus seed and fruit setting (May, 2004). This situation can be specially limiting in genotypes with already decreased pollen viability as TB. Interestingly, we also found a beneficial impact of pre-flowering rainfalls on TB reproductive performance, as it was related with reduced *coulure* rates (Fig. 6). Pre-flowering rains are key to ensure an adequate plant water status for proper flower development and further flowering and fruit set processes (Li-Mallet et al., 2016; Santos et al., 2011). In this regard, Korkutal et al. (2019) have recently indicated that early water stress hinders embryo sac development and pollen grains functionality in cv. Syrah, which could also be in agreement with the higher environmental than genetic effect observed for *coulure* variation in the comparison between TB and TT (Table 2 and Fig. 3)

Related with the effect of precipitations, our modelling results indicated that evapotranspiration was another key factor on TB grape production, as it affected the number of flowers per inflorescence, the number of seeded berries in the cluster, and cluster weight (Fig. 6). Evapotranspiration depends on several factors, including environmental conditions (Ohana-Levi et al., 2020). Accordingly, we observed significant correlations between pre-flowering and flowering evapotranspiration rates and global solar radiation, precipitations, and periods at low (below 10°C) and high (above 30°C) temperature (Fig. 5). Thus, environmental conditions that generate an increase on pre-flowering and flowering evapotranspiration values (namely, high solar radiation, low precipitations, presence of episodes above 30°C, and/or absence of episodes below 10°C) correlated with increased TB fruit set. In this line, an adequate pre-flowering sun radiation is essential for optimal photosynthesis activity (Keller, 2010a). Low pre-flowering photosynthetic rates might reduce sugar supply to the developing inflorescence, which hinders proper male and female organs formation and leads to low rates of ovule fertilisation (Lebon et al., 2005). In this regard, early vine shading is becoming a relevant practice to decrease the number of berries per cluster, an especially useful treatment for cultivars with

compact clusters (Basile et al., 2015; Domingos et al., 2015). Thus, treatments favouring radiation at pre-flowering time would seem convenient to increase cluster yield in TB. On the other hand, warm and dry days at flowering time favour pollination processes, as they benefit proper calyptra opening and drop (May, 2004) and favour anther dehiscence and pollen release and dispersal (Vasconcelos et al., 2009).

Lastly, we explored the effect of environmental conditions on TB berry weight. At a species level, berry weight variation is determined by cell division before anthesis and cell division and expansion after anthesis (Houel et al., 2013). Despite the impact of post-flowering environmental conditions on berry cell division and/or cell expansion mechanisms (Bergqvist et al., 2002; Kliewer, 1977), they have not been considered in this work, which might explain the moderate value of variance explained by the regression model of environment effect in berry weight (cR^2=40.0%, Fig. 6). Nevertheless, we detected a significant negative impact of the mean temperature registered four weeks before flowering. At that moment, flower carpels are already visible, and ovules primordia start to develop soon after (Fougère-Rifot et al., 1995). It suggests that high temperatures at an early stage of development might irreversibly limit cell division in the carpels, which would ultimately affect berry growth potential due to lower cell number. While little information is available on the effect of pre-flowering temperatures on the early grapevine carpel development and growth, findings in other crops indicate that high pre-flowering temperatures cause an underdevelopment of the pistil that derives into reduced carpel weight (Calderini et al., 1999; Rodrigo and Herrero, 2002).

In summary, the genome rearrangement that caused the loss of berry colour in TB also limits the reproductive performance of this cultivar. This might be a drawback for commercial grape production in TB, especially if vines are grown under environmental conditions than hinder fruit set (such as those observed in high altitude sites, prone to low pre-flowering temperatures and/or abundant flowering rainfalls). Nevertheless, the lower yield performance of TB compared to its black-berried counterpart can be an acceptable trade-off for high quality winemaking. A growing part of the wine industry perceives modest vine yields as a positive feature, since it is associated with more balanced grape composition and subsequent higher wine quality (Carmona et al., 2008). In fact, the observed lower number of seeded berries per cluster in TB generates a reduction in cluster compactness (Fig. 1), which can enable a more homogeneous solar radiation of individual berries (Tello and Ibáñez, 2018) that in turn benefits berry ripening and must composition (Ziegler et al., 2020).

5. Conclusions

Due to their conspicuousness and singularity, white-berried grape somatic variants have been selected and clonally propagated as a valuable source of diversity for both wine and table grape production (Carbonell-Bejerano et al., 2019; Pelsy et al., 2015; Vezzulli et al., 2012). The recent advent of whole-genome sequencing approaches eases the identification of the mutations causing somatic variation in grape colour, even if they involve complex genome rearrangements. We confirm here that the somatic genome reshuffling that originated TB did not only remove the functional gene alleles required for berry skin anthocyanin pigmentation, but it also led to a genetic decrease in gamete viability. Because sexual reproduction is not necessary for the maintenance of vegetatively propagated crops (as it is the case of grapevine cultivars), mutations decreasing gamete viability can be selected as side effects of interesting agronomic traits like it happened for the acquired trait of white berry production in TB. However, our detailed study of TB reproductive performance in different locations also shows that genetically decreased gamete viability is associated with increased susceptibility to environment-dependent fruit set disorders such as *millerandage*. Our multilocation study in TB shows that environment-sensitive productivity in this cultivar is related with decreased gamete viability being limiting for fruit set success under conditions that are unfavourable for grapevine pollination and fertilisation, including heavy rains at flowering time and low pre-flowering temperatures. Although this study was based on a limited number of seasons and vineyards, the results offer a detailed overview of the agronomic performance of this new cultivar under very different growing conditions, providing useful information to evaluate the potential for its cultivation in other regions. Such evaluations should not only consider decreased production as a drawback, but also that moderate grape yields are often linked to improved grape composition and wine quality. Altogether, our findings show that increased susceptibility to environment-dependent fruit set disorders in TB is ultimately caused by decreased gamete sterility associated to chromosome reshuffling. Because clonally propagated cultivars are prone to accumulate SV in the absence of the purifying selection that takes place during sexual cycles (Vondras et al., 2019; Zhou et al., 2019), we hypothesise that the susceptibility to fruit set disorders that characterizes several grapevine cultivars and clones could be associated in some instances with genome rearrangements ending in a fraction of unviable unbalanced gametes as in TB.

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CRediT authorship contribution statement

J. Tello: Conceptualization, Data curation, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **C. Royo:** Data curation, Writing – review & editing. **E. Baroja:** Data curation, Writing – review & editing. **E. García-Escudero:** Resources, Writing – review & editing. **J.M. Martínez-Zapater:** Writing – original draft, Writing – review & editing, Funding acquisition. **P. Carbonell-Bejerano:** Conceptualization, Data curation, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

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