



UNIVERSITÀ POLITECNICA DELLE MARCHE
Repository ISTITUZIONALE

Sensorial and nutritional quality of inter and intra—Specific strawberry genotypes selected in resilient conditions

This is the peer reviewed version of the following article:

Original

Sensorial and nutritional quality of inter and intra—Specific strawberry genotypes selected in resilient conditions / Mazzoni, L., Di Vittori, L., Balducci, F., Forbes-Hernandez, T.Y., Giampieri, F., Battino, M., Mezzetti, B., Capocasa, F.. - In: SCIENTIA HORTICULTURAE. - ISSN 0304-4238. - ELETTRONICO. - 261:(2020). [10.1016/j.scienta.2019.108945]

Availability:

This version is available at: 11566/277307 since: 2024-12-06T14:59:32Z

Publisher:

Published

DOI:10.1016/j.scienta.2019.108945

Terms of use:

The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. The use of copyrighted works requires the consent of the rights' holder (author or publisher). Works made available under a Creative Commons license or a Publisher's custom-made license can be used according to the terms and conditions contained therein. See editor's website for further information and terms and conditions.

This item was downloaded from IRIS Università Politecnica delle Marche (<https://iris.univpm.it>). When citing, please refer to the published version.

(Article begins on next page)

1 **SENSORIAL AND NUTRITIONAL QUALITY OF INTER AND INTRA – SPECIFIC**
2 **STRAWBERRY GENOTYPES SELECTED IN RESILIENT CONDITIONS**

3 Luca MAZZONI¹, Lucia DI VITTORI¹, Francesca BALDUCCI¹, Tamara Y. FORBES-
4 HERNÁNDEZ², Francesca GIAMPIERI^{2,3}, Maurizio BATTINO^{2,3}, Bruno MEZZETTI¹, Franco
5 CAPOCASA^{1*}

6 ¹Department of Agricultural, Food and Environmental Sciences, Università Politecnica delle Marche, Via
7 Brece Bianche, 60131 Ancona, Italy. LM: l.mazzoni@staff.univpm.it; LDV: lucia.divittori@gmail.com; FB:
8 francesca.balducci@staff.univpm.it; BM: b.mezzetti@staff.univpm.it; FC: f.capocasa@staff.univpm.it

9 ²Nutrition and Food Science Group, Department of Analytical and Food Chemistry, CITACA, CACTI,
10 University of Vigo - Vigo Campus, Vigo, Spain. TYFH: tforbes@uvigo.es

11 ³Department of Clinical Specialized and Odontostomatological Sciences, Università Politecnica delle Marche,
12 Via Tronto 10/a, 60020, Ancona, Italy. FG: f.giampieri@staff.univpm.it; MB: m.a.battino@staff.univpm.it

13

14 *Correspondence to: Franco Capocasa

15 Department of Agricultural, Food and Environmental Sciences (D3A), Università Politecnica delle Marche,
16 Via Brece Bianche, 60131 Ancona, Italy.

17 Tel: +39-071.220.4640

18 Fax: +39-071.220.4685

19 E-mail: f.capocasa@staff.univpm.it

20

21

22

23

24

25 **Declarations of interest: none**

26 **HIGHLIGHTS**

- 27 • The wild species *Fragaria virginiana glauca* is a source of phytochemicals
- 28 • Back-crossing with *Fragaria x ananassa* increased production and sensorial quality
- 29 • Choosing the right parents is fundamental for improving fruit quality
- 30 • Third back-cross generation showed an overall increase in fruit quality parameters
- 31

32 **ABSTRACT**

33 The strawberry breeding is now aimed to produce new cultivars combining high plant adaptability

34 and yield with high sensorial and nutritional quality of the fruit. The aim of this study is to assess the

35 breeding progress achieved in 3 backcross generations (BC1, BC2, BC3) from F1 *Fragaria x*

36 *ananassa* (Fxa) x *F. virginiana glauca* (FVG) interspecific crosses, in terms of productive parameters

37 (plant commercial yield) and the sensorial quality (Average Fruit Weight, Soluble Solids and

38 Titratable Acidity). Among those genotypes, the most interesting were selected and analyzed for

39 nutritional parameters (Total Antioxidant Capacity, Total Phenolics Content and Total Anthocyanins

40 Content by spectrophotometer; Vitamin C, specific Anthocyanins and specific Phenolic Acids

41 through HPLC). For the sensorial analyses, the BC3 genotypes showed higher Soluble Solids content

42 in respect to BC1 and BC2. Regarding the Titratable Acidity, F1 showed the highest values. Also for

43 the qualitative parameters, BC3 showed a higher value, in particular for the Total Antioxidant

44 Capacity and phenolic acids content. For the Total Phenolics Content and the Total Anthocyanins

45 Content the highest values were registered in F1 and Fxa (aimed to Industry), respectively. Finally,

46 in the case of Vitamin C, the BC1 had fruits rich in this compound. The highest values for

47 anthocyanins content were registered in Fxa (aimed to Industry). The results of the back-crossing

48 program developed at UNIVPM-D3A showed that the BC3 generation possess many improved

49 characters, in respect to Fxa genotype and the F1 generation.

50

51 **Keywords:** back-crossing; HPLC; average fruit weight; polyphenols; vitamin C; plant yield

1. INTRODUCTION

The genetic improvement of fruit since inception, was based on the selection of genotypes that demonstrated better agronomic/commercial and organoleptic attributes compared to their previous generations. Over the years, the market demand has changed in favor of new agricultural systems able to reduce environmental impacts and improve fruit quality and safety. Therefore, breeding programs have now to be oriented towards the release of new cultivars with increased resistance to pests and diseases and fruit sensorial and nutritional quality (Mezzetti et al., 2018). In most species, wild germplasm remains the major source of genes for disease resistance and quality traits. However, breeding programs that use wild germplasm to reach these goals are generally negatively influenced by the difficulties in obtaining the appropriate genetic source and the long-term timeline of the selection (Diamanti et al., 2014).

By focusing on fruit quality, the sensorial traits are the primary guide for consumer acceptance. In the evaluation of the sensorial quality, the consumers combine the aspect of the fruit (color, shape, size) with the taste of the fruit (sweetness, acidity), in order to satisfy their personal definition of the ideal fruit. Also, the flavor is one of the main attributes that the consumer appreciates in berries, and that can influence the consumer's acceptance. Among the volatile organic compounds (VOCs) responsible for the aroma of berries, a subset of about 20 volatile compounds have a high impact on the human nose (Ulrich and Olbricht, 2013). For these reasons, most of the actual cultivar breeding programs include sensory quality as an important breeding objective (Ulrich et al., 1997). As a consequence, the new cultivars need to combine high yield standards to the more appreciable and appealing fruit traits.

The other basic aspect of fruit quality is the nutritional value. In fact, a careful consumer also seeks fruits with high nutritional value, and strawberry are well known for their many health benefits for the consumers (Battino et al., 2009, 2019; Di Vittori et al., 2018; Forbes-Hernandez et al., 2017; Gasparrini et al., 2018), thanks to their high content of bioactive compounds such as vitamins, minerals, polyphenols, especially anthocyanins and phenolic acids (Battino et al., 2019; Gil et al.,

2000; Maatta-Riihinen et al., 2003, 2004; Mazzoni et al., 2016; Rice-Evans et al., 1995; Tian et al., 2017; Wu and Prior, 2005). However, the quantity and quality of bioactive compounds possessed by fruit is closely related to the genotype (Capocasa et al., 2008; Diamanti et al., 2012; Du et al., 2009; Scalzo et al., 2005a; Wu et al., 2006).

The aim of this work was to compare productive, fruit sensorial and nutritional quality of F1 inter-specific hybrid and three back-cross generations with Fxa cultivars and selections, all grown in resilient conditions, and to evaluate the progress of the breeding program in ameliorating nutritional quality, maintaining good productive and sensorial parameters.

86

87 2. MATERIALS AND METHODS

88 2.1. Plant growth, fruits harvest and sampling

Strawberry selections derived from interspecific crossing (F1) of *Fragaria x ananassa* with *Fragaria virginiana glauca*, three subsequent back-crossings (BC1, BC2, BC3) with *Fragaria x ananassa*, advanced selections and cultivars of *Fragaria x ananassa* (Fxa), and selections of *Fragaria x ananassa* adapted to the industrial transformation for their dark red coloration (Fxa Ind) were grown for three production cycles in open field, located at the UNIVPM Didactic-Experimental Farm “P. Rosati” in Agugliano, Italy (43°32'N - 13°22'E) (Table 1).

The field was characterized by not fumigated clay-silty heavy soil, pH 7.8, and was subjected to a short rotation (3-4 years) after a previous strawberry cultivation cycle.

During the harvesting seasons (spring-summer 2014, 2016 and 2017), productive parameters were analysed. Furthermore, 20 full red ripe fruits from the third, fourth and fifth harvest (the main harvesting periods) were sampled from each genotype and frozen at -20°C, then analysed for the sensorial traits.

From the sensorial data and the in-field evaluations, the more interesting genotypes were selected for fruit nutritional quality study (Table 1). Following the same harvesting and sampling procedure, another batch of 20 ripe fruits were frozen at -20°C, then extracted and analysed for nutritional

104 quality. Spectrophotometric parameters were evaluated for fruits harvested in the years 2014, 2016
 105 and 2017, while HPLC analyses were performed only in fruits harvested during the year 2016.
 106

Type	N° genotypes analyzed for productive and sensorial parameters	N° Genotypes analyzed for nutritional analyses
F1	16	16
BC1	3	3
BC2	10	10
BC3	10	10
Fxa	55	8
Fxa (Ind)	4	4

107 **Table 1: Strawberry genotypes analyzed in this study.**

108

109 **2.2. Productive parameters**

110 On the genotypes listed in Table 1, plant yield was measured considering only the fruits with
 111 homogeneous shape, colour, with a diameter bigger than 22 mm, and free of any physical or
 112 pathological damage. Results were expressed as grams/plant.

113 **2.3. Fruit sensorial Analysis**

114 Fruit sensorial quality of strawberry genotypes was analysed taking into account the following
 115 parameters:

- 116 a) Average Fruit Weight (AFW): determined by weighing 20 fruits per genotype at each commercial
 117 harvest, results were expressed as grams/fruit.
- 118 b) Solid Soluble (SS): determined using a hand-held refractometer (ATAGO, Tokio, Japan), results
 119 were expressed as °Brix.
- 120 c) Titratable Acidity (TA): determined from 10 mL of juice diluted with distilled water (1/2 v/v) and
 121 titrated with 0.1N NaOH solution, until pH 8.2, and expressed as mEQ of NaOH per 100g Fresh
 122 Weight (FW).

123 **2.4. Spectrophotometric Nutritional Analysis**

124 a) Extraction method

125 For each sample of whole raw fruit stored at -20°C, ten fruits were selected and cut in two specular
126 slices, then chopped into small pieces, weighed (10g) and placed in a Falcon tube containing methanol
127 (1:4, fruit: methanol, w/v) for initiation of extraction. This methanolic solution was homogenized by
128 Ultraturrax T25 homogenizer (Janke and Kunkel, IKA Labortechnik, Staufen, Germany). The
129 suspension was placed in continuous agitation for 30 minutes, in the dark. The suspension was
130 centrifuged at 4.000 rpm for 10 min (Centrifuge Rotofix32, Hettich Zentrifugen, Tuttlingen,
131 Germany) and the supernatant collected and stored in three amber vials. To complete the extraction,
132 the procedure was repeated as above on the residual pellet. The supernatant was collected and
133 combined with the previous ones in the same amber vials and stored at -20°C.

134 b) Total Antioxidant Capacity (TAC)

135 TAC was evaluated by the ABTS assay, according to a previously validated procedure (Miller et al.,
136 1993). Every sample was analysed three times. The results are expressed as mM of Trolox equivalent
137 per kilogram of fresh weight (mM Trolox eq/Kg fruit).

138 c) Total Phenol Content (TPH)

139 TPH was evaluated by the Folin-Ciocalteu's reagent method (Slinkard and Singleton, 1977). Results
140 were calculated and expressed as mg of gallic acid per kilogram of fresh weight (mg GA/Kg fruit).

141 d) Total Anthocyanin Content (ACY)

142 ACY assay was performed by the pH differential shift method (Giusti and Wrolstad, 2001), using the
143 anthocyanins' characteristic to change intensity of hue depending on pH shifting. The results were
144 expressed as mg of pelargonidin-3-glucoside (the compounds more representative for anthocyanins
145 in strawberry) per kilogram of fresh weight (mg PEL-3-GLU/Kg fruit).

146 **2.5. HPLC Nutritional Analysis**

147 a) Anthocyanins and phenolic acids extraction

148 The extraction method for the HPLC analysis of anthocyanins and phenolic acids is exactly the same
149 as the spectrophotometric analyses. In this case, however, before the HPLC measurement, the samples
150 were filtered with 0.45 µm hydrophobic PTFE (polytetrafluoroethylene) syringe filters.

151 b) Vitamin C extraction

152 For each strawberry genotype, 5 fruits were picked from the bulk samples stored at -20°C, and cut in
153 small pieces; then, 1g was weighed and 4 mL of extracting solution added. The extracting solution
154 consisted of MilliQ water containing 5% meta-phosphoric acid and 1 mM ethylenediaminetetraacetic
155 acid (EDTA). Vitamin C was extracted by sonication of the strawberry-extracting solution
156 suspension, during 5 min, after a previous homogenization using an Ultraturrax T25 homogenizer
157 (Janke & Kunkel, IKA Labortechnik, Germany) at medium-high speed for 2 min. After the ultra-
158 sound assisted extraction, the cell walls and proteins were precipitated by centrifugation at 2500 rpm
159 for 10 min at 4°C, the supernatant was then filtered through a 0.45 µm nylon (NY) filter into 1.8 mL
160 HPLC vials, and stored at -20°C until the analysis through HPLC.

161 c) Anthocyanins analysis

162 The HPLC program was performed as described in Terefe et al. (2013), with some modifications as
163 suggested in Fredericks et al. (2013). The three most abundant anthocyanins in strawberries
164 (pelargonidin-3-glucoside, pelargonidin-3-rutinoside and cyanidin-3-glucoside) were used as
165 standards. The final values were expressed as the sum of the three main anthocyanins, as milligrams
166 per 100 grams of fresh weight (mg/100g FW).

167 d) Phenolic acids analysis

168 Phenolic acids were analyzed according to Fredericks et al. (2013). The identification and
169 quantification of phenolic acids was performed using chlorogenic acid, caffeic acid, ellagic acid and
170 p-coumaric acid as standards for creating a calibration curve. The final values were expressed as the
171 sum of the four main phenolic acids, as milligrams per 100 grams of fresh weight (mg/100g FW).

172 e) Vitamin C analysis

173 Vit C was measured as described by Helsper et al. (2003). Results were expressed as milligrams of
174 vitamin C per 100 grams of fresh weight (mg vit C/100 g FW).

175 **2.6. Statistical analysis**

176 Results for strawberry fruit sensorial and nutritional parameters of all genotypes included in this study
177 were obtained and analyzed in triplicate. However, data are presented as mean $\pm$ standard error (SE)
178 for each crossing type, considering all the years of study together. One-way analysis of variance was
179 used to test the differences among crossing types. Statistically significant differences ($p \leq 0.05$) were
180 determined with SNK test. Statistical processing was carried out using STATISTICA software
181 (Statsoft, Tulsa, OK, USA).

182

183 **3. RESULTS**

184 **3.1. Plant yield and Average Fruit Weight**

185 Regarding Plant yield, Fxa was the crossing type with the statistically higher value, followed by BC3
186 (Figure 1a). The result was expected considering that Fxa group contains the selections and
187 commercial varieties that satisfy the productive standards required by the market. By crossing Fxa
188 with the wild genotype, productivity has been clearly lost. However, through sequential back-
189 crossings with Fxa parental, some productive capacity was recovered moving from BC1 towards BC3
190 (Figure 1a). Similarly, also the higher Average Fruit Weight was registered for the Fxa group,
191 followed by Fxa (Ind) and BC2. The BC3 group was statistically similar to Fxa (Ind) and BC2, while
192 BC1 and F1 revealed the lowest values for Average Fruit Weight (Figure 1b).

193

194

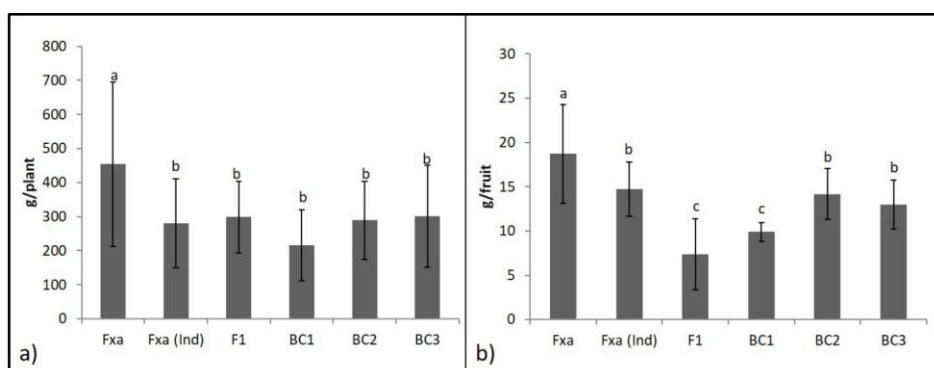
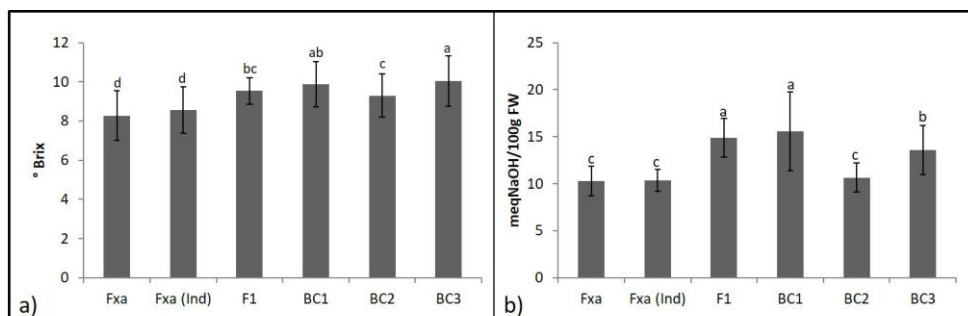


Figure 1: a) Plant Yield and b) Average Fruit Weight (AFW) for different types of crossing. Data are expressed as three-years means $\pm$ standard errors. Different letters indicate significant differences at $p < 0.05$ (SNK test).

3.2. Soluble Solids Content and Titratable Acidity

BC3 selections yielded fruits with the highest average values of fruit Soluble Solids (SS); BC1 showed lower average value, but statistically similar to BC3. This result is evidence on how successive backcrossing generations can promote the stabilization of high amount of fruit SS (Figure 2a) together with other agronomic and sensorial traits, mainly fruit yield and size, as previously showed. Fruit SS mean values in BC3 (10.05°Brix) were higher in comparison to what was detected in Fxa selections and cultivars (Figure 2a). On the contrary, the highest mean values of Titratable Acidity (TA) obtained in BC1 fruits were statistically similar to those obtained in F1 fruit (Figure 2b). There was reduced TA from fruit of subsequent backcrossing generation (BC2), but this increased again in BC3 fruit, it is therefore evident that, the parent used in any backcross generation influences fruit TA of the progeny, even increasing it to higher levels in comparison with TA values detected from Fxa selections and cultivars. In fact, consumers do not accept higher acidity levels but generally prefers balanced SS and TA values (Capocasa et al., 2016).



213 **Figure 2: a) Soluble Solids (SS) and b) Titratable Acidity (TA) for different types of crossing.**
 214 **Data are expressed as three-years means $\pm$ standard errors. Different letters indicate significant**
 215 **differences at $p < 0.05$ (SNK test).**

216

217 3.3. Fruit nutritional spectrophotometric parameters

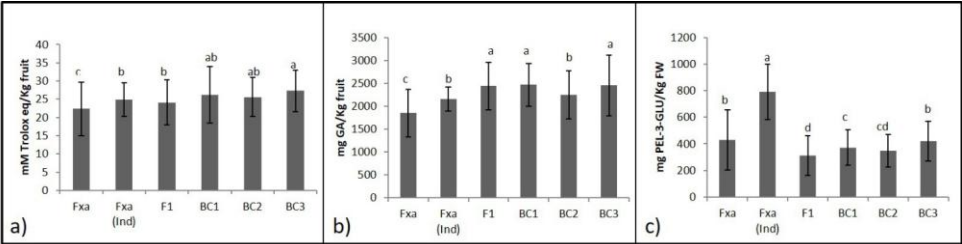
218 Regarding the fruit nutritional parameters, fruit Total Antioxidant Capacity (TAC) detected for BC3
 219 fruit was interesting as it had the highest mean value, even if it was not statistically different from
 220 values obtained from BC1 and BC2 selections. Unexpectedly, F1 fruit TAC mean values were lower
 221 and similar to TAC values obtained in Fxa genotypes currently used by processing industry (Ind),
 222 while the lowest TAC mean value was obtained in Fxa advanced selections and cultivars used for the
 223 fresh market (Figure 3a).

224 A similar behavior was observed in the results obtained on fruit Total Phenolic Content (TPH); BC1
 225 fruits recorded the highest mean value of TPH, statistically similar to values registered in F1, BC3
 226 and Fxa (Ind), all these were higher than values obtained in Fxa genotypes (Figure 3b).

227 The highest mean value of fruit total anthocyanins content was detected from Fxa breeding selections
 228 adopted from the processing industry (Ind) (Figure 3c). These results indicate that the red dark color
 229 requested for strawberry fruit processing and pasteurization is strictly correlated to high ACY
 230 contents. High ACY content positively affects the color stability, turning towards a pleasing violet
 231 rather than towards the unattractive brown color after processing (Diamanti et al., 2016). The mean
 232 values of fruit ACY contents of F1 and 3 backcrossing generations were lower than what obtained in

233 Fxa (Ind) fruit selections, with the lowest values obtained in fruits from F1 population which then
 234 increased in BC1 and BC3 fruit selections; in this last group, the mean value of ACY content were
 235 statistically similar to those of Fxa genotypes.

236



237 **Figure 3: a) Total Antioxidant Capacity (TAC), b) Total Phenolic Content (TPH) and c) Total**
 238 **Anthocyanin Content (ACY) for different types of crossing. Data are expressed as three-years**
 239 **means ± standard errors. Different letters indicate significant differences at p<0.05 (SNK test).**

240

241 3.4. Fruit nutritional HPLC parameters

242 The high ACY content from Fxa (Ind) selections was confirmed by HPLC analyses of total
 243 anthocyanins (sum of Cyanidin-3-O-glucoside, Pelargonidin-3-O-glucoside, Pelargonidin-3-O-
 244 rutinoside, the second one representing about 90% of the strawberry anthocyanins). In fact, fruit of
 245 Fxa (Ind) selections showed almost double the content of anthocyanins in comparison to all the other
 246 crossing types. These strawberry genotypes were specifically selected for the processing industry and
 247 showed dark red color, an absolute index of the abundant presence of anthocyanins. All fruits from
 248 the other cross-typologies presented statistically similar quantities of total anthocyanins and
 249 confirmed the increasing trend of content from F1 fruits towards BC3 populations, with the latter
 250 being higher than Fxa genotypes for the fresh market (Table 2).

251 Data on total Phenolic Acids content of fruit, confirmed a significant wide variability among different
 252 cross-typologies (Table 2). BC3 selections showed the highest content of phenolic acids whereas, F1
 253 generation contained less phenolic acids; this was evidence of the lower capacity of FVG in

254 increasing phenolic acids content in the first-generation fruits (Table 2). While, subsequent back
 255 crossing generations with Fxa parents produced new selections with a statistically increased value of
 256 phenolic acids, in particular fruit of BC3 population. This improvement during the back-crossing
 257 program was probably due to the positive effect of Fxa genotype used as parents, which showed quite
 258 high values of total Phenolic Acids.

259 HPLC analyses on fruit Vitamin C content showed very limited differences and almost always not
 260 statistically different (Table 2). The highest content of Vitamin C was detected in fruit of BC1
 261 population, even if not statistically different from Fxa, F1, and BC3 (Table 2). For this compound, it
 262 is not possible to note a trend linked to the type of crossing combination.

263

Type	Anthocyanins (mg/100g FW)	Phenolic Acids (mg/100g FW)	Vitamin C (mg/100g FW)
Fxa (Ind)	60.37 ± 13.25 ^a	62.9 ± 6.46 ^{bc}	54.49 ± 8.92 ^b
Fxa	33.98 ± 24.90 ^b	67.15 ± 18.47 ^b	66.45 ± 17.23 ^a
F1	32.71 ± 13.61 ^b	49.47 ± 16.74 ^d	62.26 ± 13.72 ^{ab}
BC1	32.86 ± 11.88 ^b	53.36 ± 17.94 ^{cd}	66.92 ± 13.59 ^a
BC2	34.18 ± 7.89 ^b	72.84 ± 14.42 ^b	52.72 ± 7.69 ^b
BC3	35.68 ± 9.45 ^b	84.09 ± 19.76 ^a	60.08 ± 7.69 ^{ab}

264 **Table 2: Anthocyanins, Phenolic Acids and Vitamin C content measured by HPLC for different**
 265 **type of crossing in the year 2016. Data are expressed as means ± standard errors. Different**
 266 **letters indicate significant differences at p<0.05 (SNK test).**

267

268 **4. DISCUSSION**

269 Productive results are in agreement with other studies published by our group, where the mean total
 270 yield among BC1 and BC2 were similar , but lower than the mean values of Fxa genotypes (Diamanti

et al., 2012). The presence of wild germplasm in the crossing types significantly reduced the Average Fruit Weight, but back-crossings with Fxa genotypes seem to be able to restore good levels of Average Fruit Weight from the BC2 generation onwards. AFW results confirmed what we have previously published, with fruits deriving from BC1 and BC2 plants being clearly smaller than fruits obtained from Fxa plants (Diamanti et al., 2012).

The FVG genotype seemed to increase fruit SS and TA, with both the parameters tending to be higher in wild germplasm than in Fxa genotype (Diamanti et al., 2012, 2014; Mezzetti et al., 2016); furthermore, when used in inter-specific breeding programs, the use of proper parents have an effect in reducing fruit TA but keeping higher SS fruit content. In fact, differently from all the other studies previously mentioned, in BC3 we obtained fruits with increased SS mean values combined with **not too** high TA, therefore maintaining an optimal sugar/acid ratio but with an increased sensorial perception for the consumer.

The low TAC mean values detected, in this study, in F1 generation fruit, still possessing 50% of wild FVG germplasm, has been probably determined by the genetic background of the *F. virginiana glauca* accession. Usually, the wild germplasm is believed to possess strong antioxidant activity, higher than the cultivated germplasm (Halvorsen et al, 2002; Scalzo et al., 2005b), but Wang and Lewers (2007) showed a larger variability in fruit TAC values of different wild octoploid *Fragaria* accessions. Therefore, even when starting a long-term interspecific crossing program, it is very important to choose the wild parent with the most appropriate genetic background to improve fruit TAC values. However, also the choice of high-quality recurrent Fxa parents used in the different backcross generations is important, and this aspect can explain the good levels of TAC obtained in the following generations of the inter-specific breeding program. Similar results were obtained in Diamanti et al. (2012), where BC2 and BC1 populations presented statistically higher values of TAC than Fxa selections.

The association between high amount of TPH and high TAC value has already been demonstrated in many studies (Halvorsen et al., 2002; Milivojevic et al., 2011; Proleggente et al., 2002; Scalzo et al.,

Commentato [LC(1): It is either high or low but not 'not too'

2005b). Therefore, the best crossing-types for TAC (BC3 and BC1) also showed the best crossing types for TPH. In Diamanti et al. (2014), BC2 selections presented values clearly higher than BC3 selections, which in turn presented higher values than the Fxa cultivar analyzed. In this study, all the back-crossings presented statistically higher TPH values than Fxa genotypes but, differently from the other studies in literature, BC3 selections mean value was statistically similar to the mean value of F1 and BC1 selections. In Mezzetti et al. (2016), BC1 selections showed higher TPH values than BC2 selections, which in turn showed slightly higher values than Fxa commercial varieties. These differences can be determined by the parents used and clearly also by the climatic conditions that affect the different production cycles.

The fruit content of anthocyanins is important for the contribution to the color of fresh fruit, a valuable quality attribute for the consumer appreciation, and for color stability in processed fruit (Diamanti et al., 2016). In addition, high content of ACY contributes to an increase in antioxidant capacity of the fruits and therefore their nutritional value. This study showed that strawberry genotypes selected for the processing industry are a very important genetic source for increasing the ACY content, overcoming the values of the genotypes for fresh consumption and the selections directly deriving from the wild germplasm (F1).

313

314 5. CONCLUSION

The results of the back-crossing program developed at UNIVPM-D3A showed that the BC3 generation, which are actually the most recent generation of this program, possessed many improved characters in respect to the Fxa genotype and the F1 generation. In particular, SS content was very high in BC3, but this value was counteracted by the high level of acidity. The yield in this group is not far from commercial requirements; with further crossings with an Fxa parent, also the fruit weight could be ameliorated. Regarding the nutritional parameters, BC3 showed the highest TAC values and, consequently, one of the highest values of TPH, demonstrating the efficiency of the breeding program to increase the nutritional value of strawberries in BC3. The ACY level was also very interesting, but

far from the Fxa (Ind). This was expected, given that Fxa (Ind) genotypes were elected for their intense red coloration. Finally, HPLC data confirmed the excellent nutritional value of BC3 generation, presenting good amount of Anthocyanins and the highest amount of Phenolic Acids. Regarding vitamin C, the value was statistically similar to the commercial Fxa genotypes and to the F1 generation (which presented 50% of wild germplasm).

328

329 **Acknowledgments**

Luca Mazzoni and Francesca Balducci were supported by the H2020-SFS-2014-2015 GoodBerry project No. 679303. The authors thank Dr. Cecilia Limera for revising and checking the manuscript.

332

333 **References**

- 334 Battino, M., Beekwilder, J., Denoyes-Rothan, B., Laimer, M., McDougall, G.J., Mezzetti, B., 2009.
335 Bioactive compounds in berries relevant to human health. *Nutr. Rev.* 67 (1), S145–S150. doi:
336 10.1111/j.1753-4887.2009.00178.x.
- 337 Battino, M., Forbes-Hernández, T.Y., Gasparri, M., Afrin, S., Cianciosi, D., Zhang, J.J., Manna,
338 P.P., Reboredo-Rodríguez, P., Varela-Lopez, A., Quiles, J.L., Mezzetti, B., Bompadre, S., Xiao, J.,
339 Giampieri, F., 2019. Relevance of functional foods in the Mediterranean diet: the role of olive oil,
340 berries and honey in the prevention of cancer and cardiovascular diseases. *Crit. Rev. Food Sci. Nutr.*
341 59, 893-920. doi: 10.1080/10408398.2018.1526165.
- 342 Capocasa, F., Balducci, F., Di Vittori, L., Mazzoni, L., Stewart, D., Williams, S., Hargreaves, R.,
343 Bernardini, D., Danesi, L., Zhong, C.F., Mezzetti, B., 2016. Romina and Cristina: Two New
344 Strawberry Cultivars with High Sensorial and Nutritional Values. *Int. J. Fruit Sci.* 16 (1), 207–219.
345 <https://doi.org/10.1080/15538362.2016.1219292>.
- 346 Capocasa, F., Scalzo, J., Mezzetti, B., Battino, M., 2008. Combining quality and antioxidant attributes
347 in the strawberry: the role of the genotype. *Food Chem.* 111, 872-878.
348 <https://doi.org/10.1016/j.foodchem.2008.04.068>.

349 Di Vittori, L., Mazzoni, L., Battino, M., Mezzetti, B., 2018. Pre-harvest factors influencing the quality
 350 of berries. *Sci. Hortic.* 233, 310-322. <https://doi.org/10.1016/j.scienta.2018.01.058>.
 351 Diamanti, J., Balducci, F., Di Vittori, L., Capocasa, F., Berdini, C., Bacchi, A., Giampieri, F., Battino,
 352 M., Mezzetti, B., 2016. Erratum: Corrigendum to “Physico-chemical characteristics of thermally
 353 processed purée from different strawberry genotypes” [*J. Food Compos. Anal.* (2015) 43C:106–118]
 354 (*Journal of Food Composition and Analysis* (****) ** (***_****)). *J. Food Compos. Anal.* 51, 112.
 355 <https://doi.org/10.1016/j.jfca.2016.06.011>.
 356 Diamanti, J., Capocasa, F., Balducci, F., Battino, M., Hancock, J., Mezzetti, B., 2012. Increasing
 357 strawberry fruit sensorial and nutritional quality using wild and cultivated germplasm. *PLoS One* 7,
 358 e46470. <https://doi.org/10.1371/journal.pone.0046470>.
 359 Diamanti, J., Mazzoni, L., Balducci, F., Cappelletti, R., Capocasa, F., Battino, M., Dobson, G.,
 360 Stewart, D., Mezzetti, B., 2014. Use of wild genotypes in breeding program increases strawberry fruit
 361 sensorial and nutritional quality. *J. Agric. Food Chem.* 62 (18), 3944–3953.
 362 <https://doi.org/10.1021/jf500708x>.
 363 Du, G., Li, M., Ma, F., Liang, D., 2009. Antioxidant capacity and the relationship with polyphenol
 364 and Vitamin C in Actinidia fruits. *Food Chem.* 113(2), 557-562.
 365 <https://doi.org/10.1016/j.foodchem.2008.08.025>.
 366 Forbes-Hernández, T.Y., Giampieri, F., Gasparrini, M., Afrin, S., Mazzoni, L., Cordero, M.D.,
 367 Mezzetti, B., Quiles, J.L., Battino, M., 2017. Lipid accumulation in HepG2 cells is attenuated by
 368 strawberry extract through AMPK activation. *Nutrients* 9 (6), 621. doi: 10.3390/nu9060621.
 369 Fredericks, C.H., Fanning, K.J., Gidley, M.J., Netzel, G., Zabaras, D., Herrington, M., Netzel, M.,
 370 2013. High-anthocyanin strawberries through cultivar selection. *J. Sci. Food Agric.* 93 (4), 846– 852.
 371 doi: 10.1002/jsfa.5806.
 372 Gasparrini, M., Giampieri, F., Forbes-Hernández, T.Y., Afrin, S., Cianciosi, D., Reboredo-
 373 Rodriguez, P., Varela-Lopez, A., Zhang, J.J., Quiles, J.L., Mezzetti, B., Bompadre, S., Battino, M.,
 374 2018. Strawberry extracts efficiently counteract inflammatory stress induced by the endotoxin

lipopolysaccharide in Human Dermal Fibroblast. *Food Chem. Toxicol.* 114, 128-140. doi: 10.1016/j.fct.2018.02.038.

Gil, M.I., Tomás-Barberán, F.A., Hess-Pierce, B., Holcroft, D.M., Kader, A.A., 2000. Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *J. Agric. Food Chem.* 48, 4581–4589.

Giusti, M.M., Wrolstad, R.E., 2001. Characterization and measurement of anthocyanins by UV-visible spectroscopy, in: Wrolstad, R.E., Acree, T.E., Decker, E.A., Penner, M.H., Reid, D.S., Schwartz, S.J., Shoemaker, C.F., Smith, D.M., Sporns, P. (Eds), *Current Protocols Food Analytical Chemistry*. John Wiley & Sons, Inc., Hoboken, NJ, F1.2.1-F1.2.13. <https://doi.org/10.1002/0471142913.faf0102s00>.

Halvorsen, B.L., Holte, K., Myhrstad, M.C.W., Barikmo, I., Hvattum, E., Remberg, S.F., Wold, A., Haffner, K., Baugerod, H., Andersen, L.F., Moskaug, O., Jacobs, D.R. Jr., Blomhoff, R., 2002. A systematic screening of total antioxidant in dietary plants. *J. Nutr.* 132 (3), 461–471. DOI: 10.1093/jn/132.3.461.

Helsper, J.P.F.G., de Vos, C.H.R., Maas, F.M., Jonker, H.H., van den Broeck, H.C., Jordi, W., Pot, C.S., Keizer, L.C.P., Schapendonk, A.H.C.M., 2003. Response of selected antioxidants and pigments in tissues of *Rosa hybrida* and *Fuchsia hybrida* to supplemental UV-A exposure. *Physiol. Plant.* 117 (2), 171–178. <https://doi.org/10.1034/j.1399-3054.2003.00037.x>.

Määttä-Riihinen, K.R., Kamal-Elin, A., Torronen, A.R., 2003. High performance liquid chromatography (HPLC) analysis of phenolic compounds in berries with diode array and electrospray ionization mass spectrometric (MS) detection: ribes species. *J. Agric. Food Chem.* 51, 6736-6744. DOI: 10.1021/jf0347517.

Määttä-Riihinen, K.R., Kamal-Eldin, A., Törrönen, A.R., 2004. Identification and quantification of phenolic compounds in berries of *Fragaria* and *Rubus* species (family Rosaceae). *J. Agric. Food Chem.* 52 (20), 6178–6187. <https://doi.org/10.1021/jf049450r>.

400 Mazzoni, L., Perez-Lopez, P., Giampieri, F., Alvarez-Suarez, J.M., Gasparrini, M., Forbes-
 401 Hernandez, T.Y., Quiles, J.L., Mezzetti, B., Battino, M., 2016. The genetic aspects of berries: from
 402 field to health. *J. Sci. Food Agric.* 96 (2), 365–371. doi: 10.1002/jsfa.7216.
 403 Mezzetti, B., Balducci, F., Capocasa, F., Cappelletti, R., Di Vittori, L., Mazzoni, L., Giampieri, F.,
 404 Battino, M., 2016. Can We Breed a Healthier Strawberry and Claim It? *Acta Hort.* 1117, 7-14. DOI:
 405 10.17660/ActaHortic.2016.1117.2.
 406 Mezzetti, B., Giampieri, F., Zhang, Y.T., Zhong, C.F., 2018. Status of strawberry breeding programs
 407 and cultivation systems in Europe and the rest of the world. *J. Berry Res.* 8, 205-221. DOI:
 408 10.3233/JBR-180314.
 409 Milivojević, J.M., Nikolić, M.D., Dragišić Maksimović, J.J., Radivojević, D.D., 2011. Generative
 410 and fruit quality characteristics of primocane fruiting red raspberry cultivars. *Turk. J. Agric. For.* 35,
 411 289-296. doi:10.3906/tar-1001-617.
 412 Miller, N.J., Rice-Evans, C., Davis, M.J., 1993. A novel method for measuring antioxidant capacity
 413 and its application to monitoring the antioxidant status in premature neonates. *Clin. Sci.* 84, 407-412.
 414 Proteggente, A.R., Pannala, A.S., Paganga, G., Van Buren, L., Wagner, E., Wiseman, S., Van De Put,
 415 F., Dacombe, C., Rice-Evans, C.A., 2002. The antioxidant activity of regularly consumed fruit and
 416 vegetables reflects their phenolic and vitamin C composition. *Free Radic. Res.* 36, 217-233.
 417 Rice-Evans, C.A., Miller, N.J., Bolwell, P.G., Bramley, P.M., 1995. The relative antioxidant activities
 418 of plant-derived polyphenolic flavonoids. *Free Radic. Res.* 22, 375–383.
 419 Scalzo, J., Battino, M., Costantini, E., Mezzetti, B., 2005a. Breeding and biotechnology for improving
 420 berry nutritional quality. *BioFactors* 23, 213–220.
 421 Scalzo, J., Politi, A., Mezzetti, B., Battino, M., 2005b. Plant genotype affects total antioxidant
 422 capacity and phenolic contents in fruit. *Nutrition* 21, 207–213. DOI: 10.1016/j.nut.2004.03.025.
 423 Slinkard, K., Singleton, V.L., 1977. Total Phenol analysis: automation and comparison with manual
 424 methods. *Am. J. Enol. Vitic.* 28, 49-55.

425 Terefe, N.S., Kleintschek, T., Gamage, T., Fanning, K.J., Netzel, G., Versteeg, C., Netzel, M., 2013.
 426 Comparative effects of thermal and high pressure processing on phenolic phytochemicals in different
 427 strawberry cultivars. *Innov. Food Sci. Emerg. Technol.* 19, 57-65.
 428 <https://doi.org/10.1016/j.ifset.2013.05.003>.
 429 Tian, Y., Liimatainen, J., Alanne, A.L., Lindstedt, A., Liu, P., Sinkkonen, J., Kallio, H., Yang, B.,
 430 2017. Phenolic compounds extracted by acid aqueous ethanol from berries and leaves of different
 431 berry plants. *Food Chem.* 220, 266-281. doi: 10.1016/j.foodchem.2016.09.145.
 432 Ulrich, D., Hoberg, E., Rapp, A., Kecke, S., 1997. Analysis of strawberry flavour – discrimination of
 433 aroma types by quantification of volatile compounds. *Z. Lebensm. Unters. Forsch.* 205, 218-223.
 434 Ulrich, D., Olbricht, K., 2013. Diversity of volatile patterns in sixteen *Fragaria vesca* L. accessions
 435 in comparison to cultivars of *Fragaria × ananassa*. *J. Appl. Botany Food Chem.* 86, 37–46.
 436 DOI:10.5073/JABFQ.2013.086.006.
 437 Wang, S.Y., Lewers, K.S., 2007. Antioxidant Capacity and Flavonoid Content in Wild Strawberries.
 438 *J. Am. Soc. Hortic. Sci.* 132 (5), 629–637.
 439 Wu, X., Beecher, G., Holden, J.M., Haytowitz, D.B., Gebhardt, S.E., Prior, R.L., 2006. Concentration
 440 of Anthocyanins in Common Foods in the United States and Estimation of Normal Consumption. *J.*
 441 *Agric. Food Chem.* 54, 4069-4075. DOI: 10.1021/jf060300l.
 442 Wu, X., Prior, R.L., 2005. Systematic identification and characterization of anthocyanins by HPLC-
 443 ESI- MS/MS in common foods in the United States: Fruits and berries. *J. Agric. Food Chem.* 53,
 444 2589-2599. DOI: 10.1021/jf048068b.