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1 **Biochemical and genetic implications of the slow ripening phenotype in peach fruit**

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13

14 **Highlights**

15 - Different allele combinations of the *Sr* gene influence peach ripening physiology and
16 biochemistry.

17 - The *Sr* gene affects ethylene synthesis, and therefore all the ethylene-dependent
18 ripening changes.

19 - *Sr2sr* hybrids showed a longer harvest window and improved postharvest behaviour
20 compared to *Sr1sr* and *Sr1Sr2*.

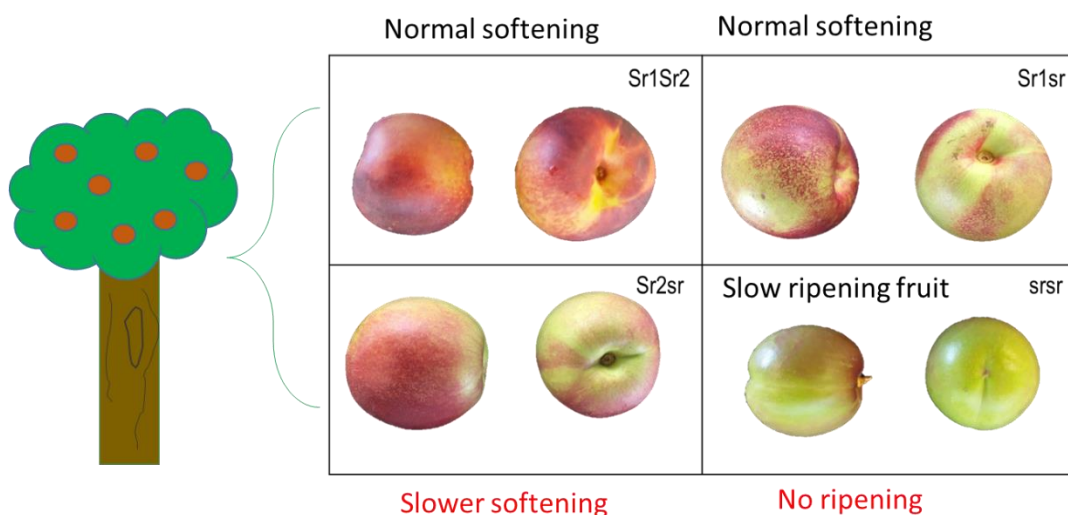
21 - Selection of *Sr2sr* hybrids could be an efficient strategy for improved shelf life in new
22 peach cultivars.

23 **Abstract**

24 The peach [*Prunus persica* L. (Batsch)] slow ripening (SR) trait is a mutation preventing
25 the normal fruit ripening process. It is determined by a single Mendelian gene (*Sr/sr*)
26 located on linkage group 4, where only homozygous individuals for a recessive allele (*sr*)
27 show the SR phenotype and are generally discarded from breeding programs. Ripening-
28 related traits such as fruit weight, firmness loss, ethylene production, ACO activity,
29 sugars and organic acids composition, malondialdehyde, antioxidant capacity and total
30 phenolic content were evaluated in a segregating population for the SR trait during two

31 consecutive harvest seasons and at different maturity stages. Although there is no
 32 commercial value for the slow ripening (*srsr*) individuals, our results demonstrate that a
 33 heterozygous combination involving *sr* and another allele at this locus (*Sr2*) showed
 34 interesting traits including a longer harvest window and improved postharvest behaviour
 35 if harvested at the appropriate maturity ($I_{AD} \geq 2$). All these traits seem to be linked to a
 36 delayed ripening behaviour mediated, in turn, by a lower ethylene production capacity
 37 and an altered sugar (mainly sucrose) and organic acid accumulation/utilisation on-tree.
 38 The selection of this allelic combination could be an easy and efficient strategy to obtain
 39 new peach cultivars with potentially improved shelf life.

40 **Graphical abstract**



41

42 **Keywords**

43 Breeding, climacteric, fruit quality, firmness, softening, texture.

44

45 **Introduction**

46 Peach is a typical climacteric fruit, with a rapid softening and short shelf life after
47 harvest that adversely affect its market value. Fruit maturation is a complex and highly
48 coordinated developmental process that affects colour, firmness, taste and flavour. Fruit
49 ethylene production rate has a clear effect on the firmness loss and senescence of the fruit,
50 and therefore in the fruit storage life (Barry and Giovannoni, 2007; Osorio et al., 2011;
51 Giné-Bordonaba et al., 2016).

52 Peach fruit showing the slow ripening (SR) phenotype fail to ripen. This character
53 was first described in some breeding populations as fruit that remained firm when mature,
54 with a very slow rate of flesh softening, a reduced CO₂ and C₂H₄ production, poor sensory
55 qualities and a high susceptibility to internal breakdown (Brecht et al., 1984, 1982). The
56 same authors also reported that exogenous C₂H₄ application failed to induce ripening as
57 normally observed in other climacteric fruit.

58 Some years later (Ramming, 1991; Tataranni et al., 2010), the slow-ripening trait
59 was proposed to be controlled by a single gene (*Sr/sr*), and when the recessive *sr* allele
60 was in homozygosis, it prevented the fruit undergoing normal ripening. More recently,
61 the *sr* gene has been mapped on linkage group 4 (G4) and molecular markers for its
62 selection have been developed (Eduardo et al., 2015; Meneses et al., 2016; Nuñez-Lillo
63 et al., 2015). These markers are based on a large deletion of 26.6 kb containing two NAC
64 transcription factors that could be the causal mutation. In fact, genes of the NAC family
65 have been shown to be involved in the regulation of ethylene-mediated ripening in tomato
66 (Osorio et al., 2011) and banana (Chen et al., 2012). A major gene/QTL for maturity date
67 (Eduardo et al., 2015; Pirona et al., 2013) and a QTL for the chilling injury symptoms of
68 fruit fresh mealiness and bleeding (Martínez-García et al., 2013) have also been identified
69 at the *Sr* genomic region. More recently, Botton et al. (2016) proposed that the peach
70 HEC3-like gene FLESHY may be involved in the SR phenotype, having an important

71 role in fruit tissue patterning at early fruit development. Information also exists on the
72 relationship between the ripening behaviour and ethylene production of different SR
73 (*srsr*) fruit (Brecht et al., 1982), but no other studies have ever investigated and compared
74 the physiological and biochemical differences occurring during ripening among *Srsr* and
75 *SrSr* genotypes.

76 Understanding the effect of the *sr* allele on the softening of peach in combination
77 with the above-mentioned and available molecular marker (Eduardo et al., 2015; Meneses
78 et al., 2016; Nuñez-Lillo et al., 2015), would offer breeders the possibility to include this
79 character in their progenies to obtain new cultivars with potentially longer shelf life. For
80 instance, a slower softening would allow a wider harvesting window, as well as an
81 extended postharvest life. In addition, a better understanding on the biochemical
82 mechanisms underlying such regulatory control of ripening would open the possibility to
83 modify certain biochemical pathways aiming to obtain peaches with extended postharvest
84 life.

85 Accordingly, the objective of this work was to deep insight the effect of each *sr*
86 allele configuration in the peach maturation/ripening physiology. To do so, we analysed
87 a series of traits such as fruit firmness, ethylene production, I_{AD}, weight, biochemical
88 compounds (glucose, sucrose, malic and citric acid) and antioxidant metabolism related
89 molecules (malondialdehyde, antioxidant capacity, and total phenolic content) along
90 ripening in a SR segregating population for two consecutive harvest seasons.

91

92 **Material and Methods**

93 Plant material

94 A segregating F1 progeny (BbxNI) from the cross between the white peach
95 cultivar ‘Belbinette’ (Bb) and the yellow nectarine ‘Nectalady’ (NI) was used in this
96 study. Trees were planted in the fields at the IRTA Experimental Station in Gimènells
97 (Lleida, Spain) initially on their own roots (2007) and later (2008) grafted on ‘Cadaman’
98 rootstock.

99 For fruit phenotyping, including physiological and biochemical measurements, a
100 subset of 25 individuals plus the parents was selected. Available information about the
101 allelic composition of the parents Belbinette (*Sr2sr*) and Nectalady (*Sr1sr*) and offspring
102 from this population (Eduardo et al., 2015; Meneses et al., 2016), was used to select the
103 subset of individuals analyzed. The difference between *Sr1* and *Sr2* could be established
104 by Eduardo et al. (2015) as they were associated with an ~10-day interval in maturity
105 dates of the parents, earlier for *Sr1* and later for *Sr2*. The selection in the progeny was
106 made in order to have represented all the genotypic classes, and the most variability of
107 maturity dates within each class:

- 108 - Slow ripening individuals (*srsr*, N=3)
- 109 - Individuals heterozygous for the *sr* allele: (*Sr1sr*, N=7) and (*Sr2sr*, N=7)
- 110 - Individuals not carrying the *sr* allele (*Sr1Sr2*, N=8)

111

112 Fruit growth

113 On-tree fruit growth (size) and non-destructive evaluation of the fruit maturity stage (DA-
114 value) both from the parents and two hybrids from each allelic class (*srsr*, *Sr1sr*, *Sr2sr*
115 and *Sr1Sr2*) was periodically monitored (every 7-10 days) from fruit set to harvest on 10
116 fruit randomly selected. Fruit size was measured with a digital calliper, as the distance
117 between the suture and the opposite side at the equatorial zone. DA-values, also known
118 as the index of Absorbance difference (I_{AD}), were measured with a commercial equipment

119 (DA-Meter, TR Turoni, Forli, Italy), which measures non-destructively Vis-spectroscopy
120 according to the I_{AD} index (index of absorbance difference = $A_{670}-A_{720}$) (Ziosi et al.,
121 2008).

122

123 Fruit ripening and ethylene measurements

124 Forty fruit were harvested from each tree at 3 different maturity stages based on fruit
125 firmness (60-80N (M1), 40-60N (M2), <30N (M3)), I_{AD} values and historical data on
126 their ripening pattern. In the case of sr fruit (srsr), firmness did never reach values <30N,
127 and therefore fruit were harvested at different maturity stages based exclusively in days
128 after full bloom (DAFB). Upon each harvest, fruit were immediately transported to the
129 lab for quality measurements (t_0). Fruit was individually weighted in a digital scale. Then,
130 I_{AD} values and firmness were measured on both sides of each fruit using 8
131 fruits/tree/harvest (total of individuals = 25). I_{AD} values were measured as described
132 above whereas flesh firmness was measured with a digital penetrometer (Model. 53205;
133 Turoni, Forlí, Italy) equipped with an 8-mm diameter plunger tip after the removal of a 1
134 mm thick slice of skin. Full bloom date for each tree was recorded to calculate days after
135 full bloom (DAFB) at the moment of harvest. In the selected individuals harvest window
136 ranged from early August to mid September.

137 Ethylene production and firmness were evaluated the same day of harvest (t_0) and after
138 1, 3 and 6 days after harvest (t_1 , t_3 , t_6). Firmness at t_1 , t_3 and t_6 was measured as
139 described for t_0 using 8 fruits/tree/harvest. Remaining fruit were stored at 20°C (70% RH)
140 for the following evaluation times. Ethylene production ($\mu\text{L kg}^{-1}\text{h}^{-1}$) was measured in an
141 acclimatized chamber at 20°C. The fruit (2 or 3 fruit per replicate, and at least 3 replicates
142 per tree and harvest) were placed in airtight 1.5 L flasks. After 2 h incubation, gas samples

143 (1 mL) were taken from the headspace of the flasks, using a 1 mL syringe, and injected
144 into a gas chromatograph (GC; Agilent Technologies 6890, Wilmington, Germany) fitted
145 with a FID detector and an alumina column F1 80/100 (2 m × 1/8 × 2.1, Tecknokroma,
146 Barcelona, Spain) following the methodology described elsewhere (Giné-Bordonaba et
147 al., 2017). Samples were taken again from the headspace of the flasks following the same
148 methodology at t1, t3 and t6.

149 The fruit flesh from each sample (8 fruits/tree) at each maturity stage (M1, M2 and M3)
150 was frozen at the time of harvest and stored at -80°C until being used for biochemical
151 measurements.

152

153 Biochemical measurements

154 The enzyme 1-Aminocyclopropane-1-carboxylic acid oxidase (ACO) was
155 extracted as described by Lindo-Garcia et al. (2019) with some modifications. The sample
156 (0.5 g of frozen tissue) was homogenized in 1 mL of buffer containing 400 mM MOPS at
157 pH 7.2, 10% glycerol, 30 mM ascorbic acid sodium salt and PVP 40000 2%. The
158 homogenized was slightly shaken for 10 min at 1°C and centrifuged at 17,000 x g for 30
159 min at 4°C. Subsequently, the supernatant was stored at -80°C until analysis of the enzyme
160 activity using the methodology described by Giné-Bordonaba et al. (2017).

161 Sugars (glucose and sucrose) and organic acids (citric and malic acid) were extracted from
162 frozen tissue as described by Giné-Bordonaba et al. (2019) with some modifications. For
163 sugars determination, 2 g of sample were diluted in 5 mL of 62.5% (v/v) aqueous methanol
164 solvent and placed in a thermostatic bath at 55°C for 15 min, mixing the solution with a
165 vortex every 5 min to prevent layering. Then, the samples were centrifuged at 24,000 x g
166 for 15 min at 20 °C. The supernatants of each sample were recovered and used for enzyme
167 coupled spectrophotometric determination of glucose (hexokinase) and sucrose (β-

168 fructosidase) using commercial kits (BioSystems S.A., Barcelona, Spain) and following
169 the manufacturer instructions.

170 Citric and malic acid were extracted by dissolving 2 g of frozen tissue in 5 mL of distillate
171 water. The solution was slightly shaken for 10 min at room temperature and then
172 centrifuged at 24,000 x g for 7 min at 20°C. The resulting supernatant was recovered and
173 used to determine the concentration of citric (citrate lyase/malate dehydrogenase) and
174 malic acid (malate dehydrogenase) using commercial kits (BioSystems S.A., Barcelona,
175 Spain) and following the manufacturer instructions.

176

177 Statistical analysis

178 All data were subjected to analysis of variance (ANOVA) using JMP® 13.1.0 SAS
179 Institute Inc. Mean comparisons for the interaction genotype * maturity was evaluated
180 using HSD test at a significance level of $p \leq 0.05$, while comparisons for specific traits
181 between genotypes along storage time or days after full bloom was done by least
182 significant difference values (LSD; $p \leq 0.05$) using critical values of t for two-tailed tests.
183 Correlations between experimental variables were made using Spearman's Rank
184 Correlations and, if required, presented as Spearman's Correlation Coefficient (r) and P
185 value based on a two-tailed test.

186 **Results**

187 *Fruit maturation*

188 As described above, the different maturity stages (M1, M2 and M3) were selected based
189 on both firmness and I_{AD} values and generally comprised fruit harvested from 140 to 188
190 DAFB yet depending on the genotypic class (Table 1). The fruit weight of all the genetic
191 classes increased progressively in the tree during maturation (Fig. 2). However, fruit
192 weight of the SR individuals (*sr/sr*) individuals increased very little, reaching very low

193 weights if compared to the other individuals. In all genotypes, the increase in fruit size
194 seemed to be intimately regulated by the fruit maturity since the increase in fruit size was
195 negatively correlated ($R^2 > 0.85$; Figure 8) to the loss of fruit firmness and the I_{AD} value.
196 The I_{AD} , which indicates the degree of chlorophyll degradation in the fruit, showed a
197 progressive decrease at different maturities for the genetic classes *Sr1Sr2*, *Sr1sr* and *Sr2sr*
198 (Table 1). In the slow ripening fruit (*sr/sr*), the I_{AD} failed, as expected, to decrease
199 indicating that the chlorophyll levels remained very high and it was not degraded as it
200 occurs in a normal ripening process. Based on the I_{AD} values, the selected maturity classes
201 (M1, M2 and M3) were comparable among the different genotypic classes except for SR
202 individuals that besides not growing (Table 1) did not show a substantial chlorophyll
203 degradation on-tree.

204 Indeed, fruit growth was similar for all the genetic classes from fruit set to approximately
205 135 days after full bloom (DAFB) (Fig. 3). However, after approximately 145 DAFB the
206 fruit growth of the SR (*sr/sr*) individuals started to slow down if compared to the rest of
207 individuals, reaching smaller sizes at the end of the fruit development (*ca.* 190 DAFB).
208 Similarly, the degradation of chlorophyll (I_{AD}) in the SR individuals started to slow down
209 at around 155 DAFB compared to the rest of individuals. It can be also observed that the
210 genetic classes with one copy of the *sr* allele (*Sr1sr* and *Sr2sr*) had a slower degradation
211 of chlorophyll at these last stages of the fruit ripening than the rest of the non-slow
212 ripening (*Sr1Sr2*) individuals.

213 *Firmness loss and ethylene production*

214 The flesh firmness of the fruit from genetic class *Sr1Sr2* rapidly began to decrease after
215 1 day of shelf life (Fig. 4), regardless the initial fruit firmness at harvest (M1, M2 and
216 M3). On the other side, and as expected, the SR genotypes (*sr/sr*) failed to soften during

217 the shelf life period (6d), and their firmness remained over 60N. The hybrids *Sr1sr* and
218 *Sr2sr* showed a different behaviour to the former genetic classes, showing mixed results
219 depending on the fruit maturity at harvest. In detail, these genotypes soften rapidly if
220 harvested at firmness $\leq 60\text{N}$ yet the loss of firmness was prevented if the fruit were
221 harvested at less mature ripening stages (firmness $> 60\text{N}$; $I_{AD} \geq 2$). In the case of the *Sr2sr*
222 individuals, fruit firmness decreased slightly after one day of shelf life (generally referred
223 as softening), but then it remained stable after 3 days of shelf life (non-melting phase)
224 contrasting with *Sr1sr* individuals, where it kept decreasing and hence experienced both
225 softening and melting. Differences on the on-tree flesh firmness reached by the
226 individuals from the four genetic classes were very clear after 190 DAFB (Fig. 4). As
227 expected, slow ripening individuals (*srsr*) showed the highest firmness, individuals
228 without the *sr* allele (*Sr1Sr2*) showed the lowest, whereas the heterozygous classes (*Sr1sr*
229 and *Sr2sr*) showed intermediate values. Fruit from *Sr1Sr2* and *Sr1sr* individuals showed
230 the highest and similar firmness loss (FL) rates (-0.95 and -1.05 N/day, respectively),
231 whereas *Sr2sr* showed a similar FL rates than *sr/sr* individuals (-0.76 and -0.62 N/day,
232 respectively).

233 We observed clear differences in the ethylene production rates among the four genetic
234 classes in the BbxNI population (Fig. 5). Ethylene production at harvest for M1 was
235 negligible for all the individuals, independently of their genetic class. However,
236 significant differences were observed in the fruit after some days of shelf life at 20°C. No
237 detectable or negligible levels were found for *srsr* and *Sr2sr* individuals during shelf life,
238 whereas fruit from *Sr1Sr2* trees produced large amounts of ethylene after 12 days of shelf
239 life at 20°C. Fruit from *Sr1sr* individuals started to produce some ethylene after 13 days
240 of shelf life. The fruit from *Sr2sr* individuals did not produce any ethylene during shelf
241 life when harvested at flesh firmness of 60N. However, they were able to produce

242 ethylene during shelf life when harvested in a more advanced maturity stage (M3, flesh
243 firmness <30N) (results not shown). On the other side, the slow ripening fruit (*sr/sr*) did
244 not produce ethylene during shelf life for any of the three maturity stages investigated
245 herein.

246 *ACC oxidase activity*

247 Similarly to what happened for ethylene production, no ACC oxidase (ACO) activity was
248 observed at harvest for any of the genetic classes when harvested at M1 (Fig. 6).
249 Significant differences were observed at more advanced maturity stages (M2 and M3).
250 At M2, ACO activity was detected in fruit from *Sr1sr* and *Sr1Sr2* individuals, whereas it
251 was almost no detectable for *Sr2sr* and *srsr* individuals. The ACO activity was negligible
252 for any of the maturity stages in the fruit from the SR individuals (*sr/sr*). However, in the
253 case of the *Sr2sr* individuals, the ACO activity significantly increased in the more mature
254 fruit (M3), although reaching much lower levels than *Sr1Sr2* and *Sr1sr* classes at similar
255 maturity stages.

256 *Sugars and organic acids*

257 The pattern of sucrose accumulation in the fruit was significantly different among
258 different genetic classes (Table 1). In general, sucrose increased as fruit ripened on the
259 tree. However, accumulation in fruit from *Sr2sr* and *sr/sr* individuals was slower,
260 reaching much lower levels than the rest of fruit, especially in the slow ripening
261 individuals (*sr/sr*). In contrast, glucose levels decreased throughout maturation in all the
262 genetic classes, except in the *sr/sr* individuals where it slightly increased (Table 1).

263 Malic acid increased during maturation on the tree for the four genetic classes (Table 1).
264 Thus said, final malate content was significantly lower in the *sr/sr* and *Sr2sr* genotypes.
265 Contrary, citric acid decreased over on-tree maturation in all cases. In this case, *Sr2sr* and

266 *sr/sr* genotypes showed a slower rate of citric acid catabolism, whereas *Sr1Sr2* and *Sr1sr*
267 genotypes showed a rapid drop in the concentration of this acid in the first stages of
268 maturation.

269 **Discussion**

270

271 Peach ripening involves dramatic changes in the colour, firmness, texture, aroma, sugars
272 and organic acids composition. As for other climacteric fruit, these changes are triggered
273 by rapid changes in the rate of C₂H₄ production (Tonutti et al., 1991; Alexander, 2002;
274 Baró-Muntel et al., unpublished). However, SR peach fruit show a failure to ripen and
275 hence do not undergo such dramatic changes (Brecht et al., 1984; Ramming, 1991). This
276 behaviour may be caused by the inability to synthesize ethylene as in anti-sense tomato
277 (Picton, 1993) and melon mutants (Ayub et al., 1996), or due to problems in the ethylene
278 perception as in the *Nr* tomato mutant (Wilkinson et al., 1995). Non-climacteric
279 phenotypes may be also due to alterations upstream the ripening cascade as in *rin*, *nor*
280 and *Cnr* tomato mutants (Giovannoni, 2001). SR fruit do not respond to exogenous
281 ethylene treatment, as it occurs with *Nr* tomato and PI 161375 melon mutants (Wilkinson
282 et al., 1995; Périn et al., 2002), which demonstrates that SR mutation blocks not only
283 ethylene synthesis but also ethylene perception (Supplementary Fig. 1).

284 In this study, the effect of some alleles of the *Sr* gene in the ripening physiology and
285 biochemistry as well as in the fruit postharvest behaviour, even when present in
286 heterozygosis, have been investigated. Whereas *Sr1Sr2* and *Sr1sr* allelic combinations
287 resulted in fruit undergoing a normal ripening process, both on- and off-tree, we observed
288 changes in several ripening related parameters in offspring with the *Sr2sr* genotype.
289 Individuals carrying the *sr* allele as *Sr1sr*, *Sr2sr* and *srsr* produced little or no ethylene
290 when harvested at $I_{AD} \geq 2$. The results observed at the firmness loss and ethylene

291 production rate in the individuals carrying the *sr* allele, and especially in *Sr2sr*
292 individuals, indicate changes at the biochemical or physiological level resulting in
293 inhibition of normal ripening. Such differences at the fruit ethylene production capacity
294 were not explained by ACO activity since all individuals at M1 (when most of the
295 differences on the postharvest ripening occurred among individuals) showed minimal
296 enzyme activity (Fig. 6). Thus said, our results clearly indicate that the *Sr* gene
297 undoubtedly affected ethylene synthesis (Fig. 5 and 6), and therefore altered all the
298 ethylene-dependent ripening changes. Such results are in agreement with Brecht et al.
299 (1984) who reported that SR fruit harvested and stored at 20°C, showed delayed and
300 reduced climacteric peaks of respiration and ethylene production if compared to non-SR
301 ‘Fantasia’ fruit. As reported by other authors (Botton et al., 2016), SR fruit maintained
302 typical immaturity traits throughout development. However, these authors suggested a
303 disturbance at the level of fruit patterning as the cause for SR phenotype.

304 Previous work by Eduardo et al., (2015) mapped the *sr* gene in the same region of linkage
305 group 4 (G4), where a NAC transcription factor candidate gene (ppa008301m) is located.
306 This gene has been proposed as a candidate gene for the maturity date (*MD*) trait (Pirone
307 et al., 2013). Genes from the NAC family have been shown to be involved in the
308 regulation of ethylene mediated ripening in tomato (Osorio et al., 2011) and banana (Shan
309 et al., 2014, 2012). Rapid softening occurring at late ripening stages of peach requires
310 significant levels of ethylene (Hayama et al., 2006, 2003), and therefore decreases in the
311 ethylene production rates as observed in *Sr2sr* fruit had a direct effect on the fruit
312 softening rate. Recently, several candidate genes for MD and mealiness (*M*) traits have
313 been identified in the same region of G4 (Nuñez-Lillo et al., 2015), which would explain
314 the high susceptibility to internal breakdown found in the SR fruit. Accordingly, Giné-

315 Bordonaba et al. (2016) also reported that a greater capacity of the fruit to produce
316 ethylene after cold storage was associated to lower mealiness incidence in peach fruit.

317 Fruit from *srsr* individuals failed to develop normal ripening traits such as fruit size, color,
318 aroma and flavour. Whether other loci are also responsible for the altered fruit weight in
319 SR individuals is still unknown, what is clear is that the SR locus clearly altered this fruit
320 attribute. In the peach ripening model (Lü et al., 2018), ethylene-activated NAC
321 transcription factor binds to the promoter of key fruit ripening genes such as those
322 involved in pigment accumulation, volatile secondary metabolite production, cell wall
323 softening and sugar accumulation. Therefore, the *srsr* allelic combination in the SR fruit
324 would disrupt the activation cascade triggered by the NAC transcription factor in some
325 way. Both the peach and melon NAC loci are located in quantitative trait loci that are
326 associated with late ripening phenotypes (Perin, 2002; Pirona et al., 2013). However, we
327 could not discard the contribution of other transcription factors or regulatory mechanisms
328 such as post-translational regulations in the ripening inhibition in the SR fruit. Indeed,
329 *Sr2sr* fruit, contrary to what occurs in *sr/sr* fruit, developed normal fruit size, normal fruit
330 colour, aroma and flavour, which would demonstrate a normal function of the
331 transcriptional ripening feedback circuit, although with certain delay likely attributed to
332 a blockage of the auxin-ethylene hormonal crosstalk (Botton et al., 2016). This delay
333 could be an interesting trait in a new peach cultivar since it would extend the shelf life of
334 the fruit and the harvest window as detailed herein (Figure 3).

335 Ethylene is synthesized from S-adenosyl-Lmethionine (SAM) via the intermediate 1-
336 aminocyclopropane-1-carboxylic acid (ACC). The conversion of SAM to ACC is
337 catalysed by ACC synthase, and the subsequent oxidation of ACC to ethylene is catalysed
338 by ACC oxidase (ACO) (Ververidis and John, 1991; Yang and Hoffman, 1984). The
339 direct effect of ethylene production in ACO activity has been proven previously by the

340 strong depression of ACO expression by the treatment with 1-MCP, and its induction by
341 ethephon (Zhang et al., 2012). It has been demonstrated that ACO is one of the proteins
342 with the largest change in relative abundance during the fruit transition from the pre-
343 climacteric (“unripe”) to the climacteric (“ripe”) phase (Prinsi et al., 2011). The small
344 increase of ACO in the *Sr2sr* genotypes observed in this work demonstrated an altered
345 pattern of ripening, although those individuals did not show the SR phenotype. In the peach
346 ripening model (Lü et al., 2018), ethylene transcription factor EIN3 activates the NAC
347 transcription factor, which binds to the ACO and ACS promoters to activate the ethylene
348 synthesis. Therefore, the lower ACO activity observed in the *Sr2sr* individuals could also
349 explain their lower ethylene levels. The same effect of a delayed ripening due to a
350 reduction in the ethylene production has been reported by the expression of the tomato
351 ACO in the antisense orientation in transgenic plants (Ayub et al., 1996; Hamilton et al.,
352 1990). RNA gel blot analyses in normal ripening fruit demonstrated that ACO transcripts
353 greatly increased at late stages of fruit development (Callahan et al., 2004).

354 Glucose and sucrose contents observed in the NlxBb progeny are similar to those reported
355 in the flesh of other peach cultivars (Moing et al., 1998; Genard et al., 1999; Famiani et
356 al., 2016; Baró-Montel et al., unpublished). As observed in our work, glucose content has
357 been reported to decrease during peach ripening as a result, among others, of the large
358 increase in the fruit volume and therefore, the dilution of the glucose content within the
359 fruit (Famiani et al., 2016). The larger increase in the glucose concentration observed in
360 the slow ripening individuals (*sr/sr*; Table 1) through ripening could be explained by the
361 scarce increase in flesh volume as well as by the lower utilization of sugars as respiratory
362 substrates since ethylene production and respiration are largely depleted in these
363 individuals. In certain fruit species, evidence suggests that sucrose accumulation is linked
364 to the initiation of the ripening process on-tree (i.e. pears; Lindo-García et al., 2019). The

365 lower sucrose content and accumulation during on-tree ripening for *sr/sr* or *Sr2sr*
366 individuals agrees with the results from Botton et al. (2016) and further suggests a
367 putative role of this compound on triggering on-tree ripening also for peach fruit.

368 Malate together with citrate accounts for a large proportion of the organic acid content of
369 peach flesh (Chapman and Horvat, 1990; Byrne et al., 1991; Moing et al., 1998; Baró-
370 Montel et al., unpublished). As reported for other peach cultivars (Chapman and Horvat,
371 1990; Byrne et al., 1991; Moing et al., 1998; Famiani et al., 2016; Baró-Muntel et al.,
372 unpublished), the concentration of malate in the NlxBb population increased throughout
373 ripening, whereas, that of citrate decreased. On the other hand, while sugars can be
374 synthesized both in fruit and leaves, acids are exclusively synthesized in leaves and then
375 translocated to sink (fruit). Alterations not only at the ethylene level but also in the
376 translocation pathways of these compounds, likely mediated by auxins (Daie et al., 1986)
377 may explain the lower levels of malic acid in *sr/sr* and *Sr2sr* individuals' fruit.
378 Accordingly, our results also support previous findings from Famiani et al. (2016) who
379 pointed out that malate and citrate account only for negligible amounts of the respiratory
380 substrates during peach ripening since no association was found between these
381 compounds and the fruit respiration pattern throughout on-tree ripening (data not shown).

382 Some other authors have reported an anomalous accumulation pattern of phenolic
383 compounds at the flesh of SR fruit, more typical of lignifying endocarp (Masia et al.,
384 1992). Our results do not support such findings and rather reveal that the fruit antioxidant
385 capacity was similar among individuals. Although there is not commercial value for the
386 slow ripening (*sr/sr*) individuals, the *Sr2sr* hybrids could be interesting due to their longer
387 shelf life and slower rate of firmness loss. Postharvest losses are not only a current
388 problem in developing countries but also in modern supply chains. Therefore, extending
389 shelf life of peach fruit without compromising flavour and texture quality attributes is a

390 desired feature in peach industry and could be accomplished by the selection of *Sr2sr*
391 allelic combination. Selection of this allelic combination through the already available
392 molecular markers (Meneses et al., 2016) could be an easy and efficient strategy to obtain
393 new peach cultivars with potentially improved shelf life. However, our results should be
394 confirmed in other populations with a different genetic background.

395 The presence of SR phenotypes in the progenies of commercial breeding programs is
396 frequent. Based on the data of Meneses et al. (2016) on a sample of 27 peach cultivars we
397 estimated that the frequency of the *sr* allele is 0.33. While this is probably an
398 overestimation of its real value because the sample of cultivars chosen included some
399 known to carry *sr* in heterozygosis, this indicates that the presence of this allele in the
400 peach elite breeding pool is very high, particularly considering that SR individuals are
401 systematically selected against. This scenario was already pointed out by Eduardo et al.
402 (2015) that proposed that the favourable selection of certain heterozygous combinations
403 of *sr* with other alleles at this locus could counteract the directional selection against *sr*,
404 leading to the maintenance of this allele at intermediate frequencies.

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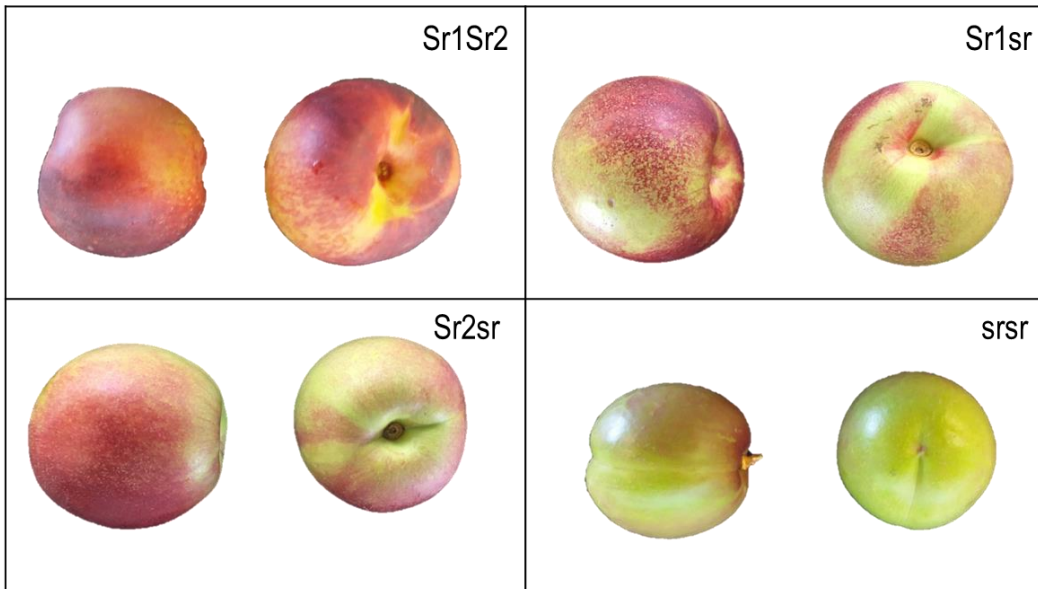
519 **Tables**

520 Table 1. Main organic acids (malic and citric acid), sugars (glucose and sucrose) and malonaldehyde (MDA) content of peach fruit at harvest from
 521 the different genetic classes in the parents and progeny from the ‘Belbinette x Nectalady’ F1 population. Fruit harvested at different maturity stages
 522 according to flesh firmness and DA-values (M1, M2 and M3) expressed in days after full bloom (DAFB). Data shown are means \pm Standard
 523 deviation.

Genotype	Maturity	DAFB	I_{AD}	Malic acid mg g ⁻¹	Citric acid mg g ⁻¹	Glucose mg g ⁻¹	Sucrose mg g ⁻¹	MDA nmol g ⁻¹	AC AU g ⁻¹
Sr1Sr2	M1	140.4 \pm 6.80	1.9 \pm 0.43ab	2.4 \pm 0.65 bcd	2.0 \pm 0.81 ab	8.1 \pm 0.03 ab	33.6 \pm 4.19 cdef	8.6 \pm 2.23	1.9 \pm 0.09
	M2	155.4 \pm 10.13	1.2 \pm 0.44cd	4.0 \pm 0.87 ab	0.5 \pm 0.12 c	7.1 \pm 1.32 bc	48.2 \pm 6.67 bc	11.5 \pm 1.40	1.7 \pm 0.09
	M3	169.8 \pm 10.52	0.7 \pm 0.32d	3.9 \pm 0.51 abc	0.6 \pm 0.16 c	5.9 \pm 0.59 bc	52.3 \pm 11.08 b	15.0 \pm 1.88	1.3 \pm 0.10
Sr1sr	M1	145.1 \pm 7.82	1.8 \pm 0.23ab	2.2 \pm 0.57 bcd	2.1 \pm 0.59 a	8.7 \pm 0.55 ab	35.5 \pm 5.62 cde	10.6 \pm 2.12	1.5 \pm 0.08
	M2	162.7 \pm 10.42	1.0 \pm 0.46cd	5.2 \pm 1.12 a	0.7 \pm 0.17 c	7.6 \pm 0.13 abc	72.5 \pm 5.86 a	12.6 \pm 3.63	1.4 \pm 0.40
	M3	181.3 \pm 8.54	0.3 \pm 0.19d	5.1 \pm 1.57 a	0.6 \pm 0.22 c	6.1 \pm 0.38 bc	53.22 \pm 4.29 b	15.7 \pm 2.24	1.2 \pm 0.20
Sr2sr	M1	143.1 \pm 5.81	2.0 \pm 0.18ab	1.5 \pm 0.52 cd	1.4 \pm 0.18 abc	8.7 \pm 0.45 ab	22.1 \pm 1.27 efg	5.2 \pm 2.11	1.6 \pm 0.06
	M2	162.6 \pm 8.04	1.3 \pm 0.47cd	3.3 \pm 0.73 abcd	1.4 \pm 0.25 abc	7.2 \pm 0.43 bc	35.5 \pm 1.39 cde	8.9 \pm 1.56	1.2 \pm 0.09
	M3	188.0 \pm 6.26	0.6 \pm 0.41d	2.6 \pm 0.12 bcd	0.9 \pm 0.28 bc	5.2 \pm 0.74 c	43.7 \pm 2.55 bcd	10.2 \pm 0.07	0.7 \pm 0.05
srsr	M1	146.0 \pm 0.00	2.0 \pm 0.03a	1.0 \pm 0.37 d	1.1 \pm 0.44 abc	8.9 \pm 0.19 ab	14.5 \pm 4.96 g	6.2 \pm 0.68	1.6 \pm 0.15
	M2	169.0 \pm 0.00	1.9 \pm 0.04b	1.7 \pm 0.55 bcd	1.3 \pm 0.42 abc	10.2 \pm 0.62 a	18.3 \pm 1.82 fg	6.2 \pm 1.25	1.4 \pm 0.14
	M3	188.0 \pm 0.00	1.7 \pm 0.06c	1.8 \pm 0.55 bcd	0.7 \pm 0.41 c	10.4 \pm 0.36 a	30.4 \pm 6.46 defg	7.1 \pm 0.70	1.2 \pm 0.03
<i>p (genotype x maturity)</i>		-	<0.0001	<0.0001	0.0005	<0.001	0.0002	ns	ns

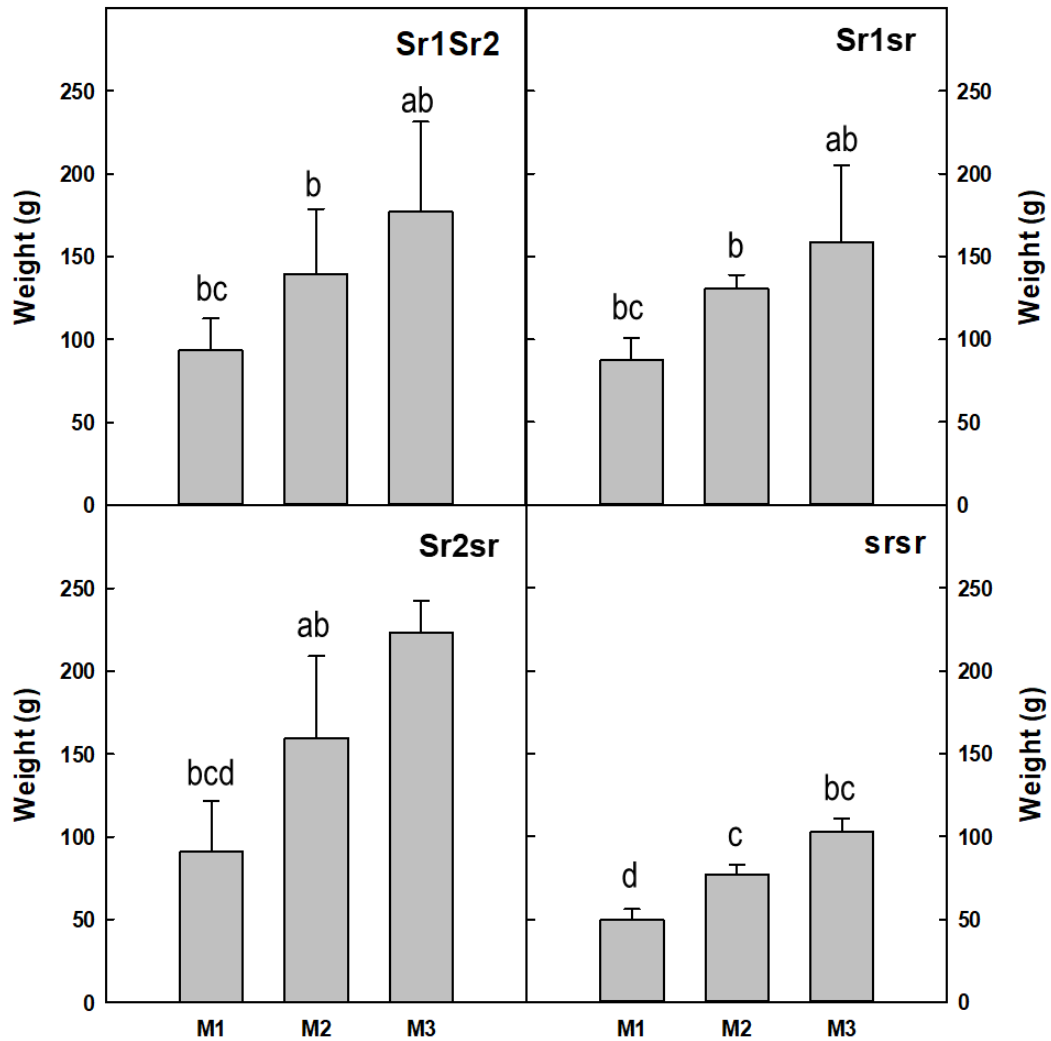
524

525 **Figures**



526

527 **Figure 1.** Visual appearance of peach fruit from the different genetic classes in the
528 progeny from the 'Belbinette x Nectalady' F1 population at the maturity stage 2 (M2).



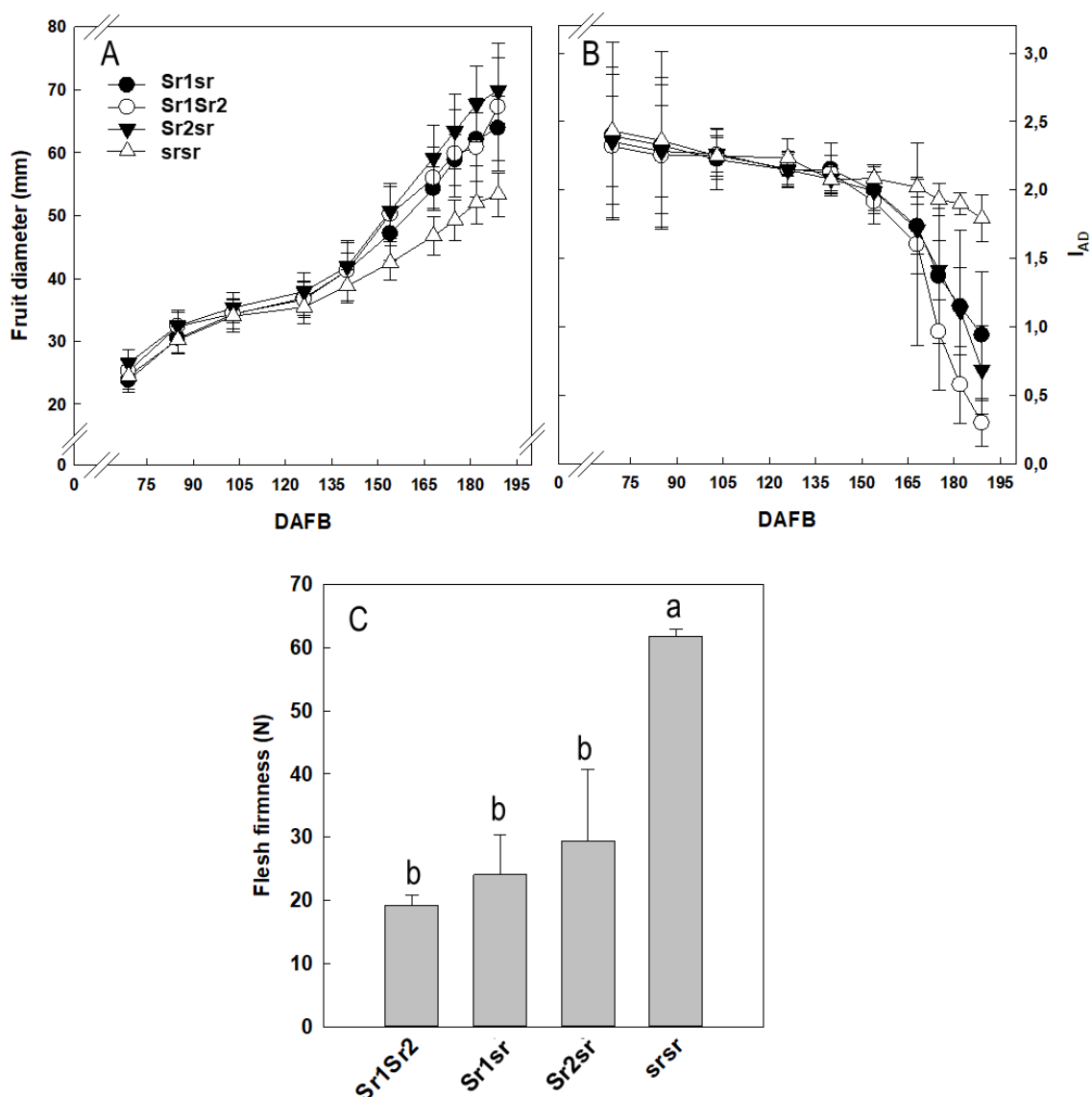
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530 **Figure 2.** Fruit weight (g), at different maturity stages (M1, M2 and M3), of peach fruit
 531 from the different genetic classes in the progeny from the ‘Belbinette x Nectalady’ F1
 532 population. Data shown are means \pm Std. dev. Means with the same letter are not
 533 significantly different according to analysis of variance (ANOVA) and Tukey’s HSD test
 534 ($p < 0.05$).

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539 **Figure 3.** Changes in fruit diameter (mm; A) and I_{AD} values (B) from fruit set to harvest
 540 in the four genetic classes of the progeny from the 'Belbinette x Nectalady' F1 population.

541 Data shown are means \pm Std. dev. LSD values for the interaction genetic class x DAFB

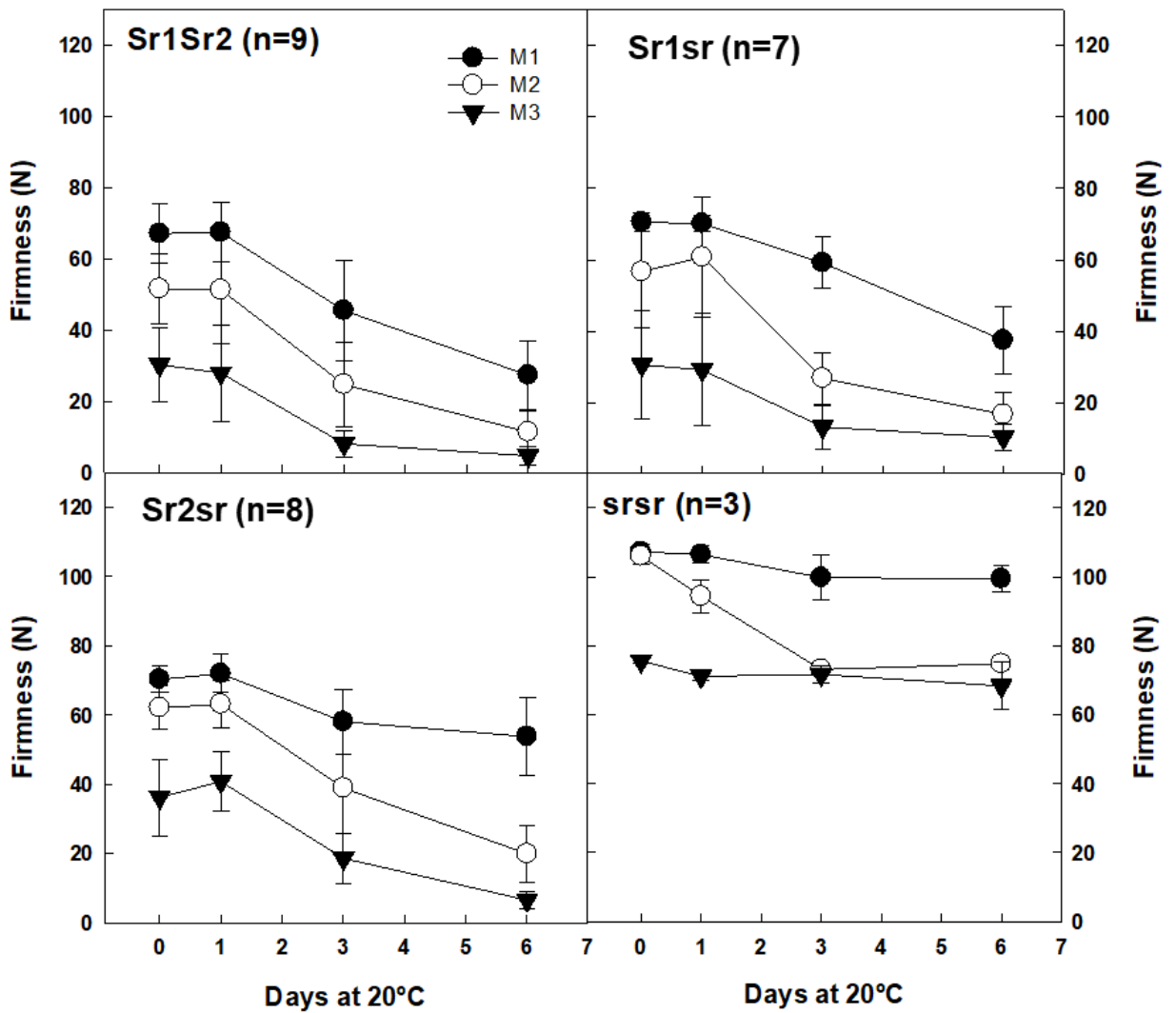
542 are 10.1 and 0.69 for (A) and (B), respectively. (C) Flesh firmness at 190 days after full

543 bloom (DAFB) for the four genetic classes of the progeny from the 'Belbinette x

544 Nectalady' F1 population. Values represent the mean mean \pm Std. dev and means with

545 the same letter are not significantly different according to analysis of variance (ANOVA)

546 and Tukey's HSD test ($p < 0.05$).



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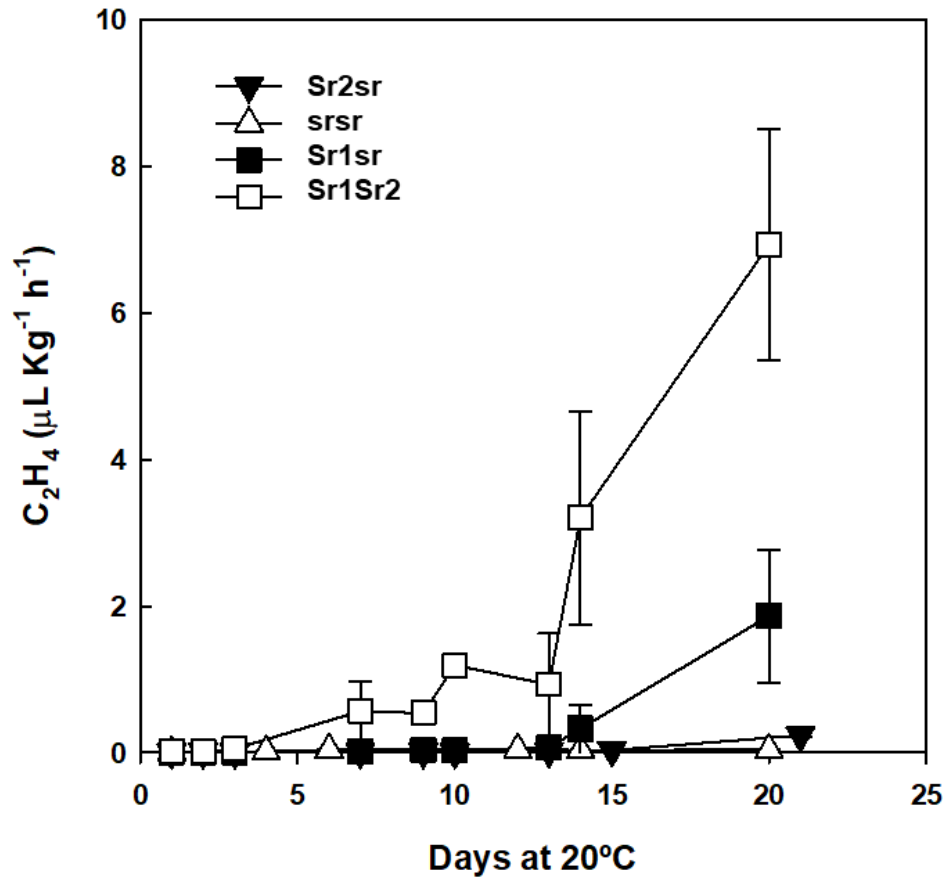
549 **Figure 4.** Changes in flesh firmness of peach fruit from the different genetic classes in
 550 the progeny from the ‘Belbinette x Nectalady’ F1 population, and harvested at different
 551 maturity stages (M1, M2 and M3), during shelf life at 20°C (70% RH). Data represents
 552 the mean ± Std. dev.

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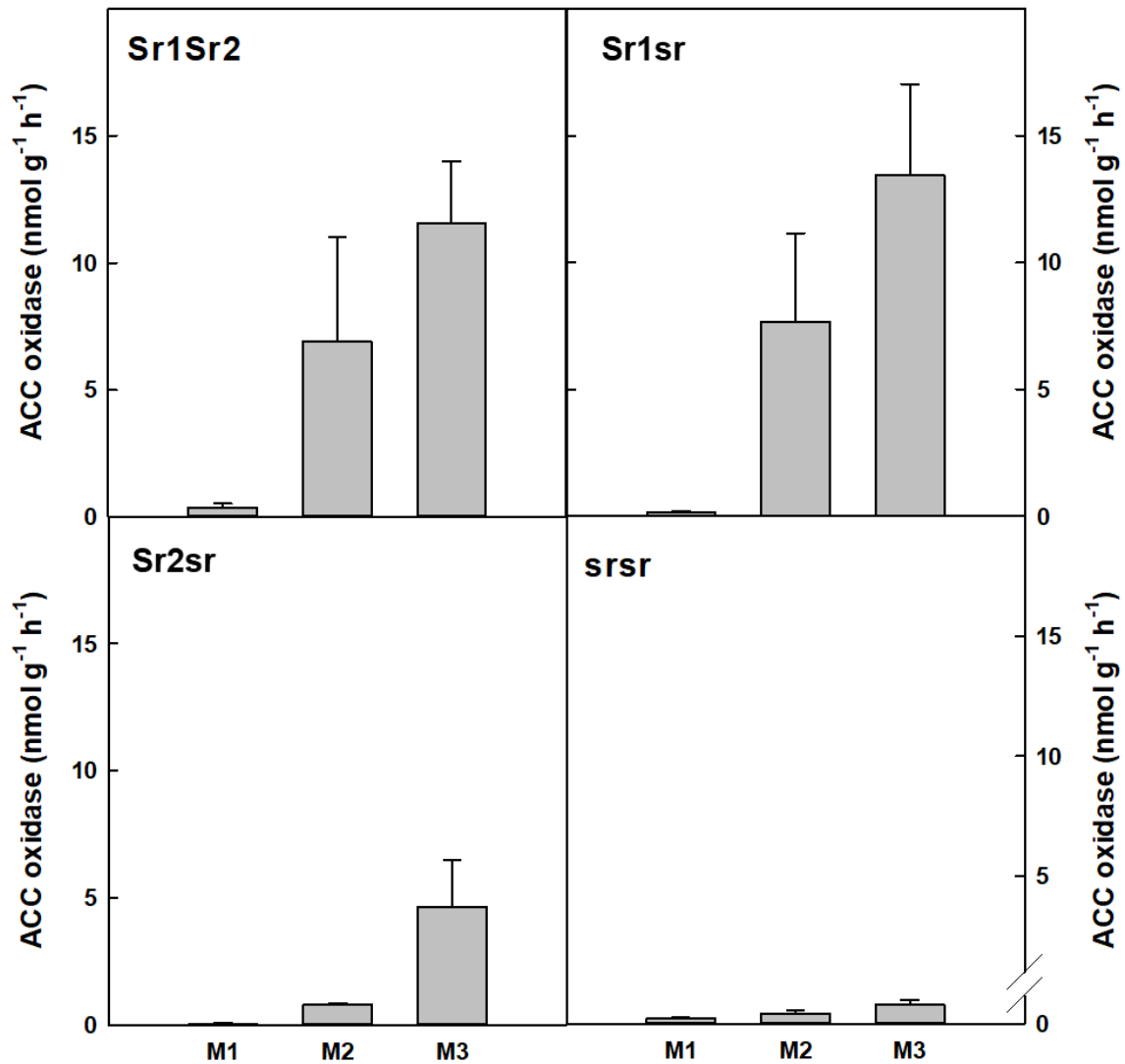


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559 **Figure 5.** Ethylene production of early harvested peach fruit (M1) from the different
 560 genetic classes in the progeny from the ‘Belbinette x Nectalady’ F1 population during
 561 shelf life. Data represents the mean ± Std. dev. LSD value for the interaction genotype x
 562 days at 20°C is 0.87.

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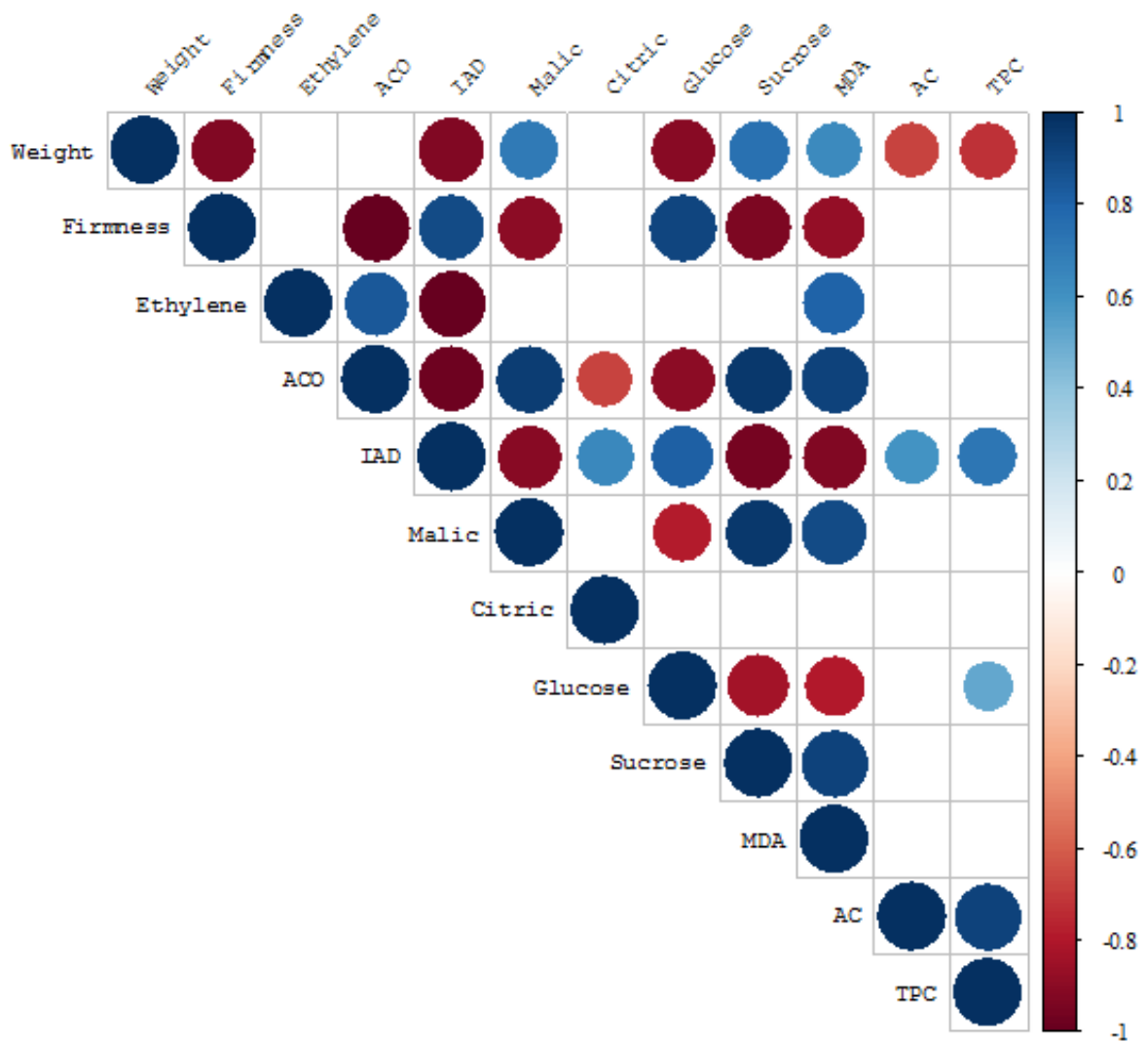


564

565 **Figure 6.** ACC oxidase activity, at different maturity stages (M1, M2 and M3), of peach
 566 fruit from the different genetic classes in the progeny from the ‘Belbinette x Nectalady’
 567 F1 population. Data shown are means \pm Std. dev. Means with the same letter are not
 568 significantly different according to analysis of variance (ANOVA) and Tukey’s HSD test
 569 ($p < 0.05$).

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574 **Figure 7.** Visualization of Spearman's rank correlation matrix (significance level $p < 0.05$)

575 between the different biochemical and quality traits analysed in the progeny from the

576 'Belbinette x Nectalady' F1 population.