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

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

Luca MAZZONI[1], Lucia DI VITTORI[1], Francesca BALDUCCI[1], Tamara Y. FORBES-HERNÁNDEZ[2], Francesca GIAMPIERI[2,3], Maurizio BATTINO[2,3], Bruno MEZZETTI[1], Franco CAPOCASA[1*]

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

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

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

*Correspondence to: Franco Capocasa

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

Tel: +39-071.220.4640

Fax: +39-071.220.4685

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

Declarations of interest: none

HIGHLIGHTS

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

ABSTRACT

The strawberry breeding is now aimed to produce new cultivars combining high plant adaptability and yield with high sensorial and nutritional quality of the fruit. The aim of this study is to assess the breeding progress achieved in 3 backcross generations (BC1, BC2, BC3) from F1 *Fragaria x ananassa (Fxa)* x *F. virginiana glauca (FVG)* interspecific crosses, in terms of productive parameters (plant commercial yield) and the sensorial quality (Average Fruit Weight, Soluble Solids and Titratable Acidity). Among those genotypes, the most interesting were selected and analyzed for nutritional parameters (Total Antioxidant Capacity, Total Phenolics Content and Total Anthocyanins Content by spectrophotometer; Vitamin C, specific Anthocyanins and specific Phenolic Acids through HPLC). For the sensorial analyses, the BC3 genotypes showed higher Soluble Solids content in respect to BC1 and BC2. Regarding the Titratable Acidity, F1 showed the highest values. Also for the qualitative parameters, BC3 showed a higher value, in particular for the Total Antioxidant Capacity and phenolic acids content. For the Total Phenolics Content and the Total Anthocyanins Content the highest values were registered in F1 and Fxa (aimed to Industry), respectively. Finally, in the case of Vitamin C, the BC1 had fruits rich in this compound. The highest values for anthocyanins content were registered in Fxa (aimed to Industry). The results of the back-crossing program developed at UNIVPM-D3A showed that the BC3 generation possess many improved characters, in respect to Fxa genotype and the F1 generation.

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.

2. MATERIALS AND METHODS

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

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

Table 1: Strawberry genotypes analyzed in this study.

2.2. Productive parameters

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

2.3. Fruit sensorial Analysis

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

a) Average Fruit Weight (AFW): determined by weighing 20 fruits per genotype at each commercial harvest, results were expressed as grams/fruit.

b) Solid Soluble (SS): determined using a hand-held refractometer (ATAGO, Tokio, Japan), results were expressed as °Brix.

c) Titratable Acidity (TA): determined from 10 mL of juice diluted with distilled water (1/2 v/v) and titrated with 0.1N NaOH solution, until pH 8.2, and expressed as mEQ of NaOH per 100g Fresh Weight (FW).

2.4. Spectrophotometric Nutritional Analysis

a) Extraction method

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

b) Total Antioxidant Capacity (TAC)

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

c) Total Phenol Content (TPH)

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

d) Total Anthocyanin Content (ACY)

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

2.5. HPLC Nutritional Analysis

a) Anthocyanins and phenolic acids extraction

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

b) Vitamin C extraction

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

c) Anthocyanins analysis

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

d) Phenolic acids analysis

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

e) Vitamin C analysis

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

2.6. Statistical analysis

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

3. RESULTS

3.1. Plant yield and Average Fruit Weight

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

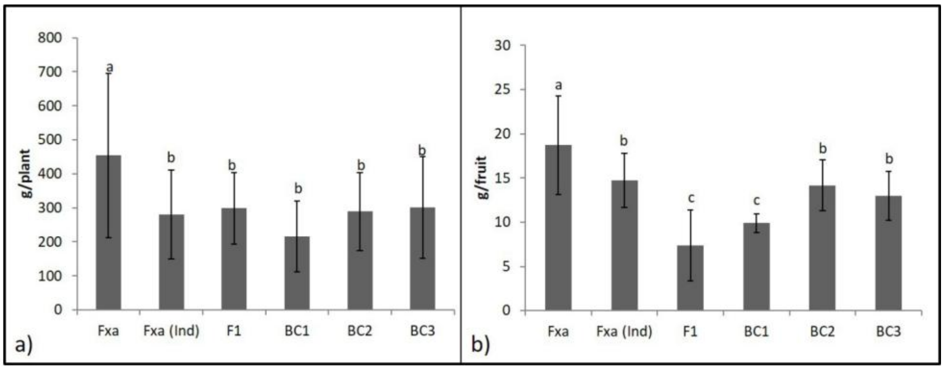

Figure 1: a) Plant Yield and b) Average Fruit Weight (AFW) for different types of crossing. Data are expressed as three-years means ± standard errors. Different letters indicate significant differences at $p<0.05$ (SNK test).

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).

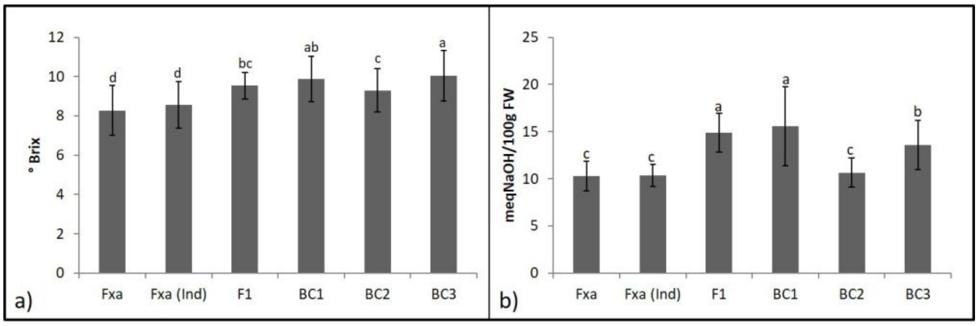

Figure 2: a) Soluble Solids (SS) and b) Titratable Acidity (TA) for different types of crossing. Data are expressed as three-years means ± standard errors. Different letters indicate significant differences at $p<0.05$ (SNK test).

3.3. Fruit nutritional spectrophotometric parameters

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

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

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

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

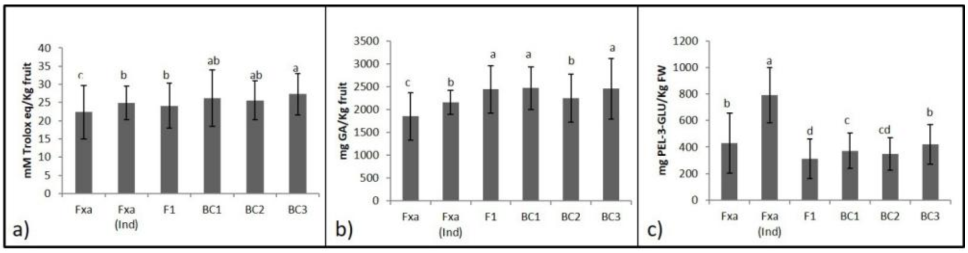

Figure 3: a) Total Antioxidant Capacity (TAC), b) Total Phenolic Content (TPH) and c) Total Anthocyanin Content (ACY) for different types of crossing. Data are expressed as three-years means ± standard errors. Different letters indicate significant differences at $p<0.05$ (SNK test).

3.4. Fruit nutritional HPLC parameters

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

Data on total Phenolic Acids content of fruit ,confirmed a significant wide variability among different cross-typologies (Table 2). BC3 selections showed the highest content of phenolic acids whereas, F1 generation contained less phenolic acids; this was evidence of the lower capacity of FVG in increasing phenolic acids content in the first-generation fruits (Table 2). While, subsequent back crossing generations with Fxa parents produced new selections with a statistically increased value of phenolic acids, in particular fruit of BC3 population. This improvement during the back-crossing program was probably due to the positive effect of Fxa genotype used as parents, which showed quite high values of total Phenolic Acids.

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

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}

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

4. DISCUSSION

Productive results are in agreement with other studies published by our group, where the mean total 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.

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

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; Proteggente et al., 2002; Scalzo et al., 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).

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 F×a 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).

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.

References

Battino, M., Beekwilder, J., Denoyes-Rothan, B., Laimer, M., McDougall, G.J., Mezzetti, B., 2009. Bioactive compounds in berries relevant to human health. Nutr. Rev. 67 (1), S145–S150. doi: 10.1111/j.1753-4887.2009.00178.x.

Battino, M., Forbes-Hernández, T.Y., Gasparrini, M., Afrin, S., Cianciosi, D., Zhang, J.J., Manna, P.P., Reboredo-Rodriguez, P., Varela-Lopez, A., Quiles, J.L., Mezzetti, B., Bompadre, S., Xiao, J., Giampieri, F., 2019. Relevance of functional foods in the Mediterranean diet: the role of olive oil, berries and honey in the prevention of cancer and cardiovascular diseases. Crit. Rev. Food Sci. Nutr. 59, 893-920. doi: 10.1080/10408398.2018.1526165.

Capocasa, F., Balducci, F., Di Vittori, L., Mazzoni, L., Stewart, D., Williams, S., Hargreaves, R., Bernardini, D., Danesi, L., Zhong, C.F., Mezzetti, B., 2016. Romina and Cristina: Two New Strawberry Cultivars with High Sensorial and Nutritional Values. Int. J. Fruit Sci. 16 (1), 207–219. https://doi.org/10.1080/15538362.2016.1219292.

Capocasa, F., Scalzo, J., Mezzetti, B., Battino, M., 2008. Combining quality and antioxidant attributes in the strawberry: the role of the genotype. Food Chem. 111, 872-878. https://doi.org/10.1016/j.foodchem.2008.04.068.

Di Vittori, L., Mazzoni, L., Battino, M., Mezzetti, B., 2018. Pre-harvest factors influencing the quality of berries. Sci. Hortic. 233, 310-322. https://doi.org/10.1016/j.scienta.2018.01.058.

Diamanti, J., Balducci, F., Di Vittori, L., Capocasa, F., Berdini, C., Bacchi, A., Giampieri, F., Battino, M., Mezzetti, B., 2016. Erratum: Corrigendum to “Physico-chemical characteristics of thermally processed purée from different strawberry genotypes” [J. Food Compos. Anal. (2015) 43C:106–118] (Journal of Food Composition and Analysis (****) ** (***–***)). J. Food Compos. Anal. 51, 112. https://doi.org/10.1016/j.jfca.2016.06.011.

Diamanti, J., Capocasa, F., Balducci, F., Battino, M., Hancock, J., Mezzetti, B., 2012. Increasing strawberry fruit sensorial and nutritional quality using wild and cultivated germplasm. PLoS One 7, e46470. https://doi.org/10.1371/journal.pone.0046470.

Diamanti, J., Mazzoni, L., Balducci, F., Cappelletti, R., Capocasa, F., Battino, M., Dobson, G., Stewart, D., Mezzetti, B., 2014. Use of wild genotypes in breeding program increases strawberry fruit sensorial and nutritional quality. J. Agric. Food Chem. 62 (18), 3944–3953. https://doi.org/10.1021/jf500708x.

Du, G., Li, M., Ma, F., Liang, D., 2009. Antioxidant capacity and the relationship with polyphenol and Vitamin C in Actinidia fruits. Food Chem. 113(2), 557-562. https://doi.org/10.1016/j.foodchem.2008.08.025.

Forbes-Hernández, T.Y., Giampieri, F., Gasparrini, M., Afrin, S., Mazzoni, L., Cordero, M.D., Mezzetti, B., Quiles, J.L., Battino, M., 2017. Lipid accumulation in HepG2 cells is attenuated by strawberry extract through AMPK activation. Nutrients 9 (6), 621. doi: 10.3390/nu9060621.

Fredericks, C.H., Fanning, K.J., Gidley, M.J., Netzel, G., Zabaras, D., Herrington, M., Netzel, M., 2013. High-anthocyanin strawberries through cultivar selection. J. Sci. Food Agric. 93 (4), 846– 852. doi: 10.1002/jsfa.5806.

Gasparrini, M., Giampieri, F., Forbes-Hernández, T.Y., Afrin, S., Cianciosi, D., Reboredo-Rodriguez, P., Varela-Lopez, A., Zhang, J.J., Quiles, J.L., Mezzetti, B., Bompadre, S., Battino, M., 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.

Mazzoni, L., Perez-Lopez, P., Giampieri, F., Alvarez-Suarez, J.M., Gasparrini, M., Forbes-Hernandez, T.Y., Quiles, J.L., Mezzetti, B., Battino, M., 2016. The genetic aspects of berries: from field to health. J. Sci. Food Agric. 96 (2), 365–371. doi: 10.1002/jsfa.7216.

Mezzetti, B., Balducci, F., Capocasa, F., Cappelletti, R., Di Vittori, L., Mazzoni, L., Giampieri, F., Battino, M., 2016. Can We Breed a Healthier Strawberry and Claim It? Acta Hort. 1117, 7-14. DOI: 10.17660/ActaHortic.2016.1117.2.

Mezzetti, B., Giampieri, F., Zhang, Y.T., Zhong, C.F., 2018. Status of strawberry breeding programs and cultivation systems in Europe and the rest of the world. J. Berry Res. 8, 205-221. DOI: 10.3233/JBR-180314.

Milivojević, J.M., Nikolić, M.D., Dragišić Maksimović, J.J., Radivojević, D.D., 2011. Generative and fruit quality characteristics of primocane fruiting red raspberry cultivars. Turk. J. Agric. For. 35, 289-296. doi:10.3906/tar-1001-617.

Miller, N.J., Rice-Evans, C., Davis, M.J., 1993. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. Clin. Sci. 84, 407-412.

Proteggente, A.R., Pannala, A.S., Paganga, G., Van Buren, L., Wagner, E., Wiseman, S., Van De Put, F., Dacombe, C., Rice-Evans, C.A., 2002. The antioxidant activity of regularly consumed fruit and vegetables reflects their phenolic and vitamin C composition. Free Radic. Res. 36, 217-233.

Rice-Evans, C.A., Miller, N.J., Bolwell, P.G., Bramley, P.M., 1995. The relative antioxidant activities of plant-derived polyphenolic flavonoids. Free Radic. Res. 22, 375–383.

Scalzo, J., Battino, M., Costantini, E., Mezzetti, B., 2005a. Breeding and biotechnology for improving berry nutritional quality. BioFactors 23, 213–220.

Scalzo, J., Politi, A., Mezzetti, B., Battino, M., 2005b. Plant genotype affects total antioxidant capacity and phenolic contents in fruit. Nutrition 21, 207–213. DOI: 10.1016/j.nut.2004.03.025.

Slinkard, K., Singleton, V.L., 1977. Total Phenol analysis: automation and comparision with manual methods. Am. J. Enol. Vitic. 28, 49-55.

Terefe, N.S., Kleintschek, T., Gamage, T., Fanning, K.J., Netzel, G., Versteeg, C., Netzel, M., 2013. Comparative effects of thermal and high pressure processing on phenolic phytochemicals in different strawberry cultivars. Innov. Food Sci. Emerg. Technol. 19, 57-65. https://doi.org/10.1016/j.ifset.2013.05.003.

Tian, Y., Liimatainen, J., Alanne, A.L., Lindstedt, A., Liu, P., Sinkkonen, J., Kallio, H., Yang, B., 2017. Phenolic compounds extracted by acid aqueous ethanol from berries and leaves of different berry plants. Food Chem. 220, 266-281. doi: 10.1016/j.foodchem.2016.09.145.

Ulrich, D., Hoberg, E., Rapp, A., Kecke, S., 1997. Analysis of strawberry flavour − discrimination of aroma types by quantification of volatile compounds. Z. Lebensm. Unters. Forsch. 205, 218-223.

Ulrich, D., Olbricht, K., 2013. Diversity of volatile patterns in sixteen Fragaria vesca L. accessions in comparison to cultivars of Fragaria × ananassa. J. Appl. Botany Food Chem. 86, 37–46. DOI:10.5073/JABFQ.2013.086.006.

Wang, S.Y., Lewers, K.S., 2007. Antioxidant Capacity and Flavonoid Content in Wild Strawberries. J. Am. Soc. Hortic. Sci. 132 (5), 629–637.

Wu, X., Beecher, G., Holden, J.M., Haytowitz, D.B., Gebhardt, S.E., Prior, R.L., 2006. Concentration of Anthocyanins in Common Foods in the United States and Estimation of Normal Consumption. J. Agric. Food Chem. 54, 4069-4075. DOI: 10.1021/jf060300l.

Wu, X., Prior, R.L., 2005. Systematic identification and characterization of anthocyanins by HPLC-ESI- MS/MS in common foods in the United States: Fruits and berries. J. Agric. Food Chem. 53, 2589-2599. DOI: 10.1021/jf048068b.