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"Improving the sensorial and nutritional quality of strawberry fruits"

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REVIEW SECTION

1 INTRODUCTION

The genetic improvement of fruit, since it started, was based on the selection of genotypes that demonstrated better agronomic/commercial and organoleptic attributes than their previous generations. Since the beginning of intensive agriculture, the main aim of reproduction was to increase plant productivity by modifying morphological characteristics, also inducing major physiological modifications (Moore and Janick, 1983). Breeders have usually investigate some specific characters in genotypes used as parents and their presence or absence in progeny, based on phenotypic expression (Moore and Janick, 1983). The selection of plant material that has or does not have a specific phenotypic trait for a breeding program was an important way to evaluate the phenotypic inheritance of progeny and produce new genotypes showing the best characters. To this end, great importance has been given to the genetic resources available for being tested as commercial cultivars or used as parents in new breeding programs (Lamb, 1974; Scott, 1974; De-jun and Peng, 1979).

Over the years, the market demand has changed in favor of evolution linked to the importance of reducing environmental impacts and the application of new cultivation techniques. This latter factor, together with the genotype and the environmental conditions, is responsible for the quality of the food product. The objectives of old breeding programs, which generated high-impact commercial varieties and populations with great genetic variability that respond to new agronomic and commercial demands, were modified to take into account the requirements for adapting to reduced impacts and new techniques. Furthermore, also qualitative and nutritional value started to be of increasing importance, especially in relation to better production efficiency (Hayward et al., 1993).

Breeding is also an important factor in the selection of genotypes that have resistance to pests and diseases. In most species, wild germplasm remains the major source of genes for disease resistance so as to lower the environmental impact of cultivating fruit crops, reducing the use of chemical products for the defense from these diseases. However, breeding programs that plan to use wild germplasm for reach these goals, are generally negatively influenced by the difficulties in obtaining the appropriate genetic source and the long-term timeline of the selection.

To date, the organoleptic quality of fruit has become one of the main objectives of many breeding programs. The organoleptic quality is the main series of features that guide the

acceptance of the fruit quality from the consumer and generally refers to recognizable attributes through the five senses. In the evaluation of the organoleptic or sensory quality, the consumers combine the aspect of the fruit (color, shape, size) with the taste of the fruit, in order to satisfy their personal definition of the ideal fruit. For this reason, it is important to note how genetic improvement is fundamental in order to create new, more appreciable and appealing varieties for many consumers' tastes. So recent breeding program aimed to find new genotypes as a compromise between productivity and quality, to satisfy the maximum number of consumers.

Nowadays, fruit consumption is extended almost all year and the consumer is increasingly accustomed to having a large differentiation of the product on the market, regardless of the harvest season. For this reason, it is important to have a huge variety of fruit on the market that can better respond to the consumer's demand.

The other basic aspect of fruit quality is the nutritional aspect; the actual consumer is increasingly attracted not only by fruits sensory quality, but also for their nutritional and healthy value. It is well known that fruit consumption is associated with general health benefits due to antioxidant activity of the many phytochemical compounds they contain (Ames et al., 1993; Rice-Evans et al., 1995; Bazzano et al., 2003; Seeram et al., 2006a,b; Boateng et al., 2007; Battino et al., 2009; Giampieri et al., 2012; Mazzoni et al., 2013; Giordani et al., 2016).

Therefore, in addition to sensory quality, fruit should have a higher nutritional quality, which is closely associated with biochemical compounds such as vitamins, minerals, polyphenols, anthocyanins and phenolic acids (Rice-Evans et al., 1995; Gil et al., 2000; Maatta-Riihinen et al., 2003, 2004; Wu and Prior, 2005; Giampieri et al., 2012; Mazzoni et al., 2016; Tian et al., 2017). Bioactive components are generally defined as compounds in foods that provide a health benefit in addition to basic nutrition. The quantity and quality of bioactive compounds possessed by fruit is closely related to the genotype of fruit (Scalzo et al., 2005a; Wu et al., 2006; Du et al., 2009).

To develop a breeding program focused on obtaining new genotypes with better nutritional quality, Welch and Graham (2004) suggest that some criteria for accepting new genotypes in the market should be considered:

- The maintenance or increase of crop productivity (i.e. yield) to ensure the widespread acceptance of farmers;
- The levels of enrichment in bioactive compounds must have a significant impact on human health;
- The characteristics and amounts of bioactive compounds must be relatively stable in various edible environments and climatic zones;

- The bioavailability of bioactive compounds enriched lines must be tested in humans to ensure that their assumption is health-effective within normal household environments.

Therefore, the main objective of the actual breeding programs is to select new strawberry (*Fragaria x ananassa*, Duch.) genotypes that respond to high qualitative parameters (agronomic, organoleptic and nutritional). The availability of new fruits with high sensory quality combined with nutritional quality is a great opportunity for the consumer to choose fruits with good flavors that, at the same time, brings health benefits.

2 FACTORS INFLUENCING THE BERRY QUALITY

2.1 Quality of berries

Among fruits, the so-called “berries” have been consumed since several years, in particular in the northern latitude countries, where there is limited availability of other fruit and vegetable species. The term “berry” or “red fruit” indicates small fruits that grow in wild bushes, could be sweet or bitter, with a juicy pulp and an intense coloration ranging from red to purple/blue, rarely it is possible to have white fruits) (Hidalgo and Almajano, 2017). The most common berries worldwide are cranberry, blackberry, blueberry, raspberry and strawberry, with elderberries, mulberries and other less common red fruits being specific of some particular environments.

During the last years, breeding programs on fruit plants focused their priorities on the obtainment of new berry cultivars adapted to different climatic conditions and cultivation systems, with high yield, fruit size and firmness, resistance to pathogens and transport damages, and longer shelf life. Besides the agronomic performance of the plant, the sensorial traits of the fruit have now an increasing priority in many breeding programs. In fact, consumer quality acceptance is generally related to specific perceived organoleptic traits such as fruit color, shape, acidity and sweetness, combined with flavor and aroma determined by volatile compounds. All these quality traits are controlled by a complex genetic background and frequently associated with negative agronomic characters, such as, for example, less fruit firmness, fruit size or productivity. Long term breeding programs can bring to the release of improved cultivars able to adapt to different climatic conditions and cultivation techniques maintain high quality standards even at long distance commercialization of the fresh product.

Recently, also the nutritional value of berries, intended as the amount of bioactive compounds with healthy effects on the final consumer, is being considered to better characterize the fruit quality. This kind of parameters belong to the so-called nutritional quality and it is of primary importance, together with all the other quality characteristics, in most of the actual berry breeding programs. In fact, the ultimate goal for the breeders is the obtainment of varieties combining agronomic, commercial, organoleptic and nutritional qualities, and that can be grown sustainably under the future climate scenarios projected, e.g. in organic farming or when the pesticides are avoided (Feliziani et al., 2016). The concentration of specific bioactive compounds in berries, responsible for the nutritional quality (such as micronutrients, ellagic acid, vitamin C, phenols and folates) could be increased through the classical genetic approach or marker-assistance and transgenic methods, but the latter is not actually allowed in Europe (Davuluri et al., 2005; Mezzetti, 2013). Micronutrients and phenolic compounds concentration

in berries, as well as commercial and organoleptic attributes, have been reported to change according to many pre-harvest conditions, such as genotype, environment, and cultivation techniques (Cordenunsi et al., 2002; Kafkas et al., 2007).

The purpose of this study is to examine all the major factors determining berry fruit nutritional quality and how are influenced by genotype, environment and cultivation system.

2.1.1 Berry plant yield efficiency

Plant yield is the amount of fruits with commercial value harvested for each production cycles. Usually, this is the first parameter that a grower takes into consideration in the decision to grow a new cultivar. Furthermore, the harvesting facility is another important parameter, which indicates if a cultivar can reduce labor costs, the main important cost for all berries. Clearly of high importance is plant adaptability to different pedo-climatic conditions and resistance to pest and pathogens. These traits are the priority to reduce inputs and increase plant yield efficiency. The traits mentioned above are more suitable for the evaluation of the plant yield efficiency. Among these, fruit size is certainly one of the most important character in the selection of new genotypes. The cultivation environment strongly influences this parameter. The contribution of the parents to the fruit size can be estimated in case of a high number of progenies (Gilbert, 1967). The commercial standard size of berry fruits obviously depends on the species of fruit. The fruit size is evaluated through the implementation of some different parameters:

- Fruit Weight: indicates the average weight of a representative sample of fruits and refers to the dimension of the fruit;
- Fruit Length: indicates the average size on the longitudinal region of a representative sample of fruits;
- Fruit Diameter: indicates the average size around the equatorial region of a representative sample of fruits.

For berries, the increase of fruit weight is important to reduce production costs, in particular labor costs. In strawberries, berry fruit has increased a lot thanks to breeding, so that for some cultivars the primary fruit can reach so high dimension not always appreciated by the consumer because considered as artificial. While, growers are requesting them so to highly reduce harvesting costs. Length and diameter are defining the final shape of the fruit, which is important because defining the appearance of the fruit requested by the consumer.

2.1.2 Berry fruit organoleptic quality

The evaluations of sensorial and organoleptic traits are based on standard evaluation method, which have been well described and well characterized. The fruit shape is strictly related to the fruit species in exams, and it is usually evaluated by descriptors in which the fruits have to be identified. The fruit color is a primary trait, which is fundamental to attract the interest of the consumer. Usually, fruits with a more vivid color are more appreciated by the consumer than pale fruits, but the color of fruit have to be representative of the specific berry species. In fact, some consumers were described as neophobic (Raudenbush et al., 1998), that means they are not confident with foodstuff which not possess typical characteristics, such as evidenced by Jaeger et al. (2002) about the case of kiwifruit with yellow flesh. In larger fruit, the color is evaluated through the analysis of the background color and the red/orange overcolor increasing during ripening by improving the appearance of the fruit. Generally, in berries fruit color shift directly from green to colored (red, orange, blue, purple, depending to the type of berry) during ripening. In strawberry, in particular, the fruit become white before to turn red and some cultivar maintain a white neck. The type of color is determined by the pigments (anthocyanin, phenols, etc,) accumulated in the fruit skin cells. Fruit color is clearly determined by the genotype but also highly influenced by the environmental conditions and maturation stage. Besides the external part of the fruit, also the flesh is valuated for its color, even if this is not a parameter directly assessable by the consumer in the market. The interest in the fruit flesh color, in fact, has only recently increased, giving that the color of the pulp is directly influenced by the amount of colored pigments like anthocyanins.

Other parameters of high interest is the firmness of fruit skin and flesh, which indicates the resistance of the fruit to mechanical damages. This feature is of priority for facilitating the harvest, transportation and post-harvest management. Furthermore, for new consumer trends more firm and crunchy fruit are more appreciated instead of a softer and juicy fruit. The fruit organoleptic quality treated until this moment do not consider all the human senses: if the shape and the color influence the eyesight, the firmness hits the touch and the hearing, then the taste is very involved in the evaluation of fruit quality, through the estimation of sourness, bitterness, astringency but, in berries, particularly sweetness and acidity. Sweetness is particularly appreciated by the consumer, and numerous breeding programs are aiming to increase the sweetness degree of berries. This parameter is usually indicated as Total Soluble Sugars, and the degree of sweetness is expressed as °Brix. Berry fruits contain high content of soluble sugars and their high level of fructose makes them valuable for individuals with diabetes (Nile and Park, 2014). In addition, the acidity of berries is a very important parameter to assess for the

evaluation of the organoleptic quality: the Total Acidity is the most common way to express the acidity of a fruit. Taken together, those characters give the sugar-acid balance of a fruit, which could be measured through the Sugar/Acidity (S/A) Ratio. This parameter describes the relative amount of sugars and acids in the berry fruit, and a balanced ratio represent a fruit that is appreciable by the consumer.

Finally, flavor is one of the main attributes that the consumer appreciates in berries, and that can influence the consumer's acceptance. The volatile organic compounds (VOCs) are the responsible for the aroma of berry fruits, and in literature a great amount of those compounds was identified (more than 300 volatile metabolites are mentioned for the strawberry) (Latrasse, 1991). However, among all these metabolites, a subset of about 20 volatiles are character impact compounds, having a high impact in the human nose, and determining the aroma of the fruits (Ulrich et al., 1997). For these reasons, most of the actual cultivar breeding programs include sensory quality as an important breeding objective (Ulrich and Olbricht, 2013).

2.1.3 Berry fruits nutritional quality

Regarding berries, in addition to the above agronomic and organoleptic traits, in the last few years the high nutritional value of their fruit has been widely studied and requested by consumers. The term nutritional quality, in the strict sense, could be defined as the amount of healthy bioactive compounds present in the fruits matrix (Diamanti et al., 2014). However, in some cases, it was defined as a combination of sensorial (mainly color, sugar, acidity, aroma) and nutritional (antioxidant, vitamins, etc.) attributes. This aspect could easily be explained by the fact that some particular bioactive compounds, such as phenolic compounds, could contribute not only to the health properties, but also to the sensory attributes of berries. For example, ellagitannins are among the more powerful antioxidant compounds, but are also the main contributors to astringency in strawberries and raspberries (Tomás-Barberán and Espín, 2001); phenols also have an important role in reducing the brightness of the fruit color, but are able to increase the color stability in fruit puree to be used for processing industry (Diamanti et al., 2015; Mazzoni et al., 2017).

The interest raised around the nutritional quality of berries is due to the fact that bioactive compounds present in these fruits could protect the human body from the onset of several chronic diseases, such as cardiovascular events, cancer, and other age-related degenerative diseases, as well as for the general health benefits they can provide (Ames et al., 1993; Scalzo et al., 2005a).

Therefore, the nutritional quality of berries is related to their high content of a diverse range of phytochemicals; phenolic molecules represent most of those compounds, but they also contain dietary fibers, vitamins, essential micronutrients. The high dietary fiber content is important because fruit pectin acts as an intestinal regulator (Ramadan et al., 2008). Berries contain a large amount of vitamin A, C, and E, and the B complex vitamins, which acts as antioxidant molecules and help to boost the immune system (Pantelidis et al., 2007).

Berries are also a good source of macro- and micronutrients. The major mineral elements found in berries are phosphorus, potassium, calcium, magnesium, iron, manganese, copper, sodium, and aluminum. These compounds are essential constituents for the human body, because of the important role they exert in development of bones and teeth, reinforcement of muscles and other physiological and biochemical processes.

Regarding phenolic compounds, anthocyanins are a subgroup of colored pigments belonging to the class of flavonoids; they act as powerful antioxidants and are widely distributed in berries, especially those with red, blue or purple pigments. Their anticancer and antiaging activities, together with the positive effects on urinary tract and blood vessels, have been well demonstrated (Moyer et al., 2002; Xue et al., 2002; Manach et al., 2005). The principal anthocyanin found in most fruits is cyanidin-3-glucoside (Paredes-López et al., 2010). Catechin is another major phytochemical belonging to flavonoids found in berries. The most common dietary catechins are catechin, gallocatechin, epicatechin, epigallocatechin, epicatechin 3-gallate, and epigallocatechin 3-gallate (Arts et al., 2000; Cieslik et al., 2006).

Quercetin is another abundant flavonoid in berries, with potent antioxidant activity, together with other important pharmacologic, biologic and medical properties (Xue et al., 2002; Seeram et al., 2003).

Together with flavonoids, the ellagic acid is one of the powerful healthy compounds that is possible to find in different berry species. It represents the 51% of the total phenolic compounds in berries, and it can exist as ellagitannins (esterified with glucose) or in free form (glucoside) (Nile and Park, 2014). Berries may have 3-fold more ellagitanins content than pecans and walnuts and about 15-fold more than other fruits and nuts (Rommel and Wrolstad, 1993; Beekwilder et al., 2005; Paredes-López et al., 2010). The great interest raised around the ellagic acid is due to the antiviral, anticancer, antibacterial and anti-inflammatory effects it demonstrated (Kalt et al., 1999; Bushman et al., 2004; Hannum, 2004). Also gallic acid, a potent antioxidant with anticancer and hepatoprotective effects, was found in good amount in berries (Rice-Evans et al., 1997; Tomás-Barberán and Clifford, 2000).

Stilbenes, a group of small phenolic compounds naturally occurring in a number of plant food sources, are also contained in good amounts in berries. Within this class of compounds, the pterostilbene is the most potent anticarcinogen and antioxidant compound, but also resveratrol and some analogues compounds showed important anti-allergenic, algicidal, antitumoral, anti-inflammatory, antiaging and antimutagenic activities (Wang et al., 2002a; Shakibaei et al., 2009). Caffeic and ferulic acids are the main phenolic acids in berries; in general, they are rarely found free, but esterified with other molecules as organic acids and carbohydrates. The most common esters of hydroxycinnamic acids are chlorogenic acid derivatives (esters of caffeic and quinic acids) (Paredes-López et al., 2010).

Finally, tannins are an important compound present in berry fruits. They can be divided into condensed non-hydrolyzable tannins (proanthocyanidins), and hydrolysable tannins (esters of gallic acid and ellagic acid). Among berries, condensed tannins are the most common (Seeram et al., 2001; Cheynier et al., 2006), while hydrolysable tannins are less frequent (Shahidi and Naczk, 2004; Tamir and Alumot, 2006). Tannins can stabilize anthocyanins by binding to them, but the main characteristic of these compounds is to influence the tart taste and the color of fruits (Puupponen-Pimia et al., 2005a,b).

2.2 Genetic factors influencing berries quality

The huge number of cultivars available on the market for each fruit specie, including berries, are generally differing for specific yield efficiency, organoleptic and nutritional characteristics. Fruit breeding programs are mostly aimed to produce new cultivars with the best compromise between yield and quality parameters. However, it is well assessed that pre-harvest factors such as cultivar, cultivation practices, plant age and environment have a significant effect on attributes associated to sensorial, commercial, and nutritional quality of various fruits, including berries (Giordani et al., 2011, 2016; Alvarez-Suarez et al., 2014a).

The genotype is the main factor that influences quality traits (Table 1), even if it is strictly related to the environmental and agronomic factors.

The fruit soluble solids and titratable acidity contents are strongly controlled by the genotype (Galletta et al., 1995). High sugars and relatively high acid content are generally required for good flavor (Shaw, 1990), and in general the fruit sweetness perception, an important factor in determining consumer's preference, is highly related to their balanced ratio. In *Fragaria vesca*, genotype effect on organoleptic quality was investigated only in very few literature studies.

GENETIC FACTOR	PARAMETER AFFECTED	REFERENCES
Different genotype	Soluble sugars and total acidity	Ruiz-Nieto et al., 1997; Caruso et al., 2004; Crespo et al., 2010; Doumett et al., 2011; Diamanti et al., 2012; Caracciolo et al., 2013
	Sugar/acidity ratio	Doumett et al., 2011
	Vitamins amount	Hakala et al., 2003; Skupien and Oszmianski, 2004; Pantelidis et al., 2007; Doumett et al., 2011
	Macro and micronutrients	Nile and Park, 2014
	Anthocyanin composition	de Ancos et al., 1999; Beekwilder et al., 2005; Pappas and Schaich, 2009; Kruger et al., 2011; Diamanti et al., 2012
	Total phenols	Halvorsen et al., 2002; Pellegrini et al., 2003; Milivojevic et al., 2011, 2013; Diamanti et al., 2012; Mikulic-Petkovsek et al., 2012; Zugic et al., 2014; Giordani et al., 2016;
	Catechin and stilbenes	Wang et al., 2002a; Lyons et al., 2003
	Flavonols	Xue et al., 2002; Seeram et al., 2003; Paredes-Lopez et al., 2010
	Hydroxycynnamic derivatives	Scalzo et al., 2005a
	Condensed tannins	Seeram et al., 2001; Cheynier et al., 2006
	Hydrolysable tannins	Shahidi and Naczk, 2004; Tamir and Alumot, 2006
	Hydroxycinnamate esters	Pappas and Schaich, 2009
	Ellagic acid	Nile and Park, 2014
	Antioxidant capacity	Halvorsen et al., 2002; Connor et al., 2002; Moyer et al., 2002; Scalzo et al., 2005a,b; Jablonska-Rys et al., 2009; Doumett et al., 2011; Diamanti et al., 2012; Giordani et al., 2016
Plant age	Fruit firmness, total phenolics and flavonoids	Wang and Lin, 2000; Siriwoharn et al., 2004; Ferreyra et al., 2007; Shin et al., 2008
	Fruit taste	Krüger et al., 2003, 2011
	Ellagitannins	Beekwilder et al., 2005
	Acidity, soluble sugars	Krüger et al., 2011
	Vitamin C content	Cordenunsi et al., 2002; Ferreyra et al., 2007; Shin et al., 2008
	Antioxidant capacity	Wang and Lin, 2000; Beekwilder et al., 2005; Kruger et al., 2011

Table 1 - main genetic factors influencing the berry fruit quality.

A significant difference was found in soluble sugars and total acidity values in different genotypes of wild strawberry (Caracciolo et al., 2013); moreover, also the ratio between these two parameters was found to be influenced by genotypes, but only in particular environments (Doumett et al., 2011).

The genetic background is the first factor defining the capacity of a fruit to accumulate bioactive compounds. This is true also for berries, where the fruit nutritional value is strongly affected by the genotype of fruit - species and variety within species - (Scalzo et al., 2005a,b), as already described in many studies carried on the evaluation of fruit nutritional parameters (Wang et al., 2002b; Scalzo et al., 2005c; Capocasa et al., 2008). Berries contain a large amount of vitamins A, C, and E, and the B complex vitamins; in particular, honeyberry and blackcurrants showed higher amount of vitamins than raspberries, gooseberries and strawberries (Hakala et al., 2003; Skupien and Oszmianski, 2004). However, strawberry showed very interesting levels of the water-soluble vitamin C together with blackcurrant (Pantelidis et al., 2007), while honeyberry was found to be also a good source of macro- and micronutrients (Nile and Park, 2014). In wild strawberry, statistical differences were found according to different genotype on the concentration of dehydroascorbic acid and total vitamin C (Doumett et al., 2011).

Vaccinium L., *Rubus* L., and *Ribes* L., are the most widely studied genus for the phenolic content, especially anthocyanins (Moyer et al., 2002; Szajdek and Borowska, 2008; Maiani et al., 2009). They act as powerful antioxidants and are widely distributed in berries, especially those with red, blue or purple pigments, such as strawberries, cranberries, raspberries, elderberries, blueberries, blackberries and blackcurrants (Nile and Park, 2014). The main anthocyanin found in most fruits is cyanidin-3-glucoside. Glycosidic derivatives of malvidin are commonly found in red grape, while cyanidin was detected in some berry species, such as elderberry, black chokeberry, and other berries of the genus *Rubus* (Pappas and Schaich, 2009). However, the anthocyanin composition of berries could be very variable according to the selected genotype (Fang, 2015). Also in berries of the same species, it is possible to find differences in anthocyanin composition according to the considered varieties: e.g., the four main raspberry anthocyanins are cyanidin-3-sophoroside (the most abundant), cyanidin-3-glucoside, cyanidin-3-glucosylrutinoside and cyanidin-3-rutinoside (Torre and Barritt, 1977; Mullen et al., 2002). However, the proportion of these four anthocyanins may vary in different cultivars, and cya-3-sophoroside do not results always the predominant ones (de Ancos et al., 1999; Beekwilder et al., 2005; Krüger et al., 2011). Total phenols can also vary according to genotype in different varieties of wild strawberry (Giordani et al., 2016). Catechin is another phenolic compound who is influenced by genotype, being abundant in caneberries. Resveratrol,

pterostilbene, and piceatannol are compounds belonging to the class of stilbenes: they have been found in deerberry, blueberry, cowberry and lingonberry (Wang et al., 2002a; Lyons et al., 2003). The flavonols quercetin and kaempferol are particularly abundant in berries, with cranberry, bilberry and chokeberry being the richest (Paredes-López et al., 2010), but quercetin is abundant also in raspberries in glucuronide form (Xue et al., 2002; Seeram et al., 2003). Blueberry and chokeberry were also characterized by high amounts of hydroxycinnamic derivatives (chlorogenic acid) (Scalzo et al., 2005a) and condensed tannins, while small amounts were detected in blackberries and honeyberries (Seeram et al., 2001; Cheiyner et al., 2006). Hydrolysable tannins are less frequent in berries, and they can be detected in strawberries, raspberries, and blackberries (Shahidi and Naczk, 2004; Tamir and Alumot, 2006). The hydroxycinnamate esters are present in whole cranberry (Pappas and Schaich, 2009). Regarding the ellagic acid, it is possible to find it in many different species, including raspberries, blackberries, strawberries and cranberries (Nile and Park, 2014).

Berries are well known to show high TAC. A huge variability of fruit antioxidant capacity and antioxidant compounds is clearly existing among different fruit species and among different cultivars of the same species. Connor et al. (2002), for example, demonstrated significant variation in TAC among 87 high-bush blueberry cultivars. Recent studies also demonstrate that strawberries, have more antioxidant capacity (from 2- to 11-fold) than, peaches, grapes, oranges, apples, pears, kiwifruit and tomatoes (Scalzo et al., 2005b). This finding was demonstrated also in another study, where analyzing the antioxidant activity of different fruit species, high level of antiradical compounds was found in berries, especially in wild strawberry (*F. vesca*) and cultivated raspberry, strawberry, blackberry, and blueberry (Scalzo et al., 2005a). These results were confirmed also in other works (Halvorsen et al., 2002; Moyer et al., 2002), underlining that in most of these berry species, the wild germplasm antioxidant content is higher with respect to the cultivated species. However, also considering only wild germplasm, it is possible to find a variation in the antioxidant capacity due to the different genotypes, as happened for different wild strawberry varieties (Giordani et al., 2016).

The difference found among cultivated and wild species is an argument of peculiar interest. Wild species have higher level of nutritional attributes when compared whit their respective cultivated species, but at the same time they may have a loss of some other important organoleptic traits (e.g. fruit size and firmness). Thus, wild germplasm has an important role as genetic source for improving fruit nutritional quality (Wang and Lewers, 2007; Diamanti et al., 2014). A clear example of this concept is the behavior of the wild strawberry *F. vesca*, which is increasing interest for its valuable nutritional properties but also for the organoleptic aspect.

In fact, previous studies indicated that *F. vesca* fruits have mean sugar concentrations twice or three times higher than those observed for *Fragaria x ananassa* cultivated strawberries (Ruiz-Nieto et al., 1997; Caruso et al., 2004; Crespo et al., 2010; Doumett et al., 2011). Regarding the nutritional aspect, several studies indicated that the mean concentration of total polyphenols in *F. vesca* is higher than that found for *Fragaria x ananassa* fruits (Pellegrini et al., 2003; Milivojevic et al., 2011, 2013; Mikulic-Petkovsek et al., 2012; Zugic et al., 2014); consequently, similar results were found also for antiradical activity (Jablonska-Rys et al., 2009; Doumett et al., 2011). Again, in strawberries, the importance of another wild species such as *F. virginiana glauca* (FVG) as source of bioactive compounds was demonstrated (Scalzo et al., 2005b; Diamanti et al., 2012). In the latter study, inter-specific back crosses (BC) of this wild strawberry with *F. x ananassa* allows to increase the fruit nutritional and organoleptic, both at the first and at the second level of crossing (BC1 and BC2), but at least two back-cross generations are needed to reach the berry plant yield values requested at the commercial level. In other berry species the introduction of the wild germplasm did not improve the nutritional quality of fruit, as in the case of wild raspberry *R. parvifolius* (Deighton et al., 2003).

There is an increasing awareness that several genetic and environmental aspects affect production and accumulation of bioactive compounds.

Plant age could be also considered another genetic factor that might influence the fruit quality (Table 1). With the aim of study this parameter, fruits of wild strawberry *F. vesca* were collected in different years on the same plants in a study of Giordani et al. (2016); fruits collected on the second year of cultivation showed higher dimension, fresh weight, acidity, sugars and better color, with low pH. Higher sugar concentrations in the second year of cultivation was found also in biennial crop of *Fragaria x ananassa* fruits, as well as happened for citric and malic acids. In addition, fruit yield resulted statistically affected by plant age, being considerably higher in the second year (Conti et al., 2014). However, it was demonstrated that the third crop cycle showed a dramatic decrease in fruit production, underlining the need of replace plants after the second year. The climatic variations registered among the years of study were found to do not seem significant for justifying the qualitative differences among different cycles of production (Moor et al., 2004; Conti et al., 2014).

2.3 Environmental and agronomic factors affecting berries quality

It has been well-assessed that agronomic, organoleptic, and nutritional traits of fruits, comprised berries, could be influenced by several pre-harvest factors (Giordani et al., 2011; Alvarez-Suarez et al., 2014b).

However, an analysis of the different factors affecting the berry quality may be made. If the genetic background could be considered as the first factor defining the quality of a fruit, other factors such as cultivation practices and environmental conditions must be taken into account for their impact on the quality of berry fruits (Wang and Lin, 2000; Kosar et al., 2004; Tulipani et al., 2011).

2.3.1 Environmental factors and berry quality

A real peculiarity of berries is their adaptability to different climatic conditions (

Temperature	**Titratable acidity, Soluble solids, Firmness, TEAC, TPH, ACY, Vitamin C, pH**	**Diamanti et al., 2009; Martinussen et al., 2010; Remberg et al., 2012, 2014; Uleberg et al., 2012, 2016; Woznicki et al., 2015; Zoratti et al., 2015a,b; Karppinen et al., 2016a**

Table 2), from very warm to very cold climates. While for some berries, blueberry in particular, soil characteristics (e.g. high pH) can result as a strong limiting factor.

ENVIRONMENTAL FACTOR	PARAMETER AFFECTED	REFERENCES
Growing location	Dry matter, total acidity, soluble solids content	Kruger et al., 2012
	Sweetness, ascorbic acid content, anthocyanin content	Cocco et al., 2015
Harvest year	Volatile aromatic compounds concentrations	Ulrich and Olbricht, 2014; Ulrich et al., 2014
Light exposition	Flavonoids content	Jaakola et al., 2002, 2004, 2010; Spayd et al., 2002; Downey et al., 2004; Kobayashi et al., 2004; Jeong et al., 2004; Zhou and Singh, 2004; Anttonen et al., 2006; Cortell and Kennedy, 2006; Fujita et al., 2006; Pereira et al., 2006; Walker et al., 2007; Matus et al., 2009; Wang et al., 2009; Niu et al., 2010; Salvatierra et al., 2010; Azuma et al., 2012; Koyama et al., 2012; Uleberg et al., 2012; Kadomura-Ishikawa et al., 2013; Zoratti et al., 2014
Temperature	Titratable acidity, Soluble solids, Firmness, TEAC, TPH, ACY, Vitamin C, pH	Diamanti et al., 2009; Martinussen et al., 2010; Remberg et al., 2012, 2014; Uleberg et al., 2012, 2016; Woznicki et al., 2015; Zoratti et al., 2015a,b; Karppinen et al., 2016a

Table 2 - main environmental factors influencing the berry fruit quality.

Some berry species reveal significant adaptability to different pedoclimatic conditions; in those cases, the quality of their fruits can be closely influenced by their growing location, in particular in open field conditions. It was demonstrated that environmental conditions clearly influence both the yield and the nutritional quality of berries. For example, Krüger et al. (2012) demonstrated that commercial and organoleptic traits like dry matter, total acidity, and soluble solids content were higher in strawberry cultivated in northern Europe than those cultivated in southern Europe. Similarly, a study on strawberries grown in northern and southern Italy showed that the interactions among genotype, climatic factors, cultivation techniques, and site significantly affected several fruit quality traits, such as sweetness, ascorbic acid content, and anthocyanins content, which were higher in southern Italy (Cocco et al., 2015). In addition, the harvest years could be considered an environmental factor, giving that in different years the environmental conditions can change in the same place. For example, in a study of Ulrich and Olbricht (2014), VOC concentration values were evaluated for three strawberry genotypes in three different years. All genotypes follow the same trend, with aroma concentrations decreasing from the first to the third year, when weather conditions were untypical for strawberry growing, being reflected in the volatile patterns (Ulrich et al., 2014).

Finally, another fundamental environmental factor to take into account for the berry fruit quality is the exposition to the light. The exclusion of both climacteric and non- climacteric fruits from sunlight has led in many cases to the suppression of flavonoid pathway genes, with a consequent reduction of flavonoid compounds. Studies on grape berries demonstrated that this fruit reacts to high light exposure by increasing the expression of flavonoid biosynthetic genes in berry skin, leading to elevated content of anthocyanins and flavonols (Jeong et al., 2004; Cortell and Kennedy, 2006; Fujita et al., 2006; Pereira et al., 2006; Matus et al., 2009; Koyama et al., 2012), also regardless of environmental temperature (Spayd et al., 2002; Azuma et al., 2012). Positive effects of light on flavonoid biosynthesis was also reported in many other berries such as cranberry (Zhou and Singh, 2004), raspberry (Wang et al., 2009), Chinese bayberry (Niu et al., 2010), bilberry (Uleberg et al., 2012), and strawberry (Anttonen et al., 2006; Kadomura-Ishikawa et al., 2013). However, not all those fruit species require strong light exposure to accumulate high amounts of flavonoids. Bilberry, for example, is an anthocyanin rich fruit, although prefers shaded growth habitats (Jaakola et al., 2004). Finally, flavonoid biosynthesis seems to be stimulated by light in a cultivar-dependent manner, as demonstrated in grapevines (Downey et al., 2004; Matus et al., 2009), while in some particular white berry cultivars, where the flavonoid pathway is genetically mutated, light do not stimulate anthocyanin production.

These kinds of mutations were observed in strawberry (Salvatierra et al., 2010), bilberry (Jaakola et al., 2002, 2010), grape berries (Kobayashi et al., 2004; Walker et al., 2007), and Chinese bayberry (Niu et al., 2010; Zoratti et al., 2014).

2.3.2 Agronomic factors and berry quality

Cultivation techniques can significantly influence the phytochemical content of berries, as demonstrated in strawberries (Skupien and Oszmianski, 2004; Fan et al., 2011; Giampieri et al., 2012; Josuttis et al., 2012; Mezzetti et al., 2016). The cultivation techniques factor covers many parameters that could be changed to manage the cultivation of a berry species (Table 3).

AGRONOMIC FACTOR	PARAMETER AFFECTED	REFERENCES
Organic farming	Total phenolics, ellagic acid, flavonols, antioxidant capacity, sugar content	Hakkinen and Torronen, 2000; Hakala et al., 2002; Olsson et al., 2006; Hargreaves et al., 2008; Conti et al., 2014; Ochmian et al., 2015
Salinity	Micronutrients and nutraceutical compounds	Keutgen and Pawelzik, 2007; Li et al., 2013
	Sensorial quality and productive parameters.	Hepaksoy et al., 2006; Li et al., 2013
Water management	Phenols content, antioxidant activity, lipid peroxidation, soluble solids, titratable acidity, firmness, plant yield, fruit weight	Terry et al., 2007; Bryla, 2009; Valentinuzzi et al., 2015; Lobos et al., 2016, 2017
Plant nutrition	Fruit weight and diameter, acidity, citric acid, malic acid, antioxidant capacity, total phenols; micronutrients content; anthocyanins, flavan-3-ols, catechin, epicatechin, benzoic acid, fruit firmness, total soluble solids	Rupp and Trankle, 2000; Angeletti et al., 2010; Castellanos-Morales et al., 2010; D'Anna et al., 2012; Pestana et al., 2012; Valentinuzzi et al., 2015; Milosevic et al., 2018
Biostimulants and microbial inoculants	Polyphenols, anthocyanins, ascorbic acid	Singh et al., 2010
Mulching	Ellagic acid	Wang et al., 2002b
	Fruit weight, length and breadth	Nagalakshmi et al., 2002; Mathad and Jholgiker, 2005; Kumar et al. 2012; Kumar and Dey, 2012
	Plant yield	Magee and Spiers, 1996; Singh and Ahmed, 2008; Kher et al., 2010
	Sugar content, total acidity, ascorbic acid content, crude protein amount, anthocyanins content	Magee and Spiers, 1996; Mathad and Jholgiker, 2005; Singh et al., 2007; Kumar and Dey, 2012; Bakshi et al., 2014; Tripathi et al., 2017
Soil management	Phenols, flavonoids, anthocyanins	Wang and Millner, 2009
	Sugars and acids, fructose, glucose, ascorbic acid, flavonoids, antioxidant capacity	Wang et al., 2002b; Akhatou et al., 2013, 2016; Signore et al., 2016; Nin et al., 2017
	Minerals	Hakala et al., 2003
Fruit harvest	Firmness, phenolic content, flavonoids content, ellagitannins content, ascorbic acid content, antioxidant capacity	Wang and Lin, 2000; Cordenunsi et al., 2002; Kosar et al., 2004; Siriwoharn et al., 2004; Beekwilder et al., 2005; Ferreyra et al., 2007; Shin et al., 2008; Kruger et al., 2011; Tulipani et al., 2011
	Titratable acidity, Soluble sugars	Krüger et al., 2011

Table 3 - main agronomic factors influencing the berry fruit quality.

A first comparison can be made between organic and conventional cultivation, and differences between the management methods both for nutritional and sensorial quality have been demonstrated (Weibel et al., 2000; Roussos and Gasparatos, 2009). In particular, Olsson et al. (2006) affirmed that organically grown strawberries possess higher levels of total phenolics, ellagic acid, and flavonols, than conventional strawberries, as also demonstrated in other fruit species by several authors (Carbonaro and Mattera, 2001; Lombardi-Boccia et al., 2004; Young et al., 2005; Del Amor et al., 2008). Contrarily, Hakkinen and Torronen (2000) reported that organic cultivation of strawberry did not lead to any improvement on the antioxidant levels in respect of conventional strawberries. In addition, the use of organic amendments did not improve some fruit quality parameters like sugar content and total antioxidant capacity compared to inorganic fertilizer amendments (Hargreaves et al., 2008).

Generally, stressor factors such as minimum salinity and reduced water can induce a stress response mechanism that promotes the production and accumulation of phytochemicals, in particular phenols. A long-term salt stress influence the content of micronutrient and nutraceutical compounds in strawberry (Keutgen and Pawelzik, 2007). However, it is necessary to be aware to exploit this stress-induced mechanism, because it could also favor the production of antinutritional compounds, such as allergens (Pühringer et al., 2000). Furthermore, some authors showed how the deficit irrigation, as well, influence the phenol content in fruit (Terry et al., 2007) and increase the antioxidant activity in strawberries (Valentinuzzi et al., 2015). Those environmental stressing conditions, among other factors, could be at the base of the impressive anthocyanin and phenolic contents, and antioxidant capacity of wild berries species (Oksman-Caldentey and Inzè, 2004; Trethewey, 2004). Also the type of soil management and the fertilization used can induce similar responses. For example, fruits from plants grown in compost socks showed higher phenolics, flavonoids, and anthocyanins contents than fruits deriving from plants cultivated in a matted row system; moreover, vinegar treatment in culture practice induces an augment of total phenolics and anthocyanins (Wang and Millner, 2009). In another study, the effect of traditional fertilization treatment (TFT) increased fruit fresh weight and diameter in *Fragaria vesca*, while the acidity showed contrasting results (Giordani et al., 2016). Regarding the nutritional quality, Effective Microorganism Technology (EMT) fertilization treatment resulted in higher citric and malic acids, antioxidant capacity and total phenolic content, probably due to the occurrence of a stress-like effect. Changes in citric and malic acids amounts, caused sugar-to-acid ratios generally higher in TFT than in EMT samples (Giordani et al., 2016). Fertilization regime was found to influence also the nutritional quality of cultivated strawberry. Recently, some authors demonstrated that nitrogen application results

in an increase of phenolics concentration in strawberry fruits (Castellanos-Morales et al., 2010). On the contrary, a reduction in iron and phosphorus intake increase the strawberry quality (Valentinuzzi et al., 2015). In particular, iron deficiency was found to imbalance the micronutrient content (Pestana et al., 2012), accumulating zinc and copper in the strawberry fruits, but without influencing the sweetness, acidity and firmness of fruits (Valentinuzzi et al., 2015). However, iron (Fe) deficiency affects strawberry bioactive compounds content, causing an increase of total phenols and anthocyanin content. In particular, fruits produced under Fe deficiency showed a significant increase of pelargonidin-3-glucoside concentration of 40% in respect to the control (Valentinuzzi et al., 2015). The increase of anthocyanins has been often related to a mechanism of response against stress conditions; in fact, anthocyanins are good scavengers of reactive oxygen, and they can be induced by several elicitors, such as ultraviolet and gamma radiation (Cantos et al., 2003; Eichholz et al., 2011), high oxygen pressure treatments (Zheng et al., 2007), high and low temperature (Crifò et al., 2011), chitosan, benzothiadiazole, harpin and 1-methylciclopropane (Liu et al., 2005), and postharvest carbon dioxide (Becatti et al., 2010). Iron deficiency also reduced the amount of flavan-3-ols, increased catechin, epicatechin, and benzoic acids concentrations. Therefore, the increase of anthocyanins, catechins, and benzoic acids led also to the increase of antioxidant capacity of strawberries grown under iron deficiency (Valentinuzzi et al., 2015). Calcium concentration was higher in fruits from phosphorus deficiency. Giving that the calcium content seems to be positively correlated with fruit firmness, it was possible to demonstrate that phosphorus deficiency also ameliorates the fruit firmness. Phosphorus shortage impact negatively on the total soluble solid content, but similarly to iron deficiency, it increased the concentration of bioactive compounds in strawberries (Valentinuzzi et al., 2015).

In the open field cultivation, the adoption of mulching strongly influences the fruit quality and the plant yield, thanks to the better conservation of soil moisture, the change of soil temperature, the better availability of soil nutrients and the protection of plants from weeds, frost injuries, and soil dirt and diseases (Sharma, 2002). Plastic mulches used to increase water use efficiency and weed control were found to impact strawberry quality (Atkinson et al., 2006), and in particular ellagic acid concentration was influenced by the mulch types (Wang et al., 2002b). Black polythene mulch seems to be the best solution for strawberry fruit quality enhancement: plants grown under this kind of mulching were very vigorous with a higher fruit weight, length and breadth in comparison to transparent polythene or no mulching (Kumar et al. 2012; Mathad and Jholgiker, 2005; Nagalakshmi et al., 2002). Black polythene mulch seems to allow the best plant yield in strawberry, thanks to the larger fruits obtained by those plants and to the

environment under mulching completely weed-free, which creates a favorable hydrothermal regime of soil (Kher et al., 2010; Singh and Ahmed, 2008). Some studies also demonstrated that the application of black polythene mulch allows the production of strawberry fruits with improved total sugars content, ascorbic acid content and crude protein amounts (Bakshi et al., 2014; Singh et al., 2007), also in association with Azotobacter bio-fertilization (Tripathi et al., 2017). When comparing to organic mulching, white over black laminated polythene mulched plants were demonstrated to stimulate higher plant yields and fruit weight. Both organic and white over black laminated polythene mulching increased TSS, TSS/acid ratio and development of anthocyanin pigmentation (Mathad and Jholgiker, 2005).

However, the mulching effects in strawberry fruits could interact with the irrigation system, as in the case of Kumar and Dey (2012), where fruit size, weight, sugar content, and anthocyanin content increased significantly under treatment with hay mulch and drip irrigation. Contrarily, total soluble solids and total acidity were highest with rainfed irrigation, without mulching.

In highbush blueberries, a study by Magee and Spiers (1996) compared different types of mulching, and they affirmed that plant growth and yields with pine bark or white-over-black plastic were not different from each other, but higher than black (woven fabric or plastic) mulches. The mulching systems did not significantly affect average fruit weight, soluble solids, titratable acidity, soluble solids/acid ratio, and anthocyanin content, in particular after a refrigerated storage of three weeks.

Akhatou et al. (2013) reported that strawberries grown in conventional crops showed higher quantity of sugars and acids in respect to the soilless conditions. The same group (Akhatou et al., 2016) describe that strawberry cultivars grown in soilless conditions revealed significant alterations of the main metabolites including sugars and organic acids, if cultivated in covered and uncovered tunnels. In addition, different varieties and different cultivation conditions significantly affect also the content of mineral components in strawberries (Hakala et al., 2003). In general, the content of soluble solids, total sugars, fructose, glucose, ascorbic acid, and titratable acidity were increased in strawberries by the hill plasticulture system compared to the matted row cultivation system. The hill plasticulture system also affects the flavonoids content and the antioxidant capacity, with better results compared to matted row system (Wang et al., 2002b).

Some berries, mainly raspberries, strawberries, and blueberries, are grown in protected cultivation, even in soilless conditions. In this case, should be selected the cultivars that perform better in terms of high yield and high-quality fruit in the protected cultivation conditions (Mazzoni et al., 2017). In protected cultivation, mainly in out of season soilless production, the

light quality and intensity is a very important factor (Atkinson et al., 2006; Van Delm et al., 2013).

Clearly, also harvest and storage conditions influence the composition of the fresh fruit. The ripening stage of the fruit at the harvest time is an important factor, because many chemical and compositional modifications occur when the fruit is still attached to the mother plant (Park and Yoon, 2013). If berries are harvested at the fully ripened stage, when anthocyanins are at the maximum concentration and aroma is fully expressed, postharvest decay can occur in case of long term storage of fruits. For counteract this problem, it is possible to harvest strawberries, for example, at white tip or three-quarter color stages, contributing to the maintenance of fruit firmness, total phenolic and flavonoids concentration for a longer storage period (Shin et al., 2008). The same factor is taken into account for raspberries, which are more tasteful at fully ripe stage but are often picked at early ripening stage to prolong their marketability (Krüger et al., 2003, 2011). This behavior is due also to the fact that some phenolic compounds, in contrast to anthocyanins, result to be higher in unripe than in ripe fruits (Wang and Lin, 2000; Kosar et al., 2004; Tulipani et al., 2011). However, the total phenols trend during ripening stages is variable according to the considered berry species: in fact, in black and red raspberries, the total phenolic content increases with the fruit ripening, while in strawberry and blackberry, less ripe fruit have higher (Wang and Lin, 2000; Shin et al., 2008) or similar (Siriwoharn et al., 2004; Ferreyra et al., 2007) contents of total phenolics than fully red fruits. Furthermore, in the *Rubus* and *Fragaria* genus, ellagitannins, that represent the biggest contribution to the antioxidant capacity, resulted up to 50% lower in early ripening red fruits compared to fully ripe red fruits (Beekwilder et al., 2005). The fruit color is genetically determined, and obviously, it changes according to the ripening stage of a fruit.

Other organoleptic parameters were investigated in raspberries, with titratable acidity increasing significantly with enhanced ripening, while the concentrations of soluble sugars remained relatively unaltered (Krüger et al., 2011). Regarding vitamin C amount in raspberries, there are not studies demonstrating its variation in different maturity stages. However, in strawberry it is possible to find some controversial data: Cordenunsi et al. (2002), and Shin et al. (2008) showed an increase of ascorbic acid during fruit development, contrarily to Ferreyra et al. (2007) that did not find any change in ascorbic acid content during fruit ripening.

Similarly, the antioxidant capacity results affected by the ripening time, giving that it depends on the anthocyanins, polyphenols and ascorbic acid content. In raspberries, Beekwilder et al. (2005) showed that ascorbic acid contributed to roughly 20%, anthocyanins to about 25% and the ellagitannins sanguiin H6 and lambertianin C to about 40% and 12% of the total antioxidant

capacity in ripe fruits, and Wang and Lin (2000) showed increased antioxidant capacity for red raspberry from the pink to the red ripe stage, while other studies did not find any TAC variation during fruit ripening (Kruger et al., 2011).

2.4 Future approaches for berry quality

Quality of berries is a complex concept, which could be defined in several different ways, according to the fruit characteristics that we take into account. Surely, fruit quality is mandatory for the success of the product in the market and for the consumer's acceptance. If for several years, the agronomic and organoleptic qualities were the main drivers of the market choice for berries, the importance in the nutritional aspects of food products in strongly increasing, mainly thanks to the increasing awareness of the consumers on the health effects related to the berry consumption. This means that nutritional quality is a parameter required by the consumers, so it must be considered when a new berry product is selected for the market. In addition, the recent breeding programs, aimed to create new fruits for the market, are focusing more and more their attention on the enhancement on nutritional quality in berries, together with all the other quality attributes.

To realize these objectives, breeding programs try to exploit firstly the genetic factors that could influence the nutritional qualities of a berry. The correct integration between genetic resources, genetic improvement and new breeding techniques is essential to take advantage of the influence of the genetic resources (including wild germplasm) for increasing the content of specific bioactive compounds in berries. But the complete profiling of metabolic components of the new genotype and biomedical studies on their health benefits, are requested.

However, the exploitation of only the genetic factors are not sufficient for ensuring a good result in the breeding program. In fact, a great importance in the influence of all the qualitative parameters of a berry fruit is ascribed to the environmental and agronomic factors, which clearly affect the fruit characteristics. Therefore, the availability on the market of a berry fruit possessing the better combination of agronomic, organoleptic or nutritional quality can become possible only if all the genetic, environmental and agronomic factors are taken into account in defining the production process.

For all these reasons, in the future scientists and breeders should focus their research to an interdisciplinary approach able to provide the information required for the obtainment of high qualitative berry fruits.

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3 STRAWBERRY (F. X ANANASSA DUCH.) GENETIC RESOURCES AND BREEDING PROGRAMS

3.1 Strawberry

Strawberry is a fruit belonging to the *Rosaceae* family, genus *Fragaria*, in which many species are included, both cultivated and spontaneous.

The plant is perennial, from 20 to 30 cm tall, characterized by an herbaceous/semi-woody rhizome stem that measures few centimeters, but in some cases, it can reach up to 10 centimeters. From the rhizome generates the radical apparatus, which is very branched and composed of primary and secondary roots in variable number depending on the species and the cultivar. This root anatomy is typical of dicotyledon plants. Primary roots originate from the crown, and then the secondary ones deepen on the ground, depending on the physical characteristics of the soil (30 cm in the case of clay soils, 50 cm in sandy soils).

The leaves fit on long petioles. They are composite, generally trilobate, of oval shape, more or less elongated, toothed and joined to rosette. Depending on the species and nutritional status, the intensity of the green color may vary.

Buds are formed at the base of the leaves; depending on the number of hours of daylight and on temperature values, they may become vegetative (runners or stolons) or productive (inflorescences).

The stolon is a long, slender sprout on the ground. It consists of two internodes and two nodes. The first node usually has a dormant or sterile gem, while the second has a gem ready to generate a new stolon. The formation of stolons is a type of propagation that allows plants to be homogeneous and with the same characteristics as the mother plant. This process of reproduction occurs in the summer during the vegetative phase, following the fruiting process.

Inflorescences are usually made up of a primary, two secondary, four tertiary and eight quaternaries axes ending with a flower. The flowers consist of 5 or more sepals and a corolla formed by 5 or more petals, that are generally white but could be also pinky in some particular genotypes. The androecium is made up of many pollen-bearing anthers. The gynaeceum is placed on the receptacle. Flowers are generally hermaphrodite (perfect flower), whose pollen is self-compatible or compatible with genetically similar flowers (of the same variety); in some species, flowers could be imperfect or unisexual: in those cases, flowers need to be fertilized through cross-pollination. Pollination can take place by passive self-pollination, by anemophilia or entomophilia.

The so-called strawberry fruit is actually a false fruit. It derives from the enlargement of the receptacle of an inflorescence because of fertilization.

The first fruit that matures on the primary axis of the inflorescence presents a larger size with respect to the other fruits that originate from lower-order inflorescences. The characteristics of fruits, including size, shape and color vary greatly depending on the variety, cultivation techniques and environmental conditions. The weight of the fruit, for example, can range from few grams to 160 grams, according to the different cultivars. The size of the fruit can vary from very small to medium, large, and very large. The fruit shape may strongly vary according to the strawberry genotype, being reniform, spheroidal, conical, oblate, cordiform, cuneiform, biconic and ovoid (Figure 1). The obtainment of regular or misshapen fruits is strongly related to the pollination process (Ariza et al., 2006). If the pollination is uniform, the fruit has a regular shape; otherwise, if the pollination is imperfect due, e.g., to unfavorable climatic conditions, a deformed fruit originates.

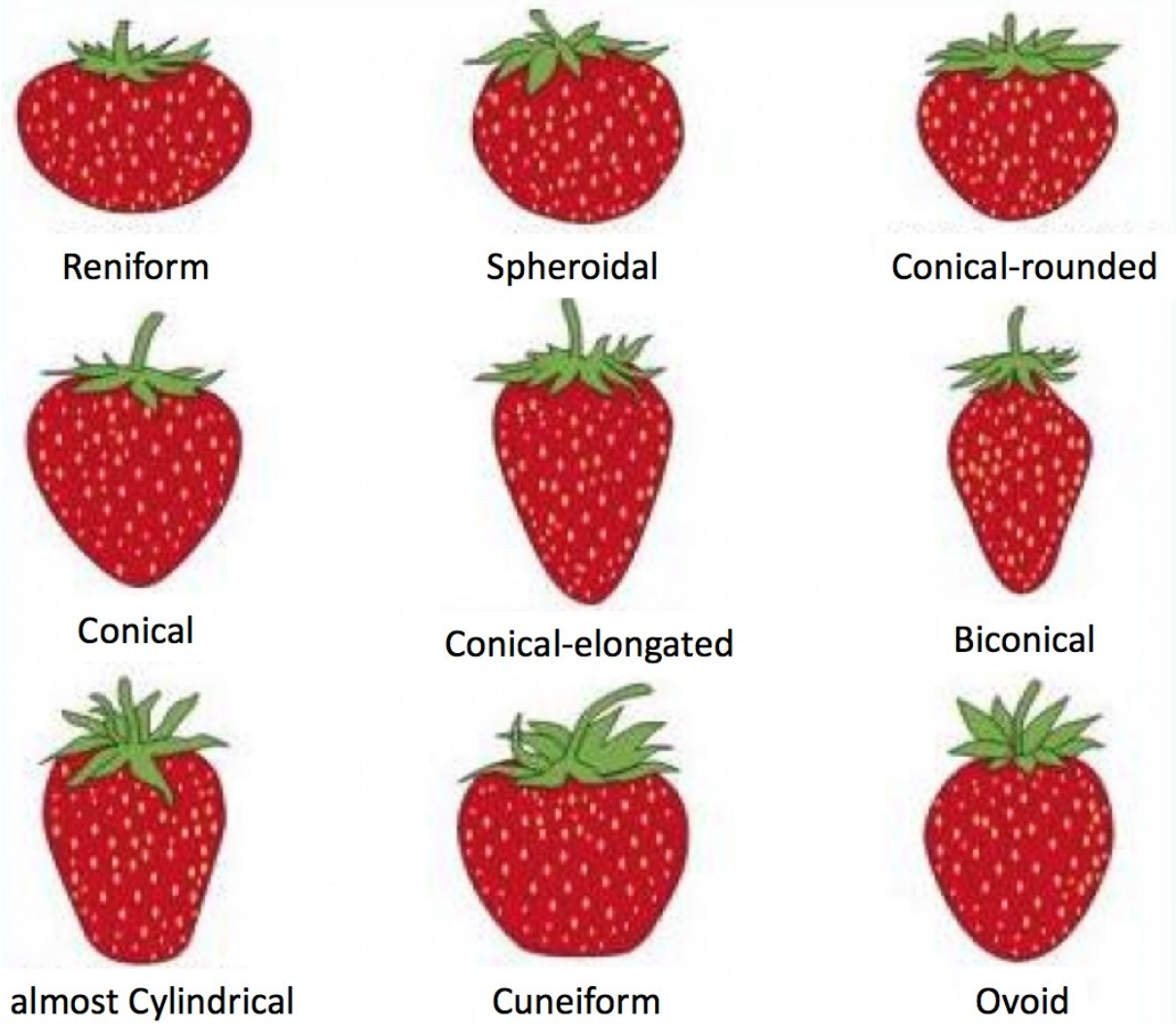

Figure 1 - Different form of strawberry fruits

The color of the fruit can range from light orange to red orange, red or dark red.
On the surface of the edible fruit there is the "real fruit" of the strawberry, called achene. The achene originates from the fertilization of the egg cell present in each pistil. Achene is a dry and variable color fruit (black, green, yellow or red).

3.2 Genetic characteristics

The various species of *Fragaria* greatly differ from the point of view of the genetic heritage: the main difference is the level of ploidy, that is, the number of chromosomes in the nucleus of eukaryotic cells. There are about 24 species with four different levels of ploidy (diploid, tetraploid, esaploid, octoploid) (Staudt 2009).
Diploid species → species characterized by a chromosomal kit represented by the presence in the cell nucleus of an equal number of chromosomes morphologically equal two to two (2n = 2x = 14 chromosomes). Among the diploid species we can locate *F. vesca* and *F. viridis*.
Tetraploid species → these species are characterized by a number of chromosomes at the cellular cell level of 28: 2n = 4x = 28 chromosomes. The tetraploid species are *F. moupinensis*. *F. orientalis* and *F. corymbosa* Losinsk.
Esaploid species → species containing 42 chromosomes at the nuclear level: 2n = 6x = 42 chromosomes. *F. moscata* is the only esaploid species.
Octoploid species → Octoploids are defined as species with 56 chromosomes: 2n = 8x = 56 chromosomes. Octoploid species, *F. ovalis*, *F. iturupensis* Stausd, *F. chiloensis* and *F. virginiana*, are characterized by a large fruit. It is from the hybridization of the last two that they have come to cultivated hybrid *F. x ananassa*.

a. Classification criteria for cultivars

The classification of the numerous strawberry cultivars available takes into account some pomological and behavioral characteristics of the plant. One of the classification criteria is how different cultivars react to the photoperiod and can be classified as follows: Remontant or Recurrent (ever-bearing or long day and day-neutral plants) and non-remontants (seasonal flowering; short day or June-bearing) types.
- Remontant cultivars → produce fruits more times per year, due to their different sensitivity to day length in relation to the temperature for flower induction. Flower initiation is possible when day length is longer than 12 h (long-day cultivars) or irrespective of photoperiod (day-neutral cultivars).

- Non-remontants cultivars → provide only one harvest in spring-summer, as a result of flower induction that took place in the preceding late summer-autumn, when their termophotoperiodic requirements for flower initiation were satisfied by short-days (less than 11-16 h) or low temperatures (9-21 °C, optimal 15-18°C). A minimum number (7-14) of short-day cycles is required for flower induction, according to cultivar, temperature and day length. Under long-day conditions, the terminal apex of the crown remains vegetative and many runners develop from the axillary buds.

3.3 Importance of Germplasm

Germplasm is the genetic material that transmits inherited characters from generation to generation. It is made up of tissues or cells capable of restoring an entire organism and represents the genetic variability available for a specific population of individuals. Plant germplasm is represented by pollen, tissues, parts of plants and seeds.

Germplasm banks are specialized centers for finding and retaining long live parts of plants containing hereditary material sufficient to reconstruct the whole plant if necessary.

There are many reasons why this material is preserved: extinction risk, significant biogeographical-ecological-landscaping meaning, potential interest in renaturalisation actions, such as restoration, recovery, retraining.

The conservation and enhancement of germplasm is an important practice of protecting biodiversity and genetic resources, which are the basis for genetic improvement.

Part of the germplasm that has come to us is the product of a selection and creation of human ecotypes of about 10000 years. The germplasm selected by man is a collection of many local ecotypes that developed in certain environments and become subject of selection and propagation through the anthropic activity.

These germplasm recovery and certification schemes had fallen off around 1900 with the 'green revolution', which generated environmental problems such as the destruction of biodiversity due to the indiscriminate use of chemistry as a fight against pathogens and weeds. Another reason that is actually leading to the extinction of varieties now no longer in trade is the rapid evolution of new commercial varieties, resulting in the perfection of modern genetic improvement. For this reason, the collection and conservation of germplasm of cultivated national varieties and spontaneous species is very important for the conservation of genes that have been, are and will be indispensable to the work of genetic improvement.

3.3.1 Germplasm banks in the world

Germplasm banks are structures that follow international protocols. The germoplasm that is stored must meet the quality requirements; the seeds must be mature and represented by several individuals to ensure the proper genetic variability. Banks are cleaned, counted, dried (15% UR, 15 °C) and finally frozen (-20 °C) to keep the seed's vitality as long as possible. Depending on the species and the environment from which they originate, the seeds have a different life span that can vary from a decade to a century. This variability in the longevity of the seeds determines a constant and repeated test of the samples over time, through germination tests.

Regarding the strawberry germplasm, activity was started in Europe in 1992-1993, with the aim of cataloging old and new strawberry cultivars. This project (Phare and Air Program 92/95), funded by the Genetic Resources Board in Paris and the Cee, was attended by 24 institutions from 16 different countries.

In the first phase, more than 900 old and new cultivars were cataloged and then classified in four groups: old genetically interesting cultivars, old interesting cultivars, recently introduced cultivar, and old cultivars of limited interest.

In the following years, to provide greater coordination and organizational efficiency among European institutions, a database containing information on conserved genotypes was established. In the last few years, an enlargement and a modification of the information gathered initially occurred.

The consolidation of the EU network on the conservation, study and enhancement of germplasm has been carried out through a number of studies, developed over the period 2005-2014, which concerned small fruits:

- Cost action 863 (2005-2010). One of the main activities of the Cost Action study called "A survey of European small fruit Germplasm" was to increase the quality and production of small fruits, with the aim of improving consumer health and maintaining sustainable development at European level. The project, called "From genome to berry fruit", set up a database for all species of small fruits observed ex situ. It is a useful tool for gene banks, breeders and other users in order to ensure germplasm security and to build a collection of small representative fruits at European level.

- a EU GenRes – GENBERRY project: "**Strawberry Genetic Resource**" database was created in Europe between 2009 and 2011. To date it contains 992 accessions represented by 1203 individuals kept by 8 institutes from 7 countries. Among these, there are 2 Italian Institutes: CRA-Research Unit for Fruitculture, and Department D3A of Università Politecnica delle Marche (http://www.bordeaux.inra.fr/eustrawberrydb/).

- FP7 EUBerry was created in Europe between 2011 and 2014 and it was focused to the sustainable improvement of European berry production, quality and nutritional value in a changing environment: Strawberries, Currants, Blackberries, Blueberries and Raspberries. In part this project has continued the characterization work of strawberry germplasm and other berries.

3.4 Genetic Improvement

The genetic improvement is a tool that allows to modify the genetic heritage of a plant, in order to improve some specific target characteristics.

The main objectives of genetic improvement are:

- Increase plant productivity and improve fruit quality (commercial, organoleptic and nutritional);
- Ameliorate the plant adaptability to specific environmental conditions (resistance to biotic and abiotic stress).

One of the main goals of genetic improvement is the increase in production yields, as agricultural products that represent basic food for the world's population have difficulty for meeting market demand. Exponential growth of the world's population, which is most present in developing countries, has led to an increase in food requirements far above agricultural production. Moreover, as plant production is closely linked to the environment where crops are grown, is fundamental to create more efficient cultivars for specific environments.

Plants are frequently subjected to external stressing conditions that negatively affect growth, development, or productivity. The application of genetic improvement could lead to the development of stress tolerant crops that maintain high plant productivity and provide substantial economic benefits.

In recent times, increasing importance is given to the fruit nutritional value for those plant species whose product is destined for fresh consumption but also for the fruit processing industry. These goals can be achieved mainly based on the knowledge of genetic control of genes determining the fruit quality traits and a thorough evaluation, selection and characterization of the new genetic variability obtained with the various available techniques (traditional and new breeding technologies).

3.4.1 Strawberry breeding programs

Thanks to the intense activity of genetic improvement, in recent years there has been a significant increase in the release and commercial diffusion of strawberry cultivars. Strawberry breeding programs could be realized on different methods. The use of a method rather than another varies according to the availability of appropriate genetic resources to the goals to achieve.

- Self-pollination (or self-intersection): it is mainly used to test the heterozygosis level of a particular parent to investigate the genetic basis of certain specific characters. This method is poorly used due to inbreeding depression.

The controlled crossing technique involves the removal of pollen from the male parent to fertilize the mother's flower ovule. When this is done within the same species, it is intraspecific crossing, whereas if it occurs between different species, we refer to hybridization (or intersection inter-specific, or intergenerational, when it occurs between different genera).

- Intra-specific crossing: consists of the use of pollen from a cultivar to fertilize the ovary of another genotype belonging to the same species; this can only occur if there is compatibility between two genotypes. This method is widely used as it utilizes combinatorial capacity that governs the transmission of quantitative characters and non-additive variance. In order to be more secure in the final result, clones of which the genealogy and the genotype are known are used. This method does not create "inbreeding depression", unless closely related parents are chosen.

If parents are utilized in successive generations of intersection, it is called a recurrent selection scheme.

- Hybridization (inter-specific crossing). It is done to increase the level of genetic variability, in addition to that naturally present within a given species. In this crossing method, plants from different species are used for the genetic improvement, in particular when the characters of interest are not present in *F. x ananassa*. Hence, other species (usually wild species) are used in combination with the same genus, or other genus as long as they are inter-compatible from the point of view of floral biology. The main characters available from wild octoploid species, often used for genetic improvement programs are: resistance to various pathogens, the dimension of the fruits (*F. chiloensis*), the resistance of the flowers to the low temperatures (*F. virginiana*), as well as the high nutritional characteristics of the fruits (Diamanti et al., 2012a). This thesis is supported by a study carried out by Wang and Lewers (2007) that showed that the fruit of F. virginiana accessions have significantly higher Total Antioxidant Capacity, Total

Phenols Content and Total Anthocyanins Content than the fruit from different lines of *F. chiloensis* and *F. x ananassa*.

In those hybridization programs there is a considerable increase in the variability of a certain species, but at the same time this method generates many drawbacks such as increased selection times, as well as possible infertility of hybrids that prevents the continuation of crossings.

The elongation of the selection times is due to the fact that often, with the transfer of positive traits, are also transmitted deleterious traits as low fertility, low consistency of the pulp, and smaller size of the fruit, low yield. For this reason, numerous recurrent selections have taken place before reaching an improved material for the characters of interest. In addition, large populations of seedlings are needed to understand the segregation of the many characters involved in an interspecific program.

- Inter-generic hybridization. This method involves many difficulties, caused by reproductive (pre-zygotic and post-zygotic) barriers that often leads to crossing failures. Hybrids that originate, if they survive, are often sterile. The main reason why research has been directed towards inter-generic hybridization has to be found in the wide range of interesting characters that can be used in genetic improvement, especially for resistance to various pathogens.

Both inter-specific and inter-generic have to be followed by a backcrossing program aimed to recover the commercial traits lost in F1 to be combined with the important new trait introduced by the wild parent (inter specific or generic). This program can be really difficult and long term depending to genetic distance of the parent used.

The number of generations and time needed for producing new genetic material having agronomic and quality standards corresponding to what requested at commercial level depends on how far from these standards are the genetic characteristics of the parents used in the cross. Therefore, an intra-cultivar cross (CCVR) can produce new genetic materials already at high commercial standards but maintaining a narrow genetic variability. While, the genetic distance and variability increases when using at least one parent that is from local germplasm, another specie (inter-specific) or when possible another genera. This as described in the following sequence:

	Increasing N. Generation Time	**Decreasing** Genetic diversity
INTRA CCVR INTRA old germplasm INTER SPECIFIC INTER GENERIC		

The backcrossing program depends to the flower biology of the species. In case of self-compatible species is generally used the standard backcrossing program. In this case commercial parent used for generating the F1 (the first generation from intersection) is used to backcrossing the top selections identified in F1 population and also in the new following generation. Generally, this scheme is used when a specific trait has to be transferred from a defined donor genotype to another recurrent genotype, agronomically qualified but lacking that trait. The final result is the creation of a new pure line, identical to the recurring parent, possessing the new trait to transfer. The method is based on a repeated series of crosses. According to the role they play, the genotypes used for backcrossing are called donor parent and recurrent parent.

The donor parent is only involved in the initial crossing (F1) and possesses the gene or genes to be transferred to the cultivar characterized by attractive commercial requirements, defined as recurrent parent. At the initial crossing, the donor parent is used as a pollinator so that the new variety not only presents almost all of the genes of the recurrent parent, but also its cytoplasm. The F1 progeny resulting from this intersection is then back-crossed. The backcrossing can be repeated several times in succession. In self-compatible species, backcrossing generations can be made using always the same recurring genotype, to determine the complete recovery of the commercially interesting characteristics. In the self-incompatible species, the pseudo-testcross strategy is generally used (Figure 2); in this strategy, the recurrent parent is not always the same, avoiding the risk of inbreeding depression, but varies in the various generations of backcrossing, choosing genotypes with characteristics similar to the parent of origin (Bassi et al., 2012).

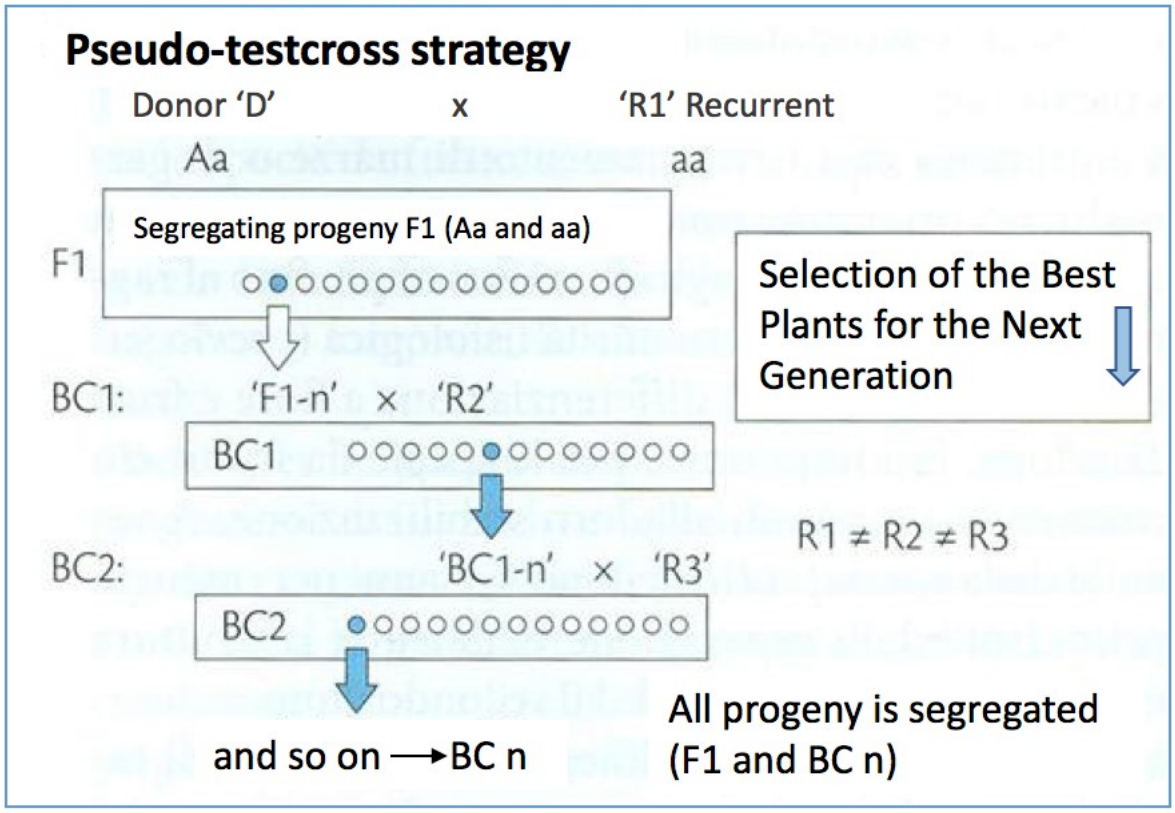

Figure 2 - 'pseudo-testcross strategy' (Bassi et al. 2012)

However, transferring individual characters through the backcross can be complicated by several factors, such as: the polygenic control of the character to transfer, the ploidy of the species, the narrow association of the character of interest with other negative characters. Furthermore, the final product will be similar to the original cultivars but resulting with a genetic combination derived by the different parents used.

3.4.2 Methodology for genetic improvement

With regard to the methodological aspect, the process of genetic improvement is articulated in the following phases:

PROGRAMMING THE CROSSES → Parents' choice must be the result of an in-depth study that leads to the knowledge of the characters a potential parent possesses and its ability to transmit them to the next generation, and therefore, it is very important to make a preliminary assessment of their combinational attitude.

General requirements for parents are productivity, rusticity, and the flowering and ripening time. Other specific requirements are related to the specific use of a parent (resistant to certain biotic and abiotic stresses, fruit integrity after manipulations, high quality).

The seedlings obtained from crossing program germinated in greenhouses and are also raised in the greenhouse, where for some traits, it is possible to make a direct (e.g with inoculum, in the case of resistance to pathogens) and indirect selection (through the use of molecular markers), or they can be directly transferred to the field for subsequent selection steps (Figure 3).

FIELD SELECTION (Box A of Figure 3) → Selection of seedlings in evaluation fields takes place 2 years after crossing. The seedlings are evaluated individually in the field. The material to be selected is evaluated through a subjective evaluation, and must be characterized by greater adaptability in terms of agronomic and pomological characters.

The subjective evaluation is intended to address the main phenotypic characteristics (dates / periods of sprouting / flowering, fruit ripening), morphological (e.g. vegetative vigor), and productive (plant yield and fruit dimension). Further information on susceptibility to abiotic (winter and spring cold) and biotic (pathogen sensitivity/resistance) adverse events are taken into account, to select only tolerant or resistant individuals.

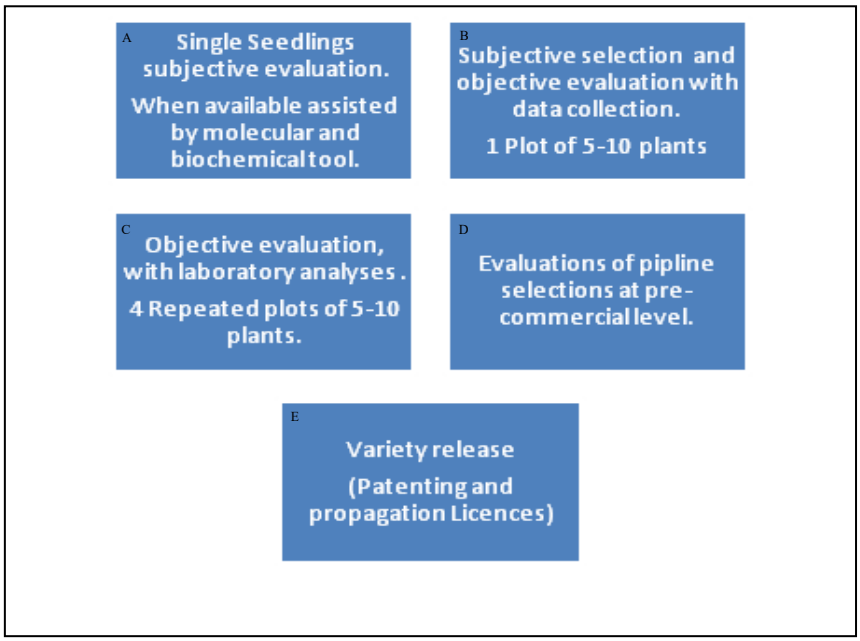

Figure 3 - Selection phase

EVALUATION OF SELECTIONS IN I LEVEL FIELDS (Box B of Figure 3) → This phase takes place 2 max 3 years after the crossing (with fresh plants we have already come from the third year). At this stage, the seedlings previously chosen (from this moment called "selections") are subjected to further evaluations in single parcel of at least 8-12 plants cloned by the original seedlings. At this stage, both the stability and homogeneity of the selected traits are assessed and the effect of environmental interactions is assessed by the evaluation in different years or by the assessment in different pedoclimatic conditions.

The evaluation of the selections in first level fields can last 2-3 to 4 years, and can be performed both with subjective evaluation and with laboratory analyses.

Transfer to the second level fields is allowed only for the selections that have confirmed interesting traits for at least a couple of years. Instead, selections that have responded negatively to the evaluation in the first level field are not keep in consideration for the next step and then discarded. Elimination can take place already in the first year of I level field evaluation.

EVALUATION OF SELECTIONS IN II LEVELS FIELDS (Box C of Figure 3) → After the evaluation in I level fields, the best selections are also assessed for several years in 2° level fields, where each selection is cultivated in 4-5 randomized parcels of 6-8 plants each. It is also possible to cultivate those selections in several fields, with different cultivation techniques compared to traditional cultivars. Further subjective and objective laboratory evaluations are carried out. The evaluations mainly take into account the following traits: ripening time, total and commercial production, average fruit weight, fruit consistency, fruit color, flavor of the fruit, fruit sweetness and acidity, nutritional quality of the fruit. All these traits are detected, parcel by parcel, through accurate objective laboratory analyses. The most interesting selections are then experimented in farms located in different environments (III levels fields), to verify their real agronomic and commercial interest in comparison with other commercially available cultivars.

EVALUATION OF SELECTIONS IN III LEVEL FIELDS - ADVANCED SELECTIONS (Box D of Figure 3) → At the third stage of evaluation, it is possible to find only the selections (advanced selections) that demonstrated, in the previous steps, the highest performance in comparison with other commercial cultivars. The third level field concerns the cultivation of plants of the advanced selections in larger plots, directly in commercial farms, in different environmental conditions. This final step enables to identify the advanced selections that really differ to the existing cultivars for some important commercial traits (mainly resiliency, yield, fruit quality) and have the potential to become a new cultivar to be introduced to the farmer and to the market (Box E of Figure 3).

RESEARCH SECTION

Aims of the study

The aim of this thesis is to improve the sensory and nutritional quality of strawberry fruit.

The study provided the implementation of an intensive crossing program (inter-and intra-specific crossings) and the selection of different progenies in order to identify strawberry genotypes suitable for being registered as commercial varieties or to be used as a parental in subsequent crossing programs.

The study was divided in 2 different parts: in the first one, which represent the main part of the thesis, the aim was to study many strawberry cultivars and selections cultivated in 3 different years to assess their adaptations to the conditions of the Mid-Adriatic area and to select the best selections deriving from the inter and intra-specific breeding programs with improved plant resilience and yield combined with high sensorial and nutritional quality of the fruit.

In the second part, a focus was made on the nutritional aspect of the strawberry through the evaluation of the anthocyanins, phenolic acids, and vitamin C content, with the aim of identify selections from the inter-specific breeding program of higher interest for the fruit nutritional traits.

The overall study has the final purpose to select new strawberry genotypes with a significant increase of the above-mentioned production and quality traits.

4 PART I: STRAWBERRY (F. X ANANASSA DUCH.) BREEDING SELECTION FOR HIGH QUALITATIVE AND NUTRITIONAL TRAITS

The UPM strawberry breeding programs was started more than 25 years ago and it is now focused on the selection of new genotypes with high commercial and agronomic characteristics, good adaptability to limiting conditions and different climates, high quality and nutritional value, through the implementation of different intra-specific and inter-specific breeding programs.

An important part of the research work carried out for the thesis was addressed to the assessment of seedlings and selections produced by specific cross combinations performed in 2011, 2012 and 2015.

During this period were produced and assessed progenies and selections originated from inter- and intra-specific cross-combinations as following described:

- From the crossing realized in 2011, several progenies from fifteen families originated from two cross-type types: backcross 2, backcross 3, were compared (in 2014). This material was compared with eight families derived from F1 of *Fragaria x ananassa* (Fxa) and *Fragaria virginiana glauca* (FVG) and with 3 families generated by backcross 1.

Data obtained from nutritional analyzes were essential for the selection of selections characterized by high antioxidant capacity which is beneficial to human health.

- From the crossing realized in 2012, progeny obtained from eight new crossing families were evaluated; two types of crossing were made: the intraspecific (*F. x ananassa* x *F. x ananassa*) and the interspecific (*F. virginiana glauca* x *F. x ananassa*). The seedlings were first evaluated in the experimental field for their morphological, agronomic characteristics and resistance to pathogens, and then subjected to an objective assessment in 2014 for the production characteristics and sensory and nutritional quality of the fruits. The results obtained are important in order to identify seedlings with high nutritional value, coupled with good qualitative and agronomic characteristics.

- Plant material with good agronomic and fruit quality standards, including high nutritional quality, was used for the subsequent back-crossing combinations that took place in the year 2015. In the year 2015, 8 crossing combinations were performed, the first two of the backcross types 2 (BC2), the third and fourth of type backcross 3 (BC3), the fifth to the eighth of the backcross type 4 (BC4).

4.1 Material and Methods

The research activity started with the identification and preparation of parents, realization of the cross combinations, seeds harvest, vernalization and germination, followed by seedlings cultivation and selection in open field conditions. Fruit quality analyses of selected seedlings was also performed.

4.1.1 Methodological phases of the traditional crossing

a. PARENTALS PREPARATION

Mother plants were grown in protected areas, specifically at the Greenhouse of the Università Politecnica delle Marche (Figure 4).

Figure 4 - Greenhouse of Università Politecnica delle Marche (AN)

The plant types used as mother plants were: frigo-plants (A +) and tray plants.

Frigo-plants of Type A are extracted from the soil during the full dormancy phase, and defoliated.

The tray plants originate from unrooted stolons, placed in polystyrene containers holes of 7-8 cm in diameter. Then they were placed at refrigerated preservation.

In September, each mother plant was placed in pots 16 cm high, with a diameter of 18 cm (Figure 5), with a soil mixture of 80% peat and 20% pumice. The first flowers start to develop after 40-45 days.

The irrigation system consists of capillary tubes, and watering time was managed by a timer set at 5 minutes of watering per day.

Figure 5 - Arrangement of mother plants to cross

b. POLLEN COLLECTION AND DRYING

Pollen collection

Pollen was obtained by collecting the anthers present in the flowers of the selected genotypes (Figure 6).

Figure 6 - Phases of picking strawberry flowers anthers

Pollen collection took place when flowers were not completely open, 1-2 days before full bloom. This operation is performed in specific places and with appropriate instruments.
Harvested anthers were placed in Petri dishes and kept at a temperature of 20-25 °C for 24 hours. In this controlled environment, the anthers opened and the pollen dehydrated to be more easily stored at low temperature. During the pollination phase, pollen is stored in a refrigerator at 4 °C temperature, while the exceeding pollen is placed at -80 °C to allow prolonged storage.

c. EMASCULATION OF SEED-BEARING PLANT

To perform pollination, mother plants were brought to flowering stage and before blooming, sepals, petals and stamens were removed by leaving only the female reproductive system intact, with the pistils completely exposed to pollination (Figure 7).
This procedure is carried out for all mother plants identified for the crossing program to avoid flowers self-pollination.

Figure 7 - Strawberry flower emasculation.

Emasculation was performed by using tweezers (Figure 7). This operation is very delicate and a lot of care must be taken not to break the anthers and thus cause self-fertilization. Tweezers are thoroughly disinfected with alcohol before operating on another cultivar, to avoid contamination. In addition, the emasculated flowers are protected by external pollen with a covering of hydrophilic cotton.

d. POLLINATION

In the next step, the brush was sprinkled with pollen, which was then spread on the stigmas of the flower of the mother plant. Pollination takes place in a very accurate manner, paying attention not to ruin the flower pistils (Figure 8). Every cultivar has its own brush to avoid contamination.

Figure 8 - Controlled pollination

e. FRUIT HARVEST AND SEEDS SEPARATION

After about 20-30 days, the first mature fruits originating from the crossing (Figure 9) were picked up and placed in plastic envelopes, and divided according to the cross-combination (Figure 10).

Figure 9 - Fruits before ripening

Figure 10 - Ripen fruits

Subsequently, the real fruits (achenes) were removed from the strawberry by using an immersion blender. The so obtained seeds were selected and cleaned from the fruit pulp. The next step was the vernalization of the seeds, through storage at 4 °C for about 1-2 months.

f. SOWING AND SEEDS GERMINATION

After the vernalization period, polystyrene boxes (40x30x20 cm) were filled with a peat blend, and then compacted. During the first decade of April, seeds were sown and covered with sifted ground, to prevent them from remaining on the surface but at the same time to avoid they lie too deep. The boxes were placed in a greenhouse, in controlled environment to prevent that external conditions negatively influence the germination process. The irrigation system was by aspersion, with three watering every day for 3 minutes. Seed germination occurred after about two weeks. When the third leaf developed, the seedlings were planted in 60-hole honeycomb polystyrene boxes. In the first decade of June, they were placed in a shady greenhouse for about two months. The seedlings were then transferred to the field in the first decade of August.

g. SEEDLINGS TRANSFER INTO THE FIELD

Well-developed seedlings were transferred from the greenhouse to the open field trial located at the UNIVPM Didactic-Experimental Farm “P. Rosati” in Agugliano (AN, 43°32'N - 13°22'E) (Figure 11).

Figure 11 - Location of Didactic-Experimental Farm "P. Rosati" UNIVPM and strawberry field.

For the aim of the breeding program to select new resilient seedlings, it was identified a cultivation field characterized by heavy soil (heavy soil clay-silty, high active lime which can compromise the absorption of phosphorus and iron with consequent chlorides, pH 7/8), not fumigated and with a short rotation (3-4 years) after a previous strawberry cultivation cycle.

h. EXPERIMENTAL FIELDS AND VEGETATIVE MATERIALS: SEEDLINGS (cross year 2012) AND SELECTIONS (cross year 2011).

Seedling field: in the summer of 2013, the largest number of seedlings were planted from the year 2012 crossing; the maximum number expected was about 160 plants for each cross combination.

In spring 2014, the first evaluation was carried out on single plants and was mainly subjective. This subjective evaluation on seedlings consisted in checking the morphological and agronomic characteristics of the plant and the resistance to major plant and soil diseases. The traits taken in consideration for the subjective selection were the vegetative habitus of the plant, foliage, vegetative vigor, plant yield and fruit quality (shape, color, firmness and flavor). The best seedlings selected by this assessment done during the harvesting period were identified, cloned and transferred in a new field for the first level evaluation carried out in 2015.

Selection field:

In summer 2014, selections deriving from 2011 crossing and selected in 2013, were assessed in the first level field. While in summer 2015, selections deriving from 2012 crossing and selected in 2014, were assessed in the first level field.

The evaluation was carried out on a single parcel of 6-8 plants combining a subjective and objective (antioxidants, polyphenols, color, consistency, conservation tests) assessment (Table 4).

Cultivation techniques

In the experimental field, normal cultivation techniques have been adopted. The main cultivation practices are listed below:

- straw shredding;
- subsoiling;
- distribution of the organic substance (manure) in the dose of about 30 ton/Ha;
- plowing at depths of 30-35 cm;
- leveling of the ground and reduction of excessive clods with superficial harrowing;
- distribution of fertilizers in the dose of 100 units of nitrogen, 70 of phosphorus and 150 of potassium per hectare.

The mulching with black plastic polyethylene film took place a week before the plantation.

Years	CROSS 2011	CROSS 2012	Analysis
2011/2012	Crossing		
2012/2013	Seedlings	Crossing	
2013/2014	Ist Level field	Seedlings	Quality & Nutritional (Cross 2011 & Seedlings 2012)
2014/2015	Ist Level field	Ist Level field	Quality & Nutritional (Not performed)
2015/2016	Ist Level field	Ist Level field	Quality & Nutritional
2016/2017	Ist Level field	Ist Level field	Quality & Nutritional

Table 4 - Cross year 2011 and 2012

4.1.2 Plant material

Crossings in Table 5 were derived by F1 hybrids obtained from interspecific crosses between commercial cultivars chosen from “strawberry varietal lists” and particularly adapted to our

growing conditions (DON, MARMOLADA, MISS, LINDA, advanced selection: 91,143,53) and wild *F. virginiana glauca*, with high nutritional value but low qualitative parameters (FVG).

For seven crossing lines, the female parental was the wild species and male parental the commercial varieties. The aim of these crosses was to obtain a progeny (F1) with commercially increased characteristics compared to female parental and at the same time endowed with high nutritional value handed down by the latter.

Three crossings originate from BC1 (backcross 1), having as female parental the F1 selection (AN94,414,52) and different male parental (ROMINA, CLERY, selection 91,143,53).

Backcross 1 was designed to maintain the nutritional values obtained from the first generation F1 by interspecific crossing (DON x FVG), and to reintroduce and maintain by recurrent male parent the qualitative and agronomic characteristics in respect of the small F1 fruits (Table 5).

Cross code	Parentals			Type
	Mother		Father	
AN94,414	DON	X	FVG	F1
AN94,467	FVG	X	MARMOLADA	F1
AN94,468	FVG	X	MISS	F1
AN94,470	FVG	X	MISS	F1
AN94,472	FVG	X	LINDA	F1
AN94,474	FVG	X	MISS	F1
AN94,490	FVG	X	MARMOLADA	F1
AN94,493	FVG	X	MARMOLADA	F1
AN00,239	AN94,414,52	X	91,143,53	BC1
AN07,003	AN94,414,52	X	ROMINA	BC1
AN07,005	AN94,414,52	X	CLERY	BC1

Table 5 - Crosses F1 and BC1

The results of the qualitative and nutritional evaluations of first generation F1 (crossing 1994) and BC1 (crosses 2000-2007) were compared with data from the selections obtained from BC2 and BC3 crossings in 2011 (Table 6).

Specifically, female parental used for crossings were advanced BC1 selections (AN07,003,52, AN07,003,51, AN07,004,51, AN07,005,53) for backcrossing 2, and advanced BC2 selections

(AN07,006,60, AN07,215,55, AN07,216,61) for backcrossing 3. Those selections, characterized by a high commercial value and nutritional characteristics, were crossed with commercial varieties as male parental (NORA, TECLA, MONTEREY).

The selections that showed the most satisfactory nutritional results were used as parentals for backcross performed in 2015.

Cross code	Parentals			Type
	Mother		Father	
AN11,002	AN07,003,52	X	NORA	BC2
AN11,004	AN07,003,52	X	TECLA	BC2
AN11,005	AN07,004,51	X	MONTEREY	BC2
AN11,006	AN07,004,51	X	NORA	BC2
AN11,008	AN07,004,51	X	TECLA	BC2
AN11,009	AN07,005,53	X	MONTEREY	BC2
AN11,012	AN07,005,53	X	TECLA	BC2
AN11,013	AN07,006,60	X	MONTEREY	BC3
AN11,014	AN07,006,60	X	NORA	BC3
AN11,017	AN07,215,55	X	MONTEREY	BC3
AN11,018	AN07,215,55	X	NORA	BC3
AN11,020	AN07,215,55	X	TECLA	BC3
AN11,021	AN07,216,61	X	MONTEREY	BC3
AN11,022	AN07,216,61	X	NORA	BC3
AN11,024	AN07,216,61	X	TECLA	BC3

Table 6 - backcrosses 2011 type BC2 and BC3

The seedlings originating from the 2012 crossing program are belong to six intraspecific (Fxa), and 2 interspecific (MONTEREY x FVG, ROMINA x FVG) crossings (Table 7).

The intraspecific crossing (Fxa) occurred between advanced selections (AN06,164,52, AN06,221,57, AN07,007,60, AN08,113,53) and commercial varieties (FORTUNA, DELY, NERINA) of high agronomic quality, in order to generate progeny with great commercial value to be used in future crossings.

The interspecific crossing (F1) between female parental of commercial varieties (MONTEREY and ROMINA) and male parental FVG (*Fragaria virginiana*) with high nutritional value has

been made to generate a progeny (F1) with higher nutritional characteristics than female parental and better commercial characteristics than the male parent. Successive backcrossing with the best recurrent female parent may led to the obtainment of selection with improved commercial characteristics.

Cross code	Parental			Type
	Mother	X	Father	
AN12,023	AN06,164,52	X	F. FORTUNA	Fxa
AN12,025	AN06,164,52	X	DELY	Fxa
AN12,027	AN06,221,57	X	NERINA	Fxa
AN12,029	AN07,007,60	X	F. FORTUNA	Fxa
AN12,044	AN08,113,53	X	F. FORTUNA	Fxa
AN12,046	AN08,113,53	X	DELY	Fxa
AN12.049	MONTEREY	X	FVG	F1
AN12,051	ROMINA	X	FVG	F1

Table 7 - Cross-Combinations of 2012, Types (Fxa) and (F1).

4.1.3 Fruit harvest and productive parameters

Table 8 lists all the strawberry genotypes analyzed in this study. On 98 different strawberry selections derived from interspecific crossing (F1) and back-crossings (BC1, BC2, BC3), varieties and selections of *Fragaria x ananassa* (Fxa), and selections of *Fragaria x ananassa* adapted to the industrial transformation for their dark red coloration (Fxa Ind) (Diamanti et al., 2016), were performed the qualitative parameters analysis: average fruit weight, total and commercial plant production, waste fruits per plant (rotten, undersized and deformed). For each entry (selections and varieties) to the third, fourth and fifth harvest of fruits (at the middle of the collection period), a homogeneous sample of 20 mature fruits was taken and the soluble solids and Titratable Acidity were detected. Of these 98 different genotypes, 46 selections from the crosses with an asterisk (specified in the last column of Table 8) and 5 varieties indicated (Nerina, Romina, Cristina, Alba and Asia) were chosen on the basis of visive evaluations performed in the field in the years prior to this study for nutritional analyses. Methanol extracts (STSM report 2008) were obtained on which were performed spectrophotometric analyses (TAC, TPH and ACY).

Type	N° genotypes obtained	Crossword code	Mother	x	Father	Genotypes analyzed for nutritional analyses
F1	16	AN12,49*	MONTEREY	X	FVG	AN12,49,52
						AN12,49,53
						AN12,49,55
						AN12,49,56
						AN12,49,57
						AN12,49,59
						AN12,49,60
						AN12,49,61
						AN12,49,62
		AN12,51*	ROMINA	X	FVG	AN12,51,52
						AN12,51,53
						AN12,51,56
						AN12,51,58
						AN12,51,59
						AN12,51,60
						AN12,51,64
BC1	3	AN07,03,59*	AN94,414,52	X	ROMINA	AN07,03,59
		AN07,05,55*	AN94,414,52	X	CLERY	AN07,05,55
		AN00,239,55*	AN94,414,52	X	91,142,5	AN00,239,55
BC2	10	AN11,06*	AN07,004,51	X	NORA	AN11,06,52
						AN11,06,58
						AN11,06,59
		AN11,05*	AN07,004,51	X	MONTEREY	AN11,05,53
						AN11,05,56
						AN11,05,57
						AN11,05,58
						AN11,05,61
						AN11,05,62
		AN11,08*	AN07,004,51	X	TECLA	AN11,08,52
BC3	10	AN11,13*	AN07,006,60	X	MONTEREY	AN11,13,51
						AN11,13,52
						AN11,13,55
						AN11,13,57
						AN11,13,58
		AN11,14*	AN07,006,60	X	NORA	AN11,14,51
		AN11,17*	AN07,215,55	X	MONTEREY	AN11,17,55

						AN11,17,56
		AN11,21*	AN07,216,61	X	MONTEREY	AN11,21,59
		AN11,22*	AN07,216,61	X	NORA	AN11,22,55
Fxa	48	AN08,113,53*	ANTEA	X	PATTY	AN08,113,53
		AN12,23	AN06,164,52	X	F.FORTUNA	
		AN12,25	AN06,164,52	X	DELY	
		AN12,29	AN07,007,70	X	F.FORTUNA	
		AN12,44	AN08,113,53	X	F.FORTUNA	
		AN12,46	AN08,113,53	X	DELY	
Fxa (Ind)	4	AN12,27*	AN06,221,57	X	NERINA	AN12,27,55 AN12,27,56 AN12,27,57 AN12,27,58
Fxa	1	AN06,164,52*				AN06,164,52
Fxa	1	AN08,108,56*				AN08,108,56
Fxa	1	NERINA*				NERINA
Fxa	1	ROMINA*				ROMINA
Fxa	1	CRISTINA*				CRISTINA
Fxa	1	ASIA*				ASIA
Fxa	1	ALBA*				ALBA

Table 8 - Strawberry genotypes analysed in this study.

4.1.4 Qualitative and nutritional parameters

- ***Chemicals for extraction and analyses***

Methanol and ethanol were purchased at Carlo Erba Reagenti, Milano (IT); ABTS (2,2′-azinobis(3-ethiolbenzothiazoline-6-sulfonic acid) diammonium salt); Trolox (6-hydroxy-2,5,7,8 tetramethychroman-2-carboxylic acid); Potassium persulfate (di-potassium peroxidisulfate); Dipotassium hydrogen phosphate; Potassium dihydrogen phosphate; Folin-Ciocalteu-Reagent; Sodium carbonate; Gallic Acid; Potassium chloride (KCl); Sodium acetate (NaAc); Hydrochloric acid (HCl); Acetic acid were purchased by Sigma- Aldrich Co. High-purity water (Milli-Q Water System, Millipore Corporation, Bedford, MA) was employed throughout.

4.1.4.1 Quality Analysis

Fruit sensorial quality of selections and parents was analyzed in the harvesting day, at each harvest (2^{nd}, 3^{rd}, 4^{th}), by taking in account the following parameters:

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

b) 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 pH8.2, and expressed as mEQ of NaOH per 100g Fresh Weight (FW).

c) Fruit Color: determined as Chroma index by a Minolta Chromameter CR 400, for two side of 10 ripe, undamaged and uniform fruits. The instruments measured three parameters: L* (luminescence), a* (red tone), b* (yellow tone). Chroma index is evaluated from a and b value $[(a^{*2}+b^{*2})1/2]$; higher Chroma value represents pale fruits and low Chroma index represents dark fruits.

d) Fruit Firmness: measured by a penetrometer 327 (Effegì, Ravenna, IT), results are expressed as grams.

4.1.4.2 Nutritional Analysis

- ***Extraction method***

For each sample of whole raw fruit stored at -20°C, ten fruits were selected and cut in two specular slices, then minced into small pieces, weighed (10g) and placed in a Falcon tube to start the extraction with methanol (1:4, fruit: methanol, w/v). In the first step, 20 ml of methanol were added to the fruits. This methanolic solution was homogenized by Ultraturrax T25 homogenizer (Janke and Kunkel, IKA Labortechnik, Staufen, DE). 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, DE) and the recovered supernatant was collected and stored in three separate amber vials corresponding to three replications for the subsequent analysis. To complete the extraction, other 20 ml of methanol were added to each pellet and the procedure repeated as above. The same volume of supernatant was collected and combined with the previous ones in the same amber vials and stored at -20°C.

- ***Total Antioxidant Capacity (TAC)***

TAC was evaluated by the ABTS assay, according to a previously validated procedure (Miller et al., 1993). ABTS, a chromogen and colorless substance, is changed into its colored

monocationic radical form ($ABTS^{\bullet+}$) by an oxidative agent. Addition of antioxidants reduces $ABTS^{\bullet+}$ into its colorless form. The extent of decolorization as percentage of inhibition of $ABTS^{\bullet+}$ is determined as a function of concentration and calculated relative to the reactivity of Trolox, a water-soluble vitamin E analogue.

The day before analysis, the stock solution of $ABTS^{\bullet+}$ was generated by oxidation of ABTS with potassium persulfate ($K_2S_2O_8$) overnight. Phosphate buffered saline (PBS) at pH 7.2-7.4 and a concentration of 5 mM was prepared with dipotassium hydrogen phosphate (K_2HPO_4) and potassium dihydrogen phosphate (KH_2PO_4).

For analysis of the samples, 100 µl of strawberry methanol extract (extraction procedure described in Extraction and HPLC analysis of anthocyanins) were mixed with 1900 µl of PBS in an Eppendorf tube. Again 100 µl of this solution were mixed with 1900 µl of $ABTS^{\bullet+}$ working solution (stock solution diluted with PBS 1:50 to 1:70, absorbance of 0.7-0.8) and vortexed. After 6 min in the dark the absorbance was measured with the UV spectrophotometer UV-1800 (Shimadzu Corp., Kyoto, JP) at a wave length of 734 nm. Every sample was analysed three times.

The standard was prepared by diluting the Trolox stock solution (2.5 mM) with PBS so that the final concentrations range from 0.025 to 0.45 mmol L^{-1}. The absorbance was measured in the same way as the samples. In the following the transformation of the measured absorbance into Trolox equivalent is explained:

Calculation of the percentage of inhibition (= ΔA) of $ABTS^{\bullet+}$:

$$\Delta A = \frac{Abs_{blank} - Abs_{sample/standard}}{Abs_{blank}} \times 100\%$$

By linear regression a standard calibration curve for each Trolox concentration (mean of absorbance for three measurements) was calculated (ΔA=ac+b).

For calculating the TAC of the strawberries the following formula was applied:

$$\text{TAC}\left(\frac{\text{mmol TE}}{\text{kg FW}}\right) = \frac{(\Delta A - b) \times F}{E \times a} * 1000$$

- ΔA = % inhibition (sample)
- a= slope (calibration line)

- b= intercept (calibration line)
- c= Trolox concentration [mmol]
- F= dilution factor (20)
- E= sample weight [g/L extracting agent]

Antioxidant activity was expressed as mmol of Trolox equivalent per g of fresh pulp weight. The calibration was calculated as linear regression from the dose response Trolox Standard. The results are expressed as mM of Trolox equivalent per kg of Fresh fruit.

- ***Total Phenol Content (TPH)***

TPH was evaluated by the Folin-Ciocaltou's reagent method (Slinkard and Singleton 1977) using gallic acid as standard for the calibration curve. Results were calculated and expressed as mg of gallic acid per kilogram of fresh fruit. Range value of standards were from 10 to 50 mg gallic acid/kg. Briefly, a test tube (glass) was filled with 7.0 ml of water. Afterwards, 1 ml of the diluted sample (1:20) was added, followed by the addition of 500 µL Folin-Ciocalteu-Reagent and vortexed. After 3 minutes, 1.5 mL sodium carbonate (0.53 mol/l) was added and the tube was mixed one more time and then stored in the dark for 60 minutes. The absorbance of the sample was measured after exactly 60 minutes at 760 nm with a spectrophotometer.

- ***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. Briefly, the samples were diluted to a ratio of 1:10 with potassium-chloride (pH 1.00) and with sodium acetate (pH 4.50) and the corresponding maximum absorbance for both solutions was measured (respectively at λ = 500 nm, and λ = 700 nm).
The results were expressed as mg of pelargonidin-3-glucoside (the compounds more representative for anthocyanins in strawberry) per kilogram of fresh weight (mg of Pel-3-Glu/Kg FW).

4.1.5 Statistical Analysis

Results for strawberry fruit production, qualitative and nutritional parameters are presented as mean ± standard error (SE) for each crossing type. Two-way analysis of variance was used to test the differences among crossing types, among harvesting years, and for the interaction

between crossing types and years. Statistically significant differences ($p \leq 0.05$) were determined with SNK test. Statistical processing was carried out using STATISTICA software (Stasoft, Tulsa, OK). Principal Component Analysis was also used to test the correlation among productive, sensorial and nutritional quality of all the 51 genotypes analyzed (46 selections + 5 varieties) indicated with an asterisk in Table 8. Based on the theoretical arguments of the PCA described by Hair et al. (2005), the significant factors loading values higher than or equal to 0.7 were used to identify the most important variables and observations in each dimension (PC). Factor loading values are the correlation of each variable with the PC. They are represented as vectors (positions) in the space resulted by the axes of the bi-plot graphic. In the graphic, variables and observations that are close to each other, and in the same geometric plan of the bi-plot, are interrelated, and distant from variables and observations to which they are not related, or even negatively related. The greater is the vector (distance from the origin of the axis), the greater is the correlation of the variable with the PC represented in that dimension (axis). In the PCA graphs, each point represent a genotype analyzed, but only on the most interesting genotypes the label name was showed.

4.2 Results and Discussions

4.2.1 Production

The analysis of variance regarding the Total Production and the Average Fruit Weight (AFW) revealed that those parameters are statistically influenced by the harvesting year, by the type of crossing and also by the interaction between harvesting year and crossing type (Table 9). Evidently, the total plant production and AFW are factors that can vary among harvesting seasons, as they are not exclusively determined by plant genetics. This aspect can be confirmed if we compare the results for Romina and Cristina cultivar, for example, with another study from our group. In that study, Romina, cultivated in the same field and in the same cultivating conditions of the present study, showed a mean value of plant production from the year 2010 to 2012 of 831 g/plant, clearly higher than in this study (605 g/plant), but it showed exactly the same AWF of this study (18.1 g). Contrarily, Cristina performed in that study a very high mean plant production (1094 g/plant), while in the present study it produced “only” 660 g/plant. Also the AFW was different, weighting 33.4 g in that study and 28.4 g in the present study (a reduction of about 12% (Capocasa et al., 2016).

Qualitative Parameters	Year	Type	Interaction Year x Type
Total Production	*	*	*
Average Fruit Weight	*	*	*

Table 9 - Analysis of variance (ANOVA 1) considering the variable cross type (Fxa, Fxa (Ind), F1, BC1, BC2 and BC3) for Total Production and Average Fruit Weight, considering the years 2016 and 2017. * = Significant interaction; N.S. = Not Significant interaction

With regard to Total Production, the SNK test with $p \leq 0.05$ showed Fxa as the crossing type with the statistically higher values, such as AN06,164.52 (1146 g/plant) and Cristina (660 g/plant), followed by Fxa (Ind) and BC3 (with AN11,17,55 presenting 456 g/plant) that were statistically similar each other and to all the other crossing types (Table 10). The result was expected if we consider that Fxa group contains the selections and commercial varieties that we have already adopted in our second level field and that we usually use in our breeding programs for their satisfaction of the productive standards required by the market. By crossing Fxa with the wild genotype, productivity has been clearly lost. As the statistical analysis shows, in fact, all the crossing types containing wild germplasm registered lower values of Total Production in respect to Fxa group; however, through sequential backcrossings with Fxa parental, we were able to recover some productive capacity moving from BC1 towards BC3 (Table 10). Those results are in agreement with other studies published by our group, where the mean total production of BC1 and BC2 were similar each other, but lower than the mean values of Fxa genotypes (Diamanti et al., 2012a).

Type	N° genotypes analyzed	Total Production (g)
Fxa	55	614.18 ± 300.19^{a}
Fxa (Ind)	4	421.63 ± 187.12^{b}
F1	16	$404.\ 8 \pm 114.03^{b}$
BC1	3	297.64 ± 110.30^{b}
BC2	10	369.21 ± 128.18^{b}
BC3	10	392.97 ± 180.33^{b}

Table 10 - Means of Total production for different types of crossing in the 3 years of study

Similarly to what we noted for the Total Production, also for the Average Fruit Weight the SNK test with $p \leq 0.05$ revealed that the higher statistical value was registered for the Fxa group, with Cristina (28.45 g) and AN08,113,53 (18.9 g) being the most interesting, followed by Fxa (Ind), BC2 (with selections AN11,05,53, 19.6 g, and AN11,08,52, 16.9 g, being sensibly higher than other selections of the same group). The BC3 group (with AN 11,17,55, 15 g) was statistically similar to Fxa (Ind) and BC2, while BC1 and F1 revealed the worst values of Average Fruit Weight (Table 11). Also in this case, among the various types of crossings, we have found Fxa group as the one with the highest Average Fruit Weight. While, the presence of wild germplasm in the other crossing types reduced significantly the Average Fruit Weight, but back-crossings with Fxa genotypes seems to be able to restore good levels of Average Fruit Weight from the BC2 generation. Similarly to what we have commented on the Total plant Production, also for the AFW our 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., 2012a).

Type	N° genotypes analyzed	Average Fruit Weight (g)
Fxa	55	18.73 ± 5.58^{a}
Fxa (Ind)	4	14.73 ± 3.07^{b}
F1	16	7.38 ± 4.02^{c}
BC1	3	9.89 ± 1.04^{c}
BC2	10	14.15 ± 2.88^{b}
BC3	10	12.97 ± 2.75^{b}

Table 11 - Average Fruit Weight for different types of crossing in the 3 years of study.

The obtainment of genotypes that produce good amount of fruits with an appropriate fruit weight is a very important aspect, because growers are searching for plant producing big fruits for reaching high yield. Furthermore, picking of small fruits is time-consuming and also the acceptance of the consumers is low for fruits of small dimensions (Hancock 2008). For all these reasons, breeding programs including wild genetic resources require different generations of backcrossing with F×a commercial cultivars for increase the average fruit weight and the total plant production, as demonstrated by our results.

4.2.2 Soluble Solids (SS) and Titratable Acidity (TA)

For the qualitative analyses, the Analysis of Variance resulted in a significant effect of the year, the type of crossing and the interaction between year and type on the Soluble Solids content. Similarly, year and crossing Type significantly influence the Titratable acidity of samples, but the interaction between Year and crossing Type is not influent on the Titratable Acidity (Table 12).

Qualitative Parameters	Year	Type	Interaction Year x Type
Soluble Solids (SS)	*	*	*
Titratable Acidity (TA)	*	*	n.s.

Table 12 - Variance Analysis (ANOVA 2) for qualitative parameters found in strawberry genotypes over the three-year period 2014, 2016 and 2017. * = Significant interaction; N.S. = Not Significant interaction.

This means that, considering the "Year" variable (2014, 2016 and 2017) and the variable "cross Type" (Fxa, Fxa (Ind), F1, BC1, BC2 and BC3), we have found variability between cross-typologies for Soluble Solids in the same year; furthermore, these types of crossing behaved differently for each year of analysis. The same result was not recorded for Titratable Acidity that showed different amounts among the different cross-typologies and among different years of study, but the differences among genotypes remained fixed over the years because of the non-significant interaction between year and genotype (Table 12).

Year	Soluble Solids (Brix°)	Titratable Acidity (meqNaOH/100g juice)
2014	9.18 ± 1.13^{b}	12.47 ± 2.72^{b}
2016	9.07 ± 1.13^{b}	12.49 ± 2.96^{b}
2017	9.78 ± 1.13^{a}	13.35 ± 3.16^{a}

Table 13 - SS and TA mean values for the 3 years of study.

Type	Soluble Solids (Brix°)	Titratable Acidity (meqNaOH/100g juice)
Fxa	8.28 ± 1.27^{d}	10.3 ± 1.58^{c}
Fxa (Ind)	8.57 ± 1.18^{d}	10.34 ± 1.16^{c}
F1	9.54 ± 0.67^{bc}	14.89 ± 2.08^{a}
BC1	9.87 ± 1.16^{ab}	15.57 ± 4.19^{a}
BC2	9.3 ± 1.11^{c}	10.66 ± 1.55^{c}
BC3	10.05 ± 1.29^{a}	13.57 ± 2.62^{b}

Table 14 - SS and TA mean values for different types of crossing in the 3 years of study.

The SNK test with $p \leq 0.05$ for Soluble Solids based on the collection years showed the highest average values for harvests carried out in 2017, even if values for 2014 and 2016 are not too far (Table 13).

The SNK test with $p \leq 0.05$ carried out considering the crossing types for Soluble Solids showed the highest average value for the BC3, in particular for AN11,21,59 (12.4 °Brix), AN11,13,55 (12 °Brix) and AN11,13,51 (11.2 °Brix); BC1 showed lower average value, but statistically similar to BC3, in particular presenting high value for the selection AN00,239,55 (12.2 °Brix); of particular interest was also the BC2 selection AN11,06,59 (10.3 ° Brix). A very positive result that emerges from this part of the study is that with the backcrossing program, generally used as a tool for the recovery of commercial characters such as the size, it was possible also to increase the quality by raising the Brix degree (Table 14).

Similarly to what observed for Soluble Sugars content, the same statistical test on Titratable Acidity on the basis of harvesting years has highlighted the highest average Acidity values for the year 2017; also in this case, values for 2014 and 2016 years do not deviate too much from 2017 value (Table 13).

The SNK test with $p \leq 0.05$ carried out considering crossing types for the Titratable Acidity showed the highest Acidity values in BC1, as AN00,239,55 (22.6 meqNaOH / 100g juice) interesting also for the high sugar content, and AN07,05,55 (15.3 meqNaOH / 100g juice). Statistically similar high Acidity values were also found in F1, particularly in selections AN12,51,53 (18.1 meqNaOH / 100g juice) and AN12,51,59 (16.3 meqNaOH / 100g juice). Those results were expected in the first generation of interspecific crossing (F1) and in the first generation of Backcrossing (BC1), because the FVG father influences the acidity of the progeny. By proceeding with the backcrossing, the acidity lost in the Fxa genotypes tends to be recovered, probably due to the type of parental used in the crossing. As predicted, Fxa are the

selections with the less acidic fruit, such as AN12,27,57 (9.2 meqNaOH / 100g juice), followed by BC2 progeny and in particular the less acid fruit of selection AN11,06,59 (9.8 meq / 100g juice). The low value of Titratable acidity in the BC2 selections is probably due to the right parents' choice. This latter selection had already been highlighted for good Soluble Solids content compared to the selections belonging to the same type of crossing; this means that this selection will result in a balanced sugar/acid ratio to the palate of a taster. In fact, as previously affirmed, only if the proportion of TA and SS is balanced, the sensation of sweetness is evoked in the final consumer. The consumer, in fact, does not accept too much acidity, but also a too low acidity causes an undesired too intense perception of sweetness (Capocasa et al., 2016).

In general, sensorial parameters tend to be higher in wild germplasm in respect to the Fxa genotype, and it was already observed before (Diamanti et al. 2012a, 2014; Mezzetti et al., 2016). This means that the backcrossing is paralleled with a loss of sugar and acidity content, but in the present study, we were able to reverse this result for the sugar content. In fact, differently from all the other studies previously mentioned, we obtain the highest SS concentration in the BC3 selections, significantly higher also than the F1 progeny. If we consider that, at the same time, we were able to decrease the acidity in BC3 in respect to F1 and BC1, we can affirm that we have potentially obtained BC3 selections with an optimal sugar/acid ratio.

TA and SS are important factors for the organoleptic quality of strawberries, and their balance will favour the strawberry acceptance by the final consumer (Diamanti et al. 2014). For these reasons, even if the final aim is to obtain a strawberry fruit with high nutritional quality, those sensorial attributes must not be neglected, giving that the costumer will give preference to a tasty than to a healthy strawberry.

4.2.3 Total Antioxidant Capacity (TAC), Total Phenol Content (TPH) and Total Anthocyanins Content (ACY).

From the ANOVA variance analysis, considering the “year” variable (2014, 2016 and 2017) and the variable “cross type” (Fxa, Fxa (Ind), F1, BC1, BC2 and BC3), it resulted that both variables are statistically significant in the variation of TAC, TPH and ACY, and also their interaction is significant in the variation of these parameters. This means that there is variability among cross-typologies for these parameters in the same year, but these types of crossing behaved differently for each year of analysis, thus not maintaining the same variability for all years examined (Table 15).

Nutritional Parameters	Year	Type	Interaction Year x Type
Total Antioxidant Capacity (TAC)	*	*	*
Total Polyphenols (TPH)	*	*	*
Total Anthocyanins (ACY)	*	*	*

Table 15 - Variance Analysis (ANOVA 2) for the nutritional parameters found in strawberry genotypes over the three-year period 2014, 2016 and 2017. * = Significant interaction; N.S. = Not Significant interaction.

Year	TAC (mM Trolox eq/Kg fruit)	TPH (mg GA/Kg fruit)	ACY (mg PEL-3-GLU/Kg FW)
2014	21.59 ± 3.64^{b}	2255 ± 500.5^{b}	574 ± 204^{a}
2016	26.65 ± 5.77^{a}	2481 ± 561^{a}	247 ± 255.5^{c}
2017	26.80 7.08[a]	2148 ± 484.5^{c}	371 ± 182.3^{b}

Table 16 - TAC, TPH and ACY mean values for years 2014, 2016, 2017.

Type	N° genotypes analyzed	TAC (mM Trolox eq/Kg fruit)	TPH (mg GA/Kg fruit)	ACY (mg PEL-3-GLU/Kg FW)
Fxa	8	22.34 ± 7.40^{c}	1849 ± 515.3^{c}	430 ± 227.7^{b}
Fxa (Ind)	4	24.90 ± 4.63^{b}	2158 ± 260.5^{b}	792 ± 206.7^{a}
F1	16	24.14 ± 6.18^{b}	2440 ± 518.2^{a}	311 ± 148.8^{d}
BC1	3	26.19 ± 7.76^{ab}	2468 ± 466.4^{a}	371 ± 133.7^{c}
BC2	10	25.63 ± 5.34^{ab}	2248 ± 526.3^{b}	347 ± 122.2^{cd}
BC3	10	27.29 ± 5.68^{a}	2455 ± 664.6^{a}	420 ± 150.6^{b}

Table 17 - TAC, TPH and ACY mean values for different types of crossing in the 3 years of study.

The SNK test with $p \leq 0.05$ performed for TAC based on the harvest years showed the highest average values for the harvests carried out in 2016 and 2017 (Table 16). The SNK test with $p \leq 0.05$ carried out considering the crossing types for TAC showed the highest value for BC3 (such as AN11,17,55 with 34.2 mM Trolox eq/Kg fruit, and AN11,13,58 with 30.8 mM Trolox eq/Kg fruit). Statistically similar values were obtained for BC1 (e.g. AN00,239,55 with 32.4 mM Trolox eq/Kg fruit) and BC2 selections (such as AN11,05,55 with 29.1 mM Trolox eq/Kg fruit). An almost unexpected lower value was recorded in the F1, while the lowest TAC value was obtained by Fxa genotypes (Table 17).

The three backcrosses generations could not be distinguished among themselves, but their TAC values were statistically higher than those of F×a genotypes. This demonstrates the successfulness of increasing TAC content by using wild germplasm for crossings, even if Fxa (Ind) genotypes, which were suitable for the industry thanks to their high content in anthocyanins, presented TAC values statistically similar to F1, BC1, BC2 and BC3, probably due to their high anthocyanins content. Similar results were obtained in Diamanti et al., 2012a, where BC2 and BC1 populations presented statistically higher values of TAC than Fxa selections. In general, values from that study were lower than data obtained in this study: e.g., the mean TAC value registered for the intra-specific cross-combination (Fxa) was 14.37 mmol Trolox Eq/kg FW.

Usually, the wild germplasm is believed to possess strong antioxidant activity, higher than the cultivated germplasm (Scalzo et al., 2005b; Halvorsen et al., 2002) as happens in this study (Table 18). In this study, however, the F1 generation that possesses 50% of wild FVG germplasm had a lower TAC than the backcrosses, in particular of BC3, which possess the highest amount of Fxa germplasm. This is presumably due to some genotypes of F1 group having a very low TAC, maybe caused by an Fxa parent with poor antioxidant capacity. One of the Fxa parent, in fact, is represented by "Monterey" cultivar, which showed a low TAC value (Table 18). This result demonstrated, once again, that the choice of the right parents for the transmission of specific traits is fundamental.

Based on the year of harvest, the highest TPH value was found in genotypes cultivated in 2016, while during 2017 harvest the lowest results were recorded (Table 16). Concerning the comparison of the different crossing-types, BC1 recorded the highest value of TPH, in particular, with fruit of the selection AN00,239,55 showing a very high content (3009.7 mg GA/Kg fruit). Statistically similar values were registered for BC3 selections, with AN11,17,56 being the more interesting (2807.7 mg GA/Kg fruit), and for F1 genotypes, with AN12,49,55 (2570.1 mg GA/Kg fruit) selection being the higher for this group. Before the beginning of the study, it was expected to find the highest value of TPH for the F1 genotypes, being the one with the highest amount of wild germplasm. However, on the light also of the results of TAC, it is logical that the best crossing-types for TAC (BC3 and BC1) resulted also the best crossing types for TPH (Table 17). The association between high amount of TPH and high TAC value was already demonstrated in many studies. However, different from the present study, in these works the TPH content of genotypes with higher amount of FVG was higher than the value registered for successive back-crossings or Fxa genotypes. In Mezzetti et al., 2016, BC1 selections showed higher TPH values of BC2 selections, which in turn showed slightly higher

values than Fxa commercial varieties. In Diamanti et al. 2014, BC2 selections presented values clearly higher than BC3 selections, which in turn presented higher values than the only Fxa cultivar analyzed (Romina). In our study, all the back-crossings presented statistically higher values of TPH 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. Considering that in BC3 we were able to recover good values for the productive parameters, the high level of TPH reached in this backcrossing is an optimum result. As an example, mean value of BC3 TPH in our study is 2455 mg GA/kg FW, while in the study of Diamanti et al., 2014, none of the BC3 selections was able to reach a value of 2000 mg GA/kg FW.

The highest Total Anthocyanins Content (ACY) was detected in 2014, followed by 2017 and then 2016 (Table 16). The cross-typology that recorded the highest value for the ACY was Fxa (Ind), with selections AN12,27,57 (954.4 mg PEL-3-GLU/Kg FW) and AN12,27,56 (793.8 mg PEL-3-GLU/Kg FW) being the richest of those compounds. This result was expected giving that Fxa (Ind) selections, as previously indicated, were destined to the industry for their dark color, which, even after transformation/pasteurization, turns towards a pleasing violet rather than towards the unattractive brown color (Diamanti et al., 2016). The trend of ACY concentration in the backcrossing programs is not always clear, and not always the same: in Mezzetti et al. (2016) and Diamanti et al. (2012a), BC1 and BC2 values are similar for the ACY concentration, while in Diamanti et al. (2014) BC3 values were higher than BC2. That trend is similar to the results of the present study: Fxa genotypes showed statistically higher values of ACY than F1, BC1 and BC2 selections, but the BC3 group demonstrated an ACY mean value statistically similar to that of Fxa genotypes. This is another important result from our breeding program, which demonstrates that we were able to recover also the ACY concentrations during the crossing process through the utilization of high-anthocyanin Fxa genotypes as parents. The high amount of ACY is important in the fruit because, in addition to possess strong antioxidant activity, they contribute to the red color, and this is an aspect particularly appreciated by the consumer. However, genotypes with very high amount of ACY (as the Fxa – Ind) showed a very dark color of the fruit, that could be not accepted by the consumer of south-Europe, but that can be very useful for the industrial processing of the fruit, as previously mentioned.

The parameters shown in Table 18 represent the average values of the TAC, TPH and ACY present in parental used for crossings. The clearest result is that the FVG genotype when used in interspecific crossing have an important effect in increasing the nutritional value of progeny,

giving its very high values of TAC and TPH in particular. Comparing these results with those one in Table 17, it is clear that crossing FVG with Fxa genotypes led to the losing of nutritional parameters, in particular in F1, but progressive Backcrossing with the best genotypes allow to recover at least a part of the initial nutritional properties of FVG. As a result, BC3 presented higher TAC and ACY in respect to F1, BC1 and BC2 (and the second highest value of TPH), indicating the quality of the breeding program in this study.

Parental	**TAC (mM Trolox eq/Kg fruit)**	**TPH (mg GA/Kg fruit)**	**ACY (mg PEL-3-GLU/Kg FW)**
MONTEREY	17.8	1190	545
FVG	41.9	4780	602
CRISTINA	18.6	1547	417
ASIA	22.6	1723	552
ALBA	23.4	2014	423
NERINA	38.6	3035	634
ROMINA	25.8	1740	690
CLERY	12.4	950	355
PATTY	10.76	1886	313
NORA	18.1	1310	455
ANTEA	17.5	2840	347
AN07,04,51	29.45	2387	509
AN07,06,60	25.9	1689	488.03
AN07,215,55	30.45	3822	808.9
AN07,216,61	29.44	3679	519
AN03,338,56	20.56	2055	190.04
AN06,221,57	16.24	1988	327.93

Table 18 - TAC, TPH and ACY mean values of 3 years of parental used in different crossings.

However, it is important to note that many genotypes evaluated in this study were previously analyzed by our group in the same field but in different years (Diamanti et al., 2012a, 2014; Mezzetti et al., 2016); the fact that on the same genotypes we have registered values of TAC, TPH and ACY quite different in different years, suggest that the TAC, and the main responsible

of its value such as vitamin C, phenolic compounds and in particular anthocyanins, are strongly influenced by a number of pre- and postharvest factors (Alvarez-Suarez et al. 2014b). It can be assumed that the conditions (especially climatic) in each season have been diverse resulting in different TAC, TPH and ACY values, influencing also, in some cases, the choice of the best genotype to utilize for the successive phases of back-crossing process.

4.2.4 PCA Analysis

4.2.4.1 PCA Analysis for Soluble Solids, Titratable Acidity, Total Production, Average Fruit Weight

The qualitative parameters Soluble Solids and Titratable Acidity were analyzed through PCA analysis together with the productive parameters Total Production and Average Fruit Weight. From Figure 12 it is possible to note that each parameter occupies a different quadrant and that those two Factors explain the 80.85% of the population variability (62.10% + 18.75%).
All the selections that approach one or more of these segments representing the parameters considered will be the most interesting to consider for that parameter.

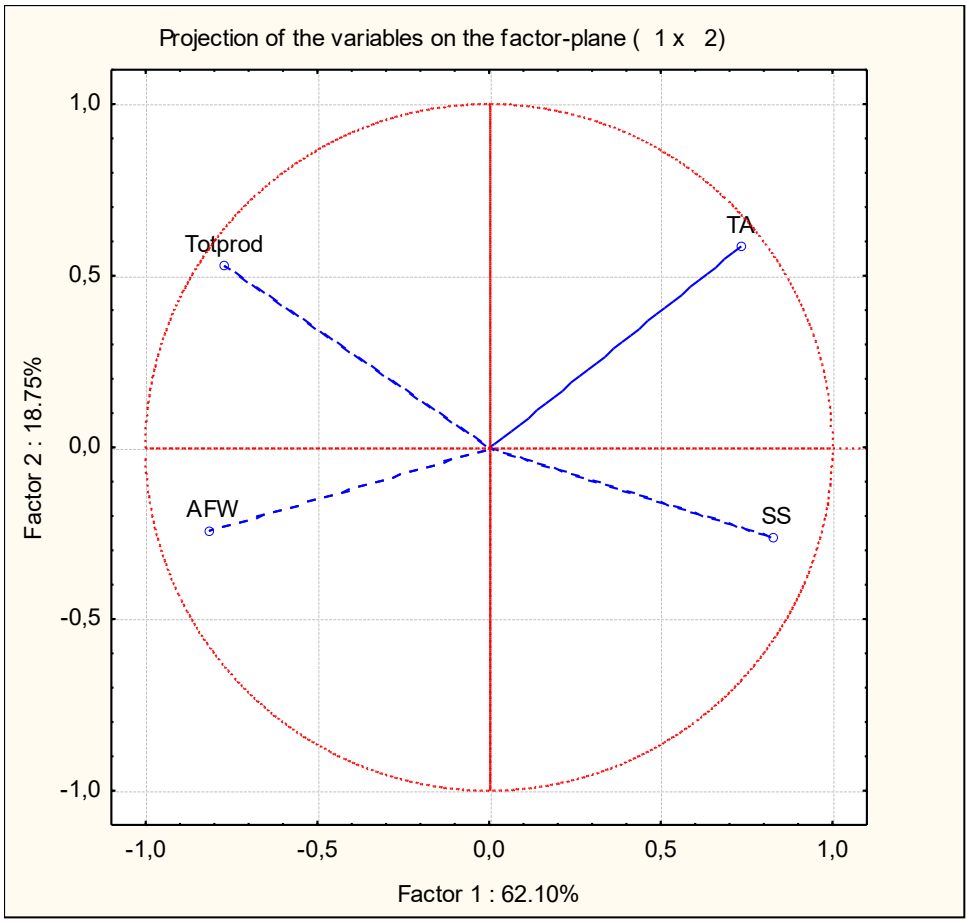

Figure 12 - PCA analysis of SS, TA, Tot Prod, AFW.

From Figure 12 it is interesting to note that Total Production and Average Fruit Weight are parameters completely opposed to Soluble Solids content and Titratable Acidity, respectively. This suggests that acidic or sweet selections will not show high values of total production or big-sized fruits, so qualitative aspects can be lost at the expense of production, and vice versa.

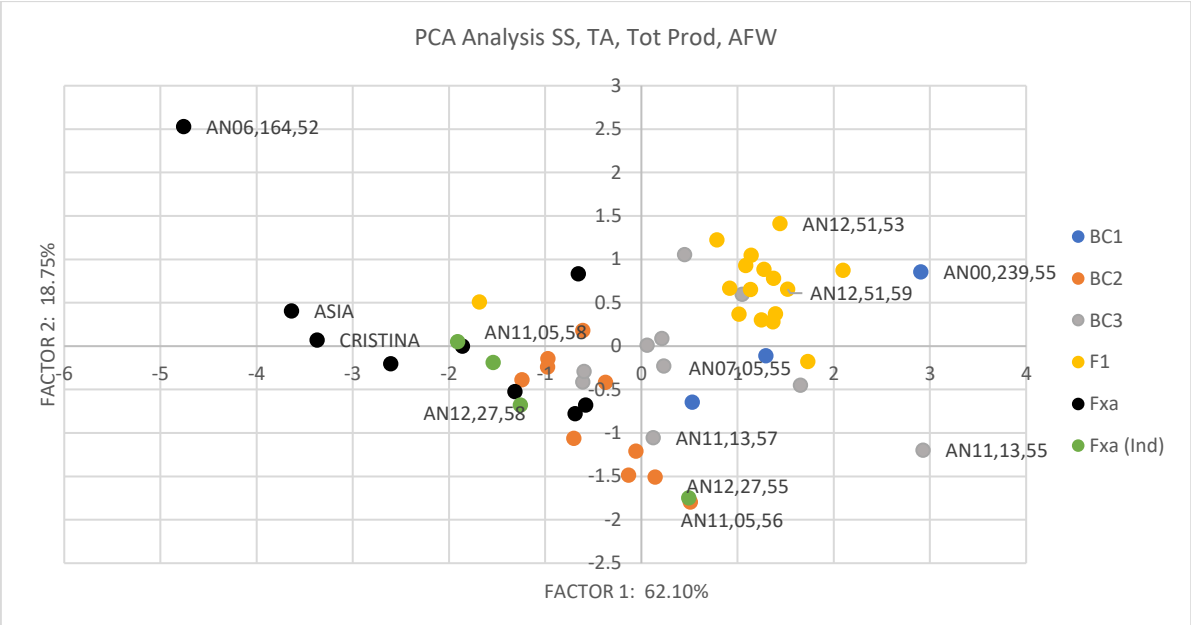

Figure 13 - PCA analysis for the observations according to SS, TA, Prod Tot, AFW, with selections in evidence.

Fxa: this type of selections is distributed only in left quadrants of the Figure 13, those regarding Total Production and Average Fruit Weight. This result was expected, considering that Fxa genotypes have already been considered interesting for the production parameters. Among the Fxa varieties, Asia and Cristina appears closer to the Total Production and Fruit Average Weight than other varieties, while the selection AN06,164,52 is clearly the most interesting for the Total Production (Figure 13).

Fxa (Ind): those selections are predominantly located in the left-lower quadrant, representing the Average Fruit Weight (as AN12,27,58), with AN12,27,55 tending to the right-lower quadrant (the "brix quadrant"). Also in this case, Fxa typology confirms characteristics that stand out above all for production parameters (Figure 13).

F1: these selections concentrate around the upper right quadrant, were the Total Acidity vector is located, such as AN12,51,53 and AN12,51,59. This result was expected because the F1 selections represent the first progeny of the wild FVG genotype, which is characterized by high acidity (Figure 13).

BC1: genotypes belonging to this group are placed in the right quadrants of the graph, that relate to Titratable Acidity and Soluble Solids, with a tendency to more acidity such as AN00,239,55 and a mid-way selection such as AN07,05,55 (Figure 13).

BC2: Going forward with the crossing, it was possible to get selections with different features, which are definitely due to the choice of different parents. In the BC2, in particular, the crossing

allows to obtain selections in the lower part of the graph (high Soluble Solids and high Average Fruit Weight) in respect to the previous BC1 generation, which presented worse productive parameters. To the BC2 group belongs the AN11,05,58 selection, with good productive parameters, up to the AN11,05,56 selection, that is located in the lower-right quadrant, where the Soluble Solids are higher (Figure 13).

BC3: With the last crossing generation, selections seems to be distributed most in the center of the graph. This means that, with this last step of crossing, the choice of the best parental is crucial for obtaining selections with certain characteristics rather than others. For example, AN11,13,55 is clearly located in the part of the graph corresponding to high Soluble Solids, while AN11,13,57 presents less sugar but a higher Average Fruit Weight (Figure 13). In general, fruits belonging to BC3 crossing showed to possess intermediate characteristics between the qualitative F1 and the productive Fxa.

4.2.4.2 PCA Analysis for Total Production, Average Fruit Weight, TAC, TPH, ACY

A second PCA analysis was performed putting in comparison productive parameters (Total Production and Average Fruit Weight) and nutritional parameters (Total Antioxidant Capacity, Total Phenol Content and Total Anthocyanin Content). From Figure 14 it is to note that the parameters occupy only the two upper quadrants and that the percentage of population variability we obtained from the two principal factors is 71.93% (44.30% + 27.63%). TAC and TPH vectors are both in the upper-right quadrant, so they result related each other in our population. Similarly, ACY, Total Production and Average Fruit Weight vectors belong to the upper-left quadrant so they result related each other.

The closer is a selection to one or more of the vectors representing the parameters, the higher will be the amount of that parameter in the selection.

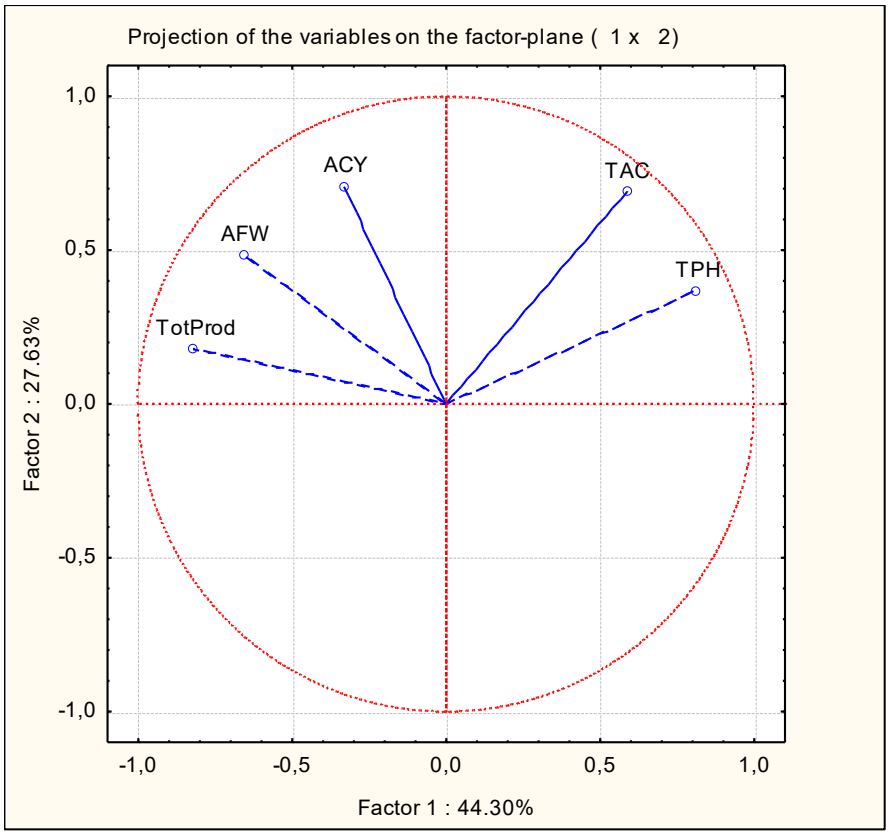

Figure 14 - PCA analysis for Tot Prod, AFW, TAC, TPH, ACY

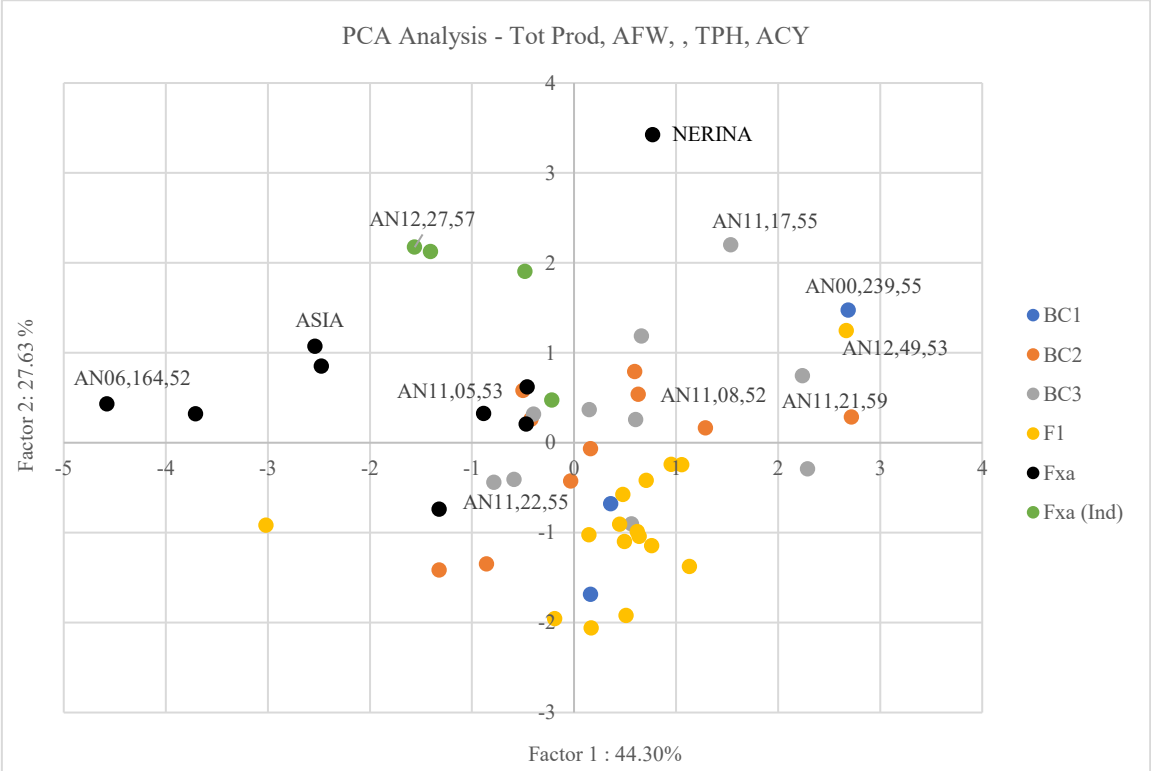

Figure 15 - PCA analysis for the observations according to Prod Tot, AFW, TAC, TPH, ACY with selections in evidence.

Fxa: as expected, selections from the Fxa crossing-type are positioned around the vectors of Total Production (such as AN06,164,52) and of Average Fruit Weight (such as Asia variety). However, the cultivar Nerina is different from all the other Fxa genotypes: in particular, it stands near the TAC and TPH vectors, confirming its great importance for the nutritional quality.

Fxa (Ind): this type of selection is mainly located around the vector corresponding to the Anthocyanins Content. Also in this case, the outcome was expected since these genotypes were selected mainly for their dark red color, indicating a strong presence of anthocyanin pigments. The selection AN12,27,57 is the nearest to the Anthocyanins vector (Figure 15).

F1: the selections belonging to the F1 type are mainly distributed in the lower-right quadrant, opposite to that occupied by the ACY, Total Production and Average Fruit Weight vectors, probably because the FVG parent negatively influences those parameters. This indicates that those parameters are very little present in these selections. However, it was possible to detect a selection closest to the vectors of TAC and TPH (AN12,49,53) (Figure 15).

BC1: those selections follow exactly the same behavior of F1, with a single selection placed in the upper-right quadrant, showing good content of antioxidant compounds (AN00,239,55). This selection is rich in sugars, has a very high acidity, contain a high amount of antioxidants but the average fruit weight is really low (<10 grams) (Figure 15).

BC2: the crossing process allow to obtain, from BC1, a BC2 generation with improved content of TPH and increased TAC, such as AN11,08,52, and also a selection (AN11,05,53) with an increased Average Fruit Weight and Total Production (Figure 15).

BC3: as happened for the first PCA performed, in BC3 selections we can obtain different characteristics strongly related to the aim and to the parental used for the crossing. For example, AN11,17,55 is positioned in the upper-right quadrant, near the vector of the TAC, but is at the same height as the anthocyanin vector; the AN11,21,59 selection is positioned near the TPH vector, but is in line with that of the Total Production. Finally, the AN11,22,55 selection is completely different from the previous BC3 selection presented, because it is located in the opposite quadrant (the lower-left quadrant) were low TAC and TPH values genotypes are located (Figure 15).

4.2.4.3 PCA Analysis for SS, TA, TAC, TPH, ACY

A third PCA was conducted in this part of the study, comparing the strawberry population for sensorial (Soluble Solids and Titratable Acidity) and nutritional parameters (TAC, TPH and ACY). From Figure 16 it can be noted that the parameters occupy three of the four quadrants: the vectors of Soluble Solids and Titratable Acidity are very close each other, and are placed in the upper-left quadrant. Opposite to them, in the lower-right quadrant, it is possible to find the ACY vector. Finally, also TPH and ACY are quite closer each other, in the lower-left quadrant. the two main factors in this case are responsible for the 74.19% of the population variability (46.70% + 27.49%).

The closer is a selection to one or more of the vectors representing the parameters, the higher will be the amount of that parameter in the selection.

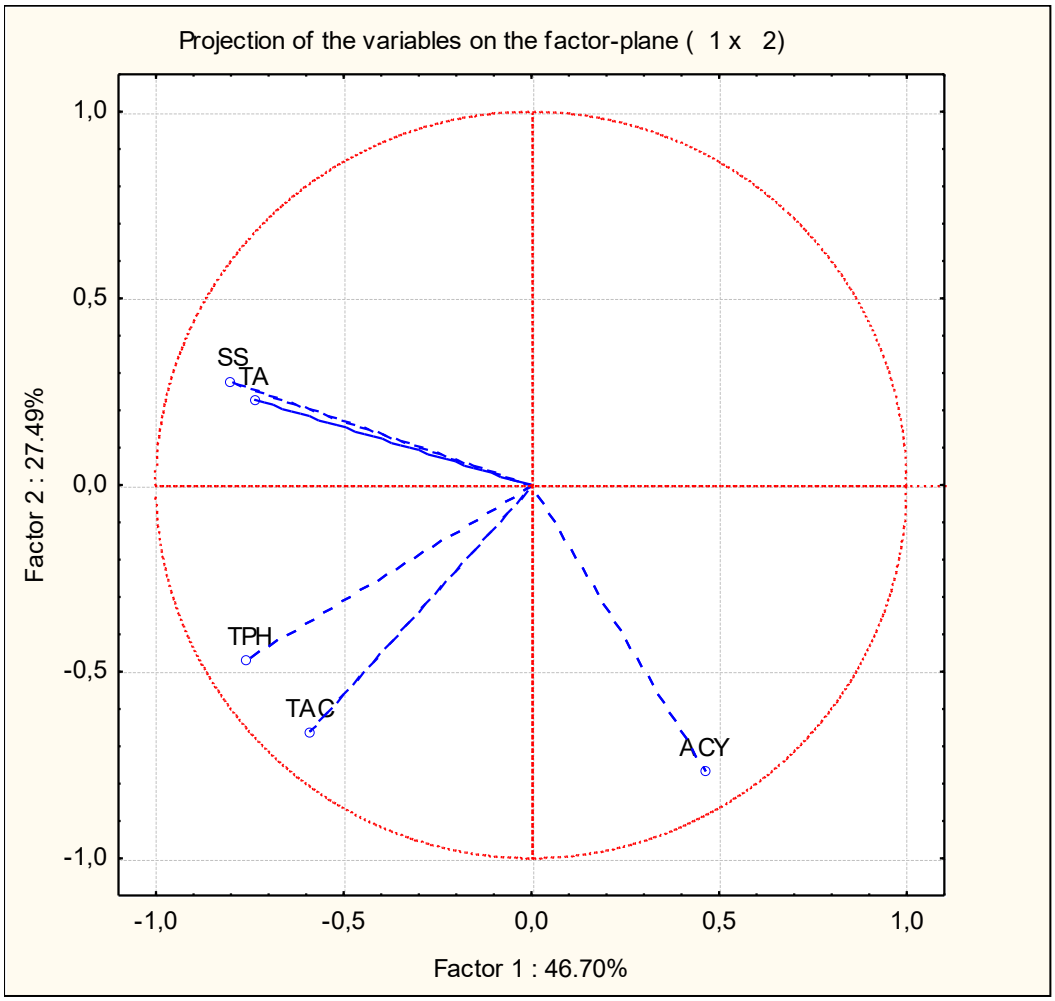

Figure 16 - PCA analysis for SS, TA, TAC, TPH, ACY

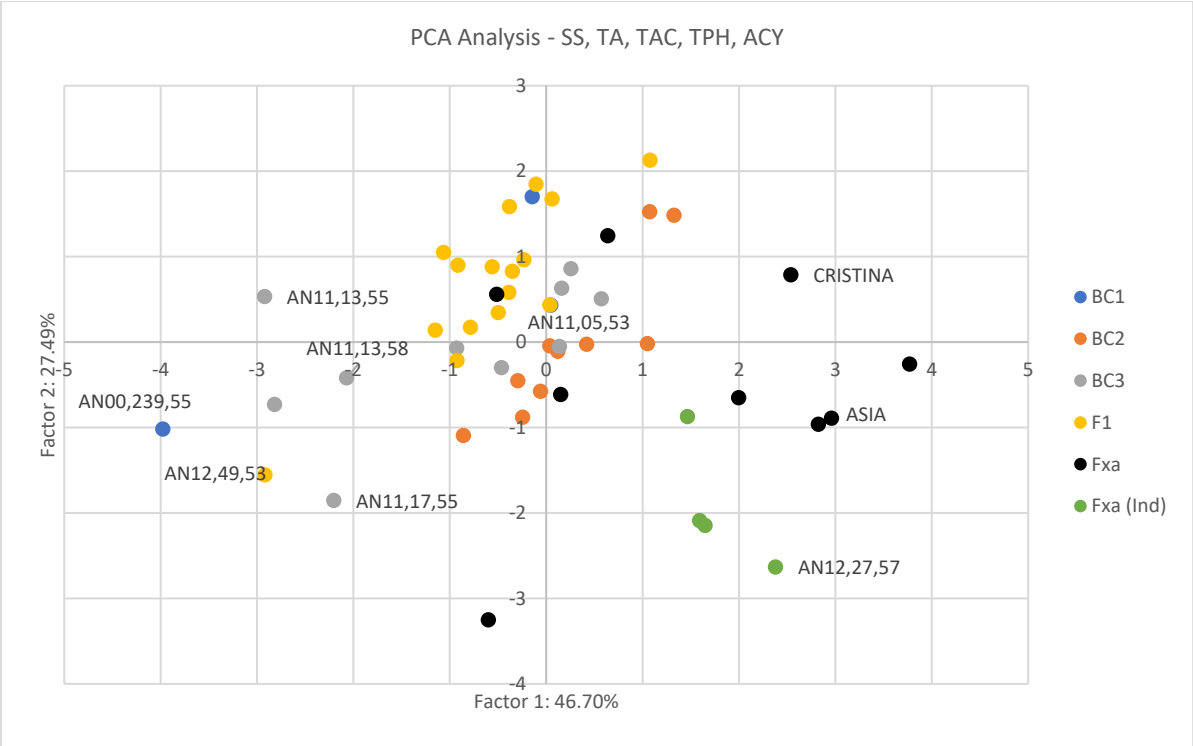

Figure 17 - PCA analysis for the observations according to SS, TA, TAC, TPH, ACY with selections in evidence.

Fxa: among the selections of this crossing type, there are none of them that stand out for one of the parameters considered. It is possible to highlight the Asia cultivar to be in line with anthocyanins and other antioxidant substances, and Cristina to be in line with the sugar and acid content, but opposite to TAC and TPH (Figure 17).

Fxa (ind): as expected, all these selections are located around the vector of the ACY, and consequently in line with the content of the other antioxidant substances. An example is the selection AN12,27,57 (Figure 17).

F1: the major part of the selections is located in the upper-left quadrant, the one where the vectors of Soluble Solids and Titratable Acidity are placed, except AN12,49,53, which is located in the lower-left quadrant around the TPH vector and very close also to TAC vector (Figure 17).

BC1: those selections are mainly located in the left part of the graph, with AN00,239,55 close to the TPH vector, and the other BC1 selections near the F1 selections (Figure 17).

BC2: these selection are located around the origin of the four Cartesian axes (as AN11,05,53), with some selections moving to the upper-right quadrant (opposite to TAC and TPH), and others moving on the opposite direction, in the same quadrant of TPH and TAC. However, no

selections stand out to possess one particular parameters among the analyzed in this PCA (Figure 17).

BC3: as happened with the previous PCAs, those selections are not grouped in one zone of the graph, but are widespread in three different quadrants. Around the TPH and TAC vectors it is possible to find the selection AN11,17,55; selection AN11,13,58 is in the lower-left quadrant, but it is closer to the Soluble Solids and Titratable Acidity. Online especially with SS and TA but also with the ACY axis we find AN11,13,55 (Figure 17).

4.3 Conclusion

From the results obtained, first of all it is clear that the harvesting year, the genotypes, and almost always their interaction too, affect the productive, sensorial, and nutritional parameters studied.

As previously mentioned, the choice of best parental genotypes for the various backcrossing generations 1, 2 and 3, allowed to obtain BC3 selections rich in antioxidant substances but also with a good recovery of productive characteristics, maintaining good sensorial properties. Among the BC3 selections, fruits of AN11,13,55 and AN11,21,59 were very interesting for their high Soluble Solids and TPH content, while AN11,17,55 showed high levels of TAC and TPH.

At this point of the breeding process, it is possible to affirm that characters for the high amount of antioxidant substances were stabilized at BC3, even with a small increase in respect to both Fxa and F1 populations; as shown above, the production in this group is not far from commercial requirements. With further increasing of the fruit weight by backcrossing with an F×a parent, which is still little due to the FVG background, some genotypes of BC3 have the potential to become commercial cultivars.

For this purpose, the best way for obtaining good productive parameters is to continue the crossing program with interesting Fxa genotypes as Nerina, Asia, Cristina, Romina and AN06,164,52 selection. For the sensorial parameters, BC3 just possess high amounts of Soluble Solids, but the Titratable Acidity should be lowered through crossing with Fxa genotypes with low acidity as Asia and Romina.

5 PART II: POLYPHENOLIC AND VITAMIN C ANALYSIS OF DIFFERENT STRAWBERRY GENOTYPES

The association between fruit and vegetable consumption and the prevention of some chronic-degenerative pathologies is a fact recognized by many parts within the scientific community (Giampieri et al., 2013). Small fruits (or berries) play an important role in the market, but above all in nutrition, both as fresh and processed products (jams, juices, jelly). Among these fruits, the strawberry is certainly the most studied from both agronomic and nutritional point of view. In addition to taste and aroma, which are particularly appreciated by the consumer, the strawberry contains many phytochemicals (vitamin C, polyphenols, folates and numerous microelements) with proven positive effects on human health (Tulipani et al 2009a; Giampieri et al., 2012). Those bioactive compounds make the strawberry by far the most wanted and consumed "berry" in the Italian market and beyond (Mazzoni et al., 2013). Among the various phytochemical compounds, polyphenols represent a large family of phenolic compounds; they are mainly represented by flavonoids (in particular anthocyanins), phenolic acids and tannins. Anthocyanins alone contribute for 25-40% to the total antioxidant capacity (Tulipani et al., 2008); consequently, it is easy to understand how important, from a nutritional and healthy point of view, this type of compounds are.

5.1 Polyphenolic compounds

Polyphenolic compounds contribute to the antioxidant properties of strawberries and belong to the group of non-nutrients. Those phytochemical molecules have a potentially positive effect on human health (Balasundram et al., 2006; Tulipani et al., 2009b; Giampieri et al. 2012, 2013; Alvarez-Suarez et al., 2014a). In plants, they occur both in vegetative and in generative parts and are non-essential compounds. As secondary metabolites, these compounds are responsible for coloration, taste and flavor of plant parts, in particular fruits, but also act as radical scavengers and metal chelators whereby antioxidant properties emerge. Furthermore, in stress situations, e.g. pathogen attack or excessive ultraviolet radiation, those antioxidant molecules play a leading role for chemical defense mechanisms (Dixon and Paiva 1995; Bravo 1998; Ozcan et al., 2014).

Polyphenolic compounds in strawberries can be basically divided into four main classes. On the basis of the amount that it is possible to find, they are: flavonoids, hydrolysable tannins (ellagitannins and gallotannins), phenolic acids and condensed tannins (proanthocyanidins) (Santos-Buelga and Scalbert 2000; Giampieri et al., 2012; Alvarez-Suarez et al., 2014a; Ozcan et al., 2014). Compounds from two of the mentioned classes, flavonoids (in particular

anthocyanins) and phenolic acids, were part of the analysis of this work and will therefore be depicted in depth below.

5.1.1 Anthocyanins

Anthocyanins belong to the class of flavonoids, the main class of phenolic compounds in strawberries, which consist of two aromatic C6 rings linked with a C3 structure. Besides the colorful anthocyanins, flavonoids comprise the colorless flavones, flavanols, flavonols and isoflavones and their glycosides (Giampieri et al., 2013; Ozcan et al., 2014). In strawberries, anthocyanins are the most represented flavonoids, followed by flavonols (especially quercetin and kaempferol derivatives) and flavanols in lower quantities (Tulipani et al., 2009b).

Anthocyanins are the glycosides of anthocyanidins. There are 23 different anthocyanidins occurring in nature, but most often glycosides of cyanidin, delphinidin, petunidin, peonidin, pelargonidin and malvidin are found in plants (Belitz et al., 2008). Pelargonidin-3-glucoside (pel-3-glu, Figure 18) is the quantitatively most important anthocyanin in strawberries, followed by pelargonidin-3-rutinoside (pel-3-rut) and cyanidin-3-glucoside (cya-3-glu). Altogether more than 25 different anthocyanins were detected in strawberries (Lopes-da-Silva et al., 2007; Diamanti et al., 2014). Cyanidin-based anthocyanins occur to a lower extent in strawberries than pelargonidin-based anthocyanins (Wang and Zheng, 2001).

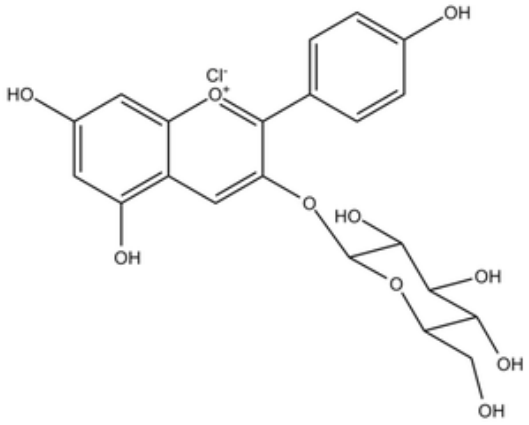

Figure 18 - Structural formula of pelargonidin-3-glucoside (www.polyphenols.com)

The main characteristic of water soluble anthocyanins is the red, blue and purple coloration of plant organs, in particular fruits. Therefore, they are the most important visible pigments in plants besides chlorophyll. Coloration attracts animals as pollinators or seed dispenser (Kong et al., 2003). Anthocyanins also protect plants from damages by excessive light, promote the

resistance against pests and accelerate the re-mobilization of nitrogen from older plant parts (Hoch et al., 2003; Tomas-Barberan and Gil, 2008). Plants' anthocyanin synthesis is dependent on several factors: among those, genetics, soil composition, ultraviolet radiation and stress such as pathogenic attack or cold temperatures (Chalker-Scott, 1999).

From the point of view of biological activity, anthocyanins have a strong antioxidant effect against reactive oxygen species. Anthocyanins are free radical scavengers compounds not only in the plant itself, but also in human body, so that foods with a high anthocyanin content have a high antioxidant potential and their consumption can help preventing cardiovascular disease, stroke and cancer (Pandey and Rizvi, 2009; Algarra et al., 2014; Rimbach et al., 2015). Typically, anthocyanin content of strawberries varies between 15 and 60 mg/100 g FW, but also higher values up to 80 mg/100 g FW were measured by some researchers (Giampieri et al., 2012).

5.1.2 Phenolic acids

Another group of phenolic compounds in strawberries is the phenolic acids. On the basis of their chemical structure, they can be divided into two subgroups. The first group consists of hydroxybenzoic acids to which belong -among others- gallic, *p*-hydroxybenzoic and vanillic acid. They have a C6-C1 skeleton in common, while the second group of hydroxycinnamic acids show a C6 ring with a C3 side chain. They occur more abundantly in strawberries. Important representatives of this aromatic group are caffeic, chlorogenic (5-caffeoylquinic acid), *p*- Coumaric and sinapic acid (Ozcan et al. 2014). Fruits rich in phenolic acids are e.g. cranberries, blackberries or dried fruits such as raisins (Balasundram et al., 2006), whereas only small quantities can be found in strawberries (Määttä-Riihinen et al., 2004; Mattila et al., 2006; Giampieri et al., 2012, 2013).

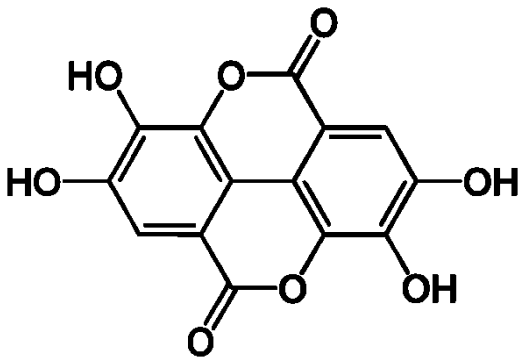

Figure 19 - Structural formula of ellagic acid (www.sigmaaldrich.com)

One of the most scarcely found phenolic acid in food is ellagic acid. However, strawberries are besides few other berry species, pomegranate and nuts, one of the fruits with a relevant quantity of this compound. Ellagic acid is a gallic acid dimer and is rarely present in its free form (Figure 19). More often, it occurs bound in ellagitannins, which belong to the phenolic group of hydrolysable tannins. Through acid hydrolysis those complex compounds decay and ellagic acid is released (Koponen et al., 2007; Tulipani et al., 2008; Nile and Park, 2014).

Biological activities of phenolic acids are ascribed to the peculiar antioxidant and anti-inflammatory properties, but they also possess anti-mutagenic, anti-carcinogenic and anti-allergy actions (Koponen et al., 2007; Giampieri et al., 2012, 2013).

5.2 Vitamin C

Research on fruit and berry nutritional value has been particularly focused on antioxidant compounds, a natural defense against aging and cell degeneration present in fruits, especially in small fruits and strawberry (Battino et al., 2009; Alvarez-Suarez et al., 2014a; Giampieri et al., 2014a,b; Forbes-Hernandez et al., 2015; Mazzoni et al., 2016). The total antioxidant capacity (TAC) represents the ability of a food matrix to exert anti-oxidative activity; it is highly dependent on the genetic basis (species-cultivar) and is influenced by cultivation techniques and growing conditions (Scalzo et al., 2005c; Battino and Mezzetti, 2006; Capocasa et al., 2008; Tulipani et al., 2008; Diamanti et al., 2012, 2014; Alvarez-Suarez et al., 2014b).

In past decades, strong attention has been given to the antioxidant power of fruit as an eligible parameter for quality. This parameter is strictly correlated to the presence of efficient oxygen radical scavengers, such as vitamin C (vit C) and phenolic compounds. In particular, a study of Tulipani et al. (2008) on different strawberry cultivars analyzed the antioxidant capacity of these fruits, investigating also the groups of compounds responsible of this activity. Interestingly, the main contribution to the TAC of extracts derived from vitamin C and other polar compounds. This evidence suggested that improving vitamin C in strawberries could be an instrument for enhancing the antioxidant capacity and for sustaining the human body functions, giving that humans do not produce this vitamin.

Vitamin C, or L-ascorbic acid, is the common name for the six carbon sugar derivative L- threo-hex-2-enono-1,4-lactone. Vitamin C is an essential metabolite for plants and animals although humans, non-human primates and a few other animals cannot synthesize this vitamin and have to obtain their vitamin C from the foods they eat. In humans, it has important biological functions: first, as previously indicated, it is a very reactive and effective antioxidant molecule,

which acts either directly or indirectly by neutralizing reactive oxygen species through enzymatic and non-enzymatic reactions (Giampieri et al., 2012). Even when present in small quantities, it plays an important role in the protection of essential biomolecules of the human body (proteins, lipids, carbohydrates, DNA and RNA) from damage by free radicals generated during the normal metabolism or in pathological conditions or through exposure to toxins and pollutants (Giampieri et al., 2012). Secondly, vitamin C is also required as an essential cofactor for the synthesis of collagen, hormones, and neurotransmitters. Furthermore, its presence seems to favor the activity of the immune system. In Europe, the recommended daily allowance (RDA) has been recently revised and increased to 80 mg daily (http://eur-lex.europa.eu/legal-content/IT/TXT/PDF/?uri=CELEX:32011R1169&qid=1492866596807&from=IT). The RDA is calculated as the daily amount needed to prevent disorders related to a deficiency in vitamin C rather than the defense of chronic diseases or the improvement of the health of the individual (Giampieri et al., 2012). Plants, the main source of vitamin C for humans, also require this compound (Pastori et al., 2003). In plants, vitamin C is a part of the antioxidant system and is important for photosynthesis and for detoxifying the free radicals generated as side products from this process. Vitamin C is also a cofactor for cell division and expansion (Noctor and Foyer, 1998; Arrigoni and De Tullio, 2000; Smirnoff and Wheeler, 2000; Pastori et al., 2003), and for many enzymes (Arrigoni and De Tullio, 2000). It also has a role in the ripening of climacteric fruits (De Tullio et al., 2004; Green and Fry, 2005) in the production of other fruit acids (Loewus, 1999; Debolt et al., 2007) and in the cellular redox system in response to plant senescence, defense, and stress (Barth et al., 2004; López-Carbonell et al., 2006; Noctor, 2006). Strawberry fruits are particularly rich sources of vitamin C, being one of the richest natural sources of this compound. A handful of strawberries is sufficient to cover the vitamin C RDA (Carr and Frei, 1999). There are several known and proposed alternative pathways for the production of vitamin C in plants, but most of these pathways in different developmental stages and tissues are not completely understood (Davey et al., 2000; Jain and Nessler, 2000; Valpuesta and Botella, 2004; Cruz-Rus et al., 2011).

5.3 Aims

With the aim of improving the amount of bioactive substances in strawberries, a more in-depth analysis on anthocyanins, phenolic acids, and vitamin C content was implemented through High Pressure Liquid Chromatography (HPLC). This part of the study was performed with 51 genotypes (46 selections + 5 varieties) already analyzed in the first part of the study. For the HPLC analysis, only fruits harvested in the year 2016 were analyzed. Among the strawberries

studied, there are commercial cultivars and UnivPM selections derived from inter-specific and intra-specific crossings. The goal was to scan these genotypes and identify their fitness for further backcrossing to increase the nutritional quality of strawberries by increasing the amount of health-promoting compounds. Even if the same genotypes were already analyzed for the TAC, TPH and ACY through spectrophotometric assays, those methods were not very specific for the compounds of interest, and they just gave an idea of the nutritional quality of the genotypes. The HPLC analysis is absolutely more specific and precise for the analysis of anthocyanins, phenolic acids, and vitamin C, and will give an exact estimation of the amount of these compounds and, therefore, of the nutritional value.

Furthermore, the genotypes will be statistically evaluated through PCA analysis by considering the anthocyanin, phenolic acids and vitamin C content together with the available productive and sensorial characters. The results of the study was the identification of some genotypes deriving from the breeding program with fruit having increased content of vitamin C combined with other important commercial parameters.

5.4 Material and methods

5.4.1 Plant material

The nutritional quality of different strawberry selections originated by the breeding program described in Chapter 3 were compared taking into account the content of anthocyanins, phenolic acids, and vitamin C. The analyzed genotypes were the same already analyzed in the I Part for the nutritional quality, and can be classified as follow:

- 16 clones of the F1 generation resulting from the cross combinations of 'Monterey' or 'Romina' with the wild species FVG (Table 19).

Genotype	Type	Mother	Father
AN12,049,52	*F1*	'Monterey'	FVG
AN12,049,53	*F1*	'Monterey'	FVG
AN12,049,55	*F1*	'Monterey'	FVG
AN12,049,56	*F1*	'Monterey'	FVG
AN12,049,57	*F1*	'Monterey'	FVG
AN12,049,59	*F1*	'Monterey'	FVG
AN12,049,60	*F1*	'Monterey'	FVG
AN12,049,61	*F1*	'Monterey'	FVG
AN12,049,62	*F1*	'Monterey'	FVG
AN12,051,52	*F1*	'Romina'	FVG
AN12,051,53	*F1*	'Romina'	FVG
AN12,051,56	*F1*	'Romina'	FVG
AN12,051,58	*F1*	'Romina'	FVG
AN12,051,59	*F1*	'Romina'	FVG
AN12,051,60	*F1*	'Romina'	FVG
AN12,051,64	*F1*	'Romina'	FVG

Table 19 - Genotypes of the first filial generation (F1): Crossings of the commercial cultivar 'Monterey' or 'Cristina' with the wild strawberry Fragaria virginiana glauca (FVG).

- 3 genotypes of the BC1 generation (Table 20).

Genotype	Type	Mother	Father
AN00,239,55	*BC1*	AN94,414,52	91,143,5
AN07,003,59	*BC1*	AN94,414,52	' Romina '
AN07,005,55	*BC1*	AN94,414,52	'Clery'

Table 20 - Genotypes of the type backcross 1 (BC1) and respective parents.

- 10 genotypes of the BC2 generation (Table 21).

Genotype	Type	Mother	Father
AN11,005,53	*BC2*	AN07,004,51	'Monterey'
AN11,005,56	*BC2*	AN07,004,51	'Monterey'
AN11,005,57	*BC2*	AN07,004,51	'Monterey'
AN11,005,58	*BC2*	AN07,004,51	'Monterey'
AN11,005,61	*BC2*	AN07,004,51	'Monterey'
AN11,005,62	*BC2*	AN07,004,51	'Monterey'
AN11,006,52	*BC2*	AN07,004,51	'Nora'
AN11,006,58	*BC2*	AN07,004,51	'Nora'
AN11,006,59	*BC2*	AN07,004,51	'Nora'
AN11,008,52	*BC2*	AN07,004,51	'Tecla'

Table 21 - Genotypes of the type backcross 2 (BC2) and respective parents.

- 10 genotypes of the backcross 3 (BC3) generation (Table 22).

Genotype	Type	Mother	Father
AN11,013,51	*BC3*	AN07,006,60	'Monterey'
AN11,013,52	*BC3*	AN07,006,60	'Monterey'
AN11,013,55	*BC3*	AN07,006,60	'Monterey'
AN11,013,57	*BC3*	AN07,006,60	'Monterey'
AN11,013,58	*BC3*	AN07,006,60	'Monterey'
AN11,014,51	*BC3*	AN07,006,60	'Nora'
AN11,017,55	*BC3*	AN07,215,55	'Monterey'
AN11,017,56	*BC3*	AN07,215,55	'Monterey'
AN11,021,59	*BC3*	AN07,216,61	'Monterey'
AN11,022,55	*BC3*	AN07,216,61	'Nora'

Table 22 - Genotypes of backcross 3 (BC3) and respective parents.

- 4 F×a advanced selections of the UnivPM breeding program for the industry (Fxa – Ind) (Table 23).

Genotype	Type	Mother	Father
AN12,027,55	*Fxa*	AN06,221,57	'Nerina'
AN12,027,56	*Fxa*	AN06,221,57	'Nerina'
AN12,027,57	*Fxa*	AN06,221,57	'Nerina'
AN12,027,58	*Fxa*	AN06,221,57	'Nerina'

Table 23 - Analysed advanced breeding selections of Fragaria × ananassa (F×a) from the Università Politecnica delle Marche breeding program, with specification of their parents.

- 5 commercial cultivar of Fragaria × ananassa (F×a): Nerina, Romina, Cristina, Asia, Alba (Table 24). Some nutritional data on these cultivars are available from Table 18.

Varieties	Description of strawberry varieties involved in the study
Nerina	Originating in Hungary. June bearing variety with regular conic fruits of dark red, suitable for industrial transformation
Romina	Originating by UNIVPM, June bearing variety with early maturation. Short or biconical fruit with high consistency and flavor. Bright red color fruits, of high antioxidant capacity.
Cristina	Originating by UNIVPM, with late ripening, with high productivity and high fruit weight, and conical fruit. It is distinguished by its particular organoleptic characteristics. It has a high content of bioactive compounds.
Asia	Originating by New Fruits Italy. High productivity even in the autumn, scalar maturation, easy to loosen, elongated cylindrical or conical fruits, with good size, bright red coloring, sweet and very aromatic flavor. It presents some critical point, as disorders of vegetative development as a result of diseases of the root system, limited consistency of the pulp and surface resistance, the coloring tends to get bogged down in the presence of high temperatures.
Alba	Precocity of ripening, high productivity, ease of harvesting fruits, very bright color, appreciable conical-elongated shape. Low consistency of the pulp and low surface strength, limited organoleptic characteristics.

Table 24 - Commercial cultivar Fragaria × ananassa (F×a)

- 3 selections Fxa: AN06,164,52 and AN08,108,56 (Table 25).

Selections	Description of strawberry selections involved in the study
AN06,164,52	IRMA x 97,169,7. Early-to-mid-ripening selection. Rustic plant with high productivity. Regular conical fruit of medium/poor size. Average quality and consistency of the fruit. Average/poor flavor.
AN08,108,56	AN00,269,51 x Sonata. Early-to-mid-ripening selection. Good growth of the plant. Primary large conical fruits, poor consistency, light color, good taste.
AN08,113,53	ANTEA x PATTY. Good shape and color of the fruits, medium fimness and high acidity.

Table 25 - Selections of Fragaria × ananassa (F×a) from the Università Politecnica delle Marche breeding program, with specification of their parents.

5.4.2 Chemicals and instruments

All chemicals and standards were purchased from Sigma Aldrich Co. (St. Louis, MO, USA), Carlo ERBA Reagents SAS (Val-de-Reuil, FR) or Merck KgaA (Darmstadt, DE). Pel-3-glu, pel-3-rut and cya-3-glu were obtained from Extrasynthese SAS (Genay, FR).

For the preparation of all aqueous solutions, ultrapure Milli-Q® (Millipore Corporation, Billerica, MA, USA) water was used (in the following simply called 'water').

Mobile phases for the high-performance liquid chromatography (HPLC) were made in the laboratory with solvents of HPLC grade and filtered with 8-12 µm particle retention paper filter discs (grade 389, Munktell Filter AB, Falun, SE) on vacuum before used.

For identification and quantification of the nutritional compounds, a Jasco HPLC system (Jasco Inc., Easton, MD, USA) was utilized. It consists of a quaternary gradient pump PU2089, the hardware interface between the PC and the system LC-Net II/ADC, a HPLC autosampler AS 4050, an ultraviolet (UV) detector UV-2070 Plus, and a computer with the ChromNAV2.0 software installed.

For anthocyanin and phenolic acid separation a Luna C18 (2) reverse phase column 250 x 4.6 mm, 5 µm particle size (part no. 00G-4252-E0, Phenomenex Inc., Torrance, CA, USA) was used. For vitamin C separation a MediterraneaSEA18 reverse phase column 150 x 4.6 mm, 5 µm particle size (Teknokroma, Sant Cugat del Vallés, Barcelona, Spain) were used. The columns was equipped with a matching precolumn (SecurityGuardTM cartridge, part no. AJO-4286, Phenomenex Inc., Torrance, CA, USA) in order to prevent the penetration of micro-

particles and to avoid the pollution of the column. For each measurement 20 µl of the sample were injected.

5.4.3 Extraction and HPLC analysis

5.4.3.1 Extraction and HPLC analysis of anthocyanins

Ten frozen strawberries of each sample were chopped into small parts and 10 g of those pieces were weighed in a Falcon® tube. The extraction was performed with 100 % methanol in proportion of 1:4 (fruit: methanol, w/v) in two steps. First, an extraction with 20 ml of 100 % methanol was conducted, followed by a homogenization with the Ultra-Turrax T 25. The suspension was then stirred continuously in the dark for 30 min at room temperature. Then, after 10 min of centrifugation at 4500 rpm (Heraeus Megafuge 16, Thermo Fisher Scientific, Waltham, USA), the supernatant was collected and stored into three amber vials. This procedure was repeated in the same way re-suspending the centrifugated pellet with further 20 ml of methanol. The second surnatant was added to the first and stored in the amber vials at – 20 °C until analysis (Diamanti et al. 2012b).

Before the HPLC measurement, the samples were filtered with 0.45 µm hydrophobic PTFE (polytetrafluoroethylene) syringe filters. The HPLC program was performed as described in Terefe et al. (2013) (changed according to Fredericks et al., 2013), through the gradient elution with mobile phase A and mobile phase B. Mobile phase A consisted of 87% water, 10% formic acid and 3% acetonitrile (v/v/v) while eluent B was 40% water, 10% formic acid and 50% acetonitrile (v/v/v). The eluting gradient was as follows: start gradient with 10% eluent B, 10 min from 10 to 25% eluent B, 5 min from 25 to 31% eluent B, 5 min from 31 to 40% eluent B, 10 min from 40 to 50% eluent B, 10 min from 50 to 100% eluent B, 100% eluent B isocratic for 5 min and from 100 to 10% eluent B in 1 min, 6 minutes of 10% eluent B for equilibrate the column. Total run time was 50 min. Flow rate was 0.8 ml min^{-1} and anthocyanins were detected through the UV detector with a wavelength set at 520 nm.

The three most abundant anthocyanins in strawberries (pel-3-glu, pel-3-rut and cya-3-glucoside) were used as standards. The identification was based on the retention time of the different anthocyanin standards. The quantification was made through the interpolation of the peaks area of the samples with the calibration curve of the anthocyanins standards, made by injecting increasing known concentrations of the standards. The sum of the concentration of the three anthocyanins results in the total anthocyanin content. All anthocyanins were calculated as

milligrams per 100 grams of fresh weight (mg anthocyanin/100 g FW). Each sample was analyzed in triplicate in the HPLC.

5.4.3.2 Extraction and HPLC analysis of phenolic acids

The samples for measuring phenolic acids were extracted and filtered in the same way as for anthocyanin analysis and analyzed according to Fredericks et al. (2013). Also in this case, phenolic acids were identified and quantified through HPLC analysis under a gradient elution with mobile phase A and mobile phase B. Eluent A consisted of 2% acetic acid in water, whereas eluent B consisted of 1% acetic acid, 49% water and 50% acetonitrile (v/v/v). The HPLC program was as follows: start gradient with 10% eluent B, from 10 to 55% eluent B (50 min), from 55% to 100% eluent B (10 min), 100% to 10% eluent B (1 min) and 10% eluent B for 5 min for re-equilibrate the column. The HPLC running was set at a flow rate of 1.0 ml min^{-1}, and phenolic acids were detected at 320 nm wavelength through UV detector. The identification and quantification of phenolic acids were performed similarly to anthocyanins, using chlorogenic acid, caffeic acid, ellagic acid and *p*-Coumaric acid as standards for creating a calibration curve. The total phenolic acid content was calculated as the sum of chlorogenic, caffeic, *p*-Coumaric and ellagic acids. All results are expressed in milligrams of the phenolic acid per 100 grams of fresh weight (mg phenolic acid/100 g FW) and each sample was analyzed in triplicate.

5.4.3.3 Extraction and HPLC analysis of vitamin C

For each strawberry genotypes, 5 fruits were picked from the -20°C storage and cut in small pieces, 1 g was weighted and 4 mL of extracting solution were added. The extracting solution consisted in MilliQ water containing 5% meta-phosphoric acid and 1 mM 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) 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 filtered through a 0.45 µm NY filter into 1.8 mL HPLC vials, and immediately analyzed through HPLC.

Vitamin C was identified and measured as described by Helsper et al. (2003). Strawberry fruits extracts were subjected to HPLC analysis immediately after the extraction procedure. The HPLC system comprised a Jasco PU-2089 Plus controller, a Jasco UV-2070 Plus ultraviolet

(UV) detector set at absorbance of 244 nm, a Jasco AS4050 autosampler and a HPLC column Mediterranea Sea C18 150×4.6 mm, 5µm particle size. The elution was in gradient with phase A (50 mM potassium phosphate buffer in MilliQ water, leading to pH 3.2 by adding orthophosphoric acid) and phase B (acetonitrile). The HPLC program was as follows: start gradient with 100% eluent A, kept for 5 minutes; than from 100 to 50% eluent A (5 min), from 50% to 100% eluent A (4 min). Vitamin C eluted at RT≈3.5 min. Quantification of the vitamin C content was carried out through a calibration curve prepared by running standard concentrations of vitamin C similarly prepared in respect to the extracts, and measured in duplicate at the beginning and the end of the analysis. Results are expressed as mg vitamin C 100 g^{-1} of fresh weight of strawberry fruits.

5.4.4 Statistical analysis

A one-way analysis of variance (ANOVA) was performed to compare the means and to find out if significant differences between the groups exist. The post-hoc Student-Newman-Keuls Test (SNK) was performed to identify the groups and the more interesting genotypes that differ significantly from each other. The significance level was always $\alpha = 0.05$ ($p < 0.05$). Principal Component Analysis was also used to test the correlation among productive, sensorial and nutritional quality of all the 51 genotypes analyzed (46 selections + 5 varieties) indicated with an asterisk in Table 8. Based on the theoretical arguments of the PCA described by Hair et al. (2005), the significant factors loading values higher than or equal to 0.7 were used to identify the most important variables and observations in each dimension (PC). Factor loading values are the correlation of each variable with the PC. They are represented as vectors (positions) in the space resulted by the axes of the bi-plot graphic. In the graphic, variables and observations that are close to each other, and in the same geometric plan of the bi-plot, are interrelated, and distant from variables and observations to which they are not related, or even negatively related. The greater is the vector (distance from the origin of the axis), the greater is the correlation of the variable with the PC represented in that dimension (axis). In the PCA graphs, each point represent a genotype analyzed, but only on the most interesting genotypes the label name was showed.

All the statistical analyses were run with STATISTICA 7.0 software (Statsoft Inc., Tulsa, OK, USA).

5.5 Results and Discussions

5.5.1 Anthocyanins and Phenolic acids

From the ANOVA analysis, considering the variable cross-type (Fxa, Fxa (Ind), F1, BC1, BC2 and BC3), both for Anthocyanins and Phenolic Acids, statistically significant results were obtained.

The SNK test with $p \leq 0.05$ was performed for total anthocyanins (sum of Cyanidin-3-O-glucoside, Pelargonidin-3-O-glucoside, Pelargonidin-3-O-rutinoside, the second one representing about the 90% of the strawberry anthocyanins). As expected, the Fxa (Ind) resulted as the group with the higher content of anthocyanins, almost double of all the other crossing types. Those strawberry genotypes were specifically selected for the processing industry (mostly progeny from Romina and Nerina cultivars) and resulted dark red in color, an absolute index of the abundant presence of anthocyanins. Following, all the other cross-typologies present statistically similar quantities of total anthocyanins, even if there is an interesting increasing trend moving from the F1 toward the BC3 population (Table 26).

The SNK test with $p \leq 0.05$ for the total content of Phenolic Acids confirmed a significant wide variability among different cross-typologies. The selections resulting to have the highest content of phenolic acids are those belonging to BC3. The typology that contains less phenolic acids is the F1 generation, which derives from crossings related to the FVG (Table 26). In this case, it is very evident how from the subsequent back crossing generation can be produced new selections with a statistically increased value of phenolic acids moving from F1 towards BC3. This improvement during the back-crossing program is probably due to the positive effect of the Fxa genotype used as parents, which showed quite high values of total Phenolic Acids.

Type	Anthocyanins (mg/100g FW)	Phenolic Acids (mg/100g FW)
Fxa (Ind)	60.37 ± 13.25^{a}	62.9 ± 6.46^{bc}
Fxa	33.98 ± 24.90^{b}	67.15 ± 18.47^{b}
F1	32.71 ± 13.61^{b}	49.47 ± 16.74^{d}
BC1	32.86 ± 11.88^{b}	53.36 ± 17.94^{cd}
BC2	34.18 ± 7.89^{b}	72.84 ± 14.42^{b}
BC3	35.68 ± 9.45^{b}	84.09 ± 19.76^{a}

Table 26 - Anthocyanins and Phenolic Acids for different type of crossing.

Focusing the attention on the total anthocyanins content detected by HPLC in different genotypes, the most interesting varieties and selections were indicated in Figure 20, together with the average total anthocyanins amounts in the different crossing types. The best genotype for the anthocyanin content resulted the Fxa variety Nerina (94.90 mg/100g FW), which is characterized by a very dark and intense red color, typical of a high anthocyanin content. This genotype is considered as a reference for the development of breeding programs aimed to increase the anthocyanin content. However, other 2 Fxa (Ind) genotypes showed a high anthocyanin content (higher than the average value of the Fxa (Ind) group), precisely AN12,27,56 (63.89 mg/100g FW) and AN12,27,57 (77.66 mg/100g FW). The first generation of the inter-specific crossing program, F1, showed three interesting selections for the ACY content, namely AN12,51,53 (57.68 mg/100g FW), AN12,49,59 (55.49 mg/100g FW) and AN12,49,57 (50.42 mg/100g FW). Among the BC1 selections analyzed, it was not possible to indicate any interesting selection for the amount of anthocyanin, giving that all the genotypes belonging to this group registered ACY concentrations lower than 50 mg/100g FW. In the successive back-crossing step, it was possible to find one BC2 selection with interesting ACY content, the AN11,05,58, that presented a value really close to 50 mg/100g FW (47.47 mg/100g FW). Regarding the last crossing generation obtained in this study, BC3, two genotypes were identified as particularly interesting for the anthocyanin content, that are AN11,13,51 (53.85 mg/100g FW) and AN11,13,52 (47.12 mg/100g FW).

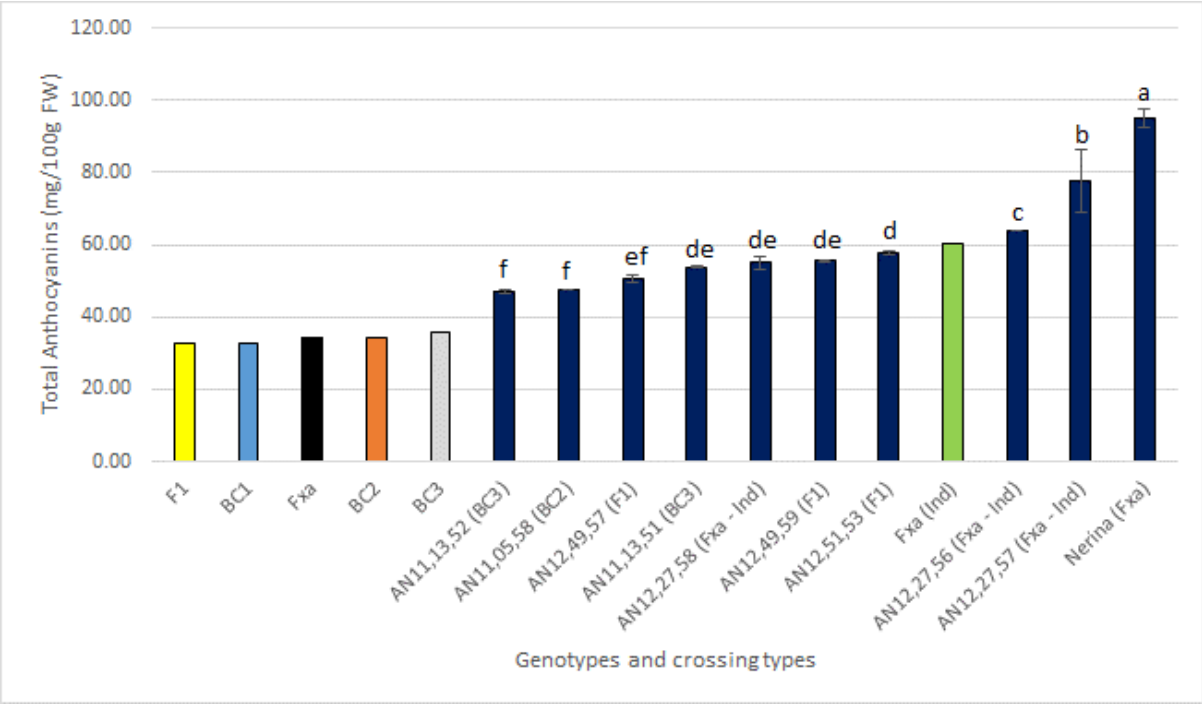

Figure 20 - Total Anthocyanins content in different type of crossing and in the most interesting genotypes

If we compare the results obtained from the ACY content of HPLC with data on TAC and TPH, we cannot find a strong relation between these two parameters. Only Nerina and AN12,49,59 can be found among the genotypes with both high ACY content and high TAC. Similarly, only AN12,51,53 and again Nerina showed high amount of both ACY with HPLC and TPH content. The HPLC analysis of total Phenolic Acids showed (Figure 21) how the breeding program was successful for obtaining BC3 selections with very high amount of this class of compounds. In fact, BC3 not only showed the highest mean value of Phenolic Acids among the different crossing types, but also produced many interesting selections with high phenolic acids content. In particular, fruit of selection AN11,13,57 showed the significantly highest content of total Phenolic acids (123.89 mg/100g FW), followed by fruit of another selection belonging to BC3 group (AN11,13,52 with 102.05 mg/100g FW) and a F1 selection (AN12,49,53 with 102.83 mg/100g FW). Fruit of other 2 selections belonging to BC3 crossing group showed very good amounts of total Phenolic Acids, in particular AN11,17,55 (96.97 mg/100g FW) and AN11,22,55 (86.80 mg/100g FW). These results mean that the breeding program have generated some selections that, from the point of view of the Phenolic Acids amount, can be considered as very interesting. If the aim is to go further with other back-crossing generation, the Fxa genotypes to cross with BC3 were not so interesting for maintaining high Phenolic Acids content. Only fruit of cultivar Alba (88.35 mg/100g FW) and Nerina (83.78 mg/100g

FW) showed high amount of Phenolic Acids and can be considered interesting as parents for segregation of progenies with fruit high content of Phenolic Acids. No selections belonging to BC1 group were detected as interesting for the Phenolic Acids content, and this is due also to the fact that, together with F1, this is the worst group for the content of this class of compounds. Differently, BC2 genotypes, the second most abundant group of Phenolic compounds, showed interesting selections for the amount of these phytochemicals, in particular AN11,08,52, AN11,05,58 and AN11,06,58, with 93.32, 88.37, and 85.54 mg/100g FW, respectively.

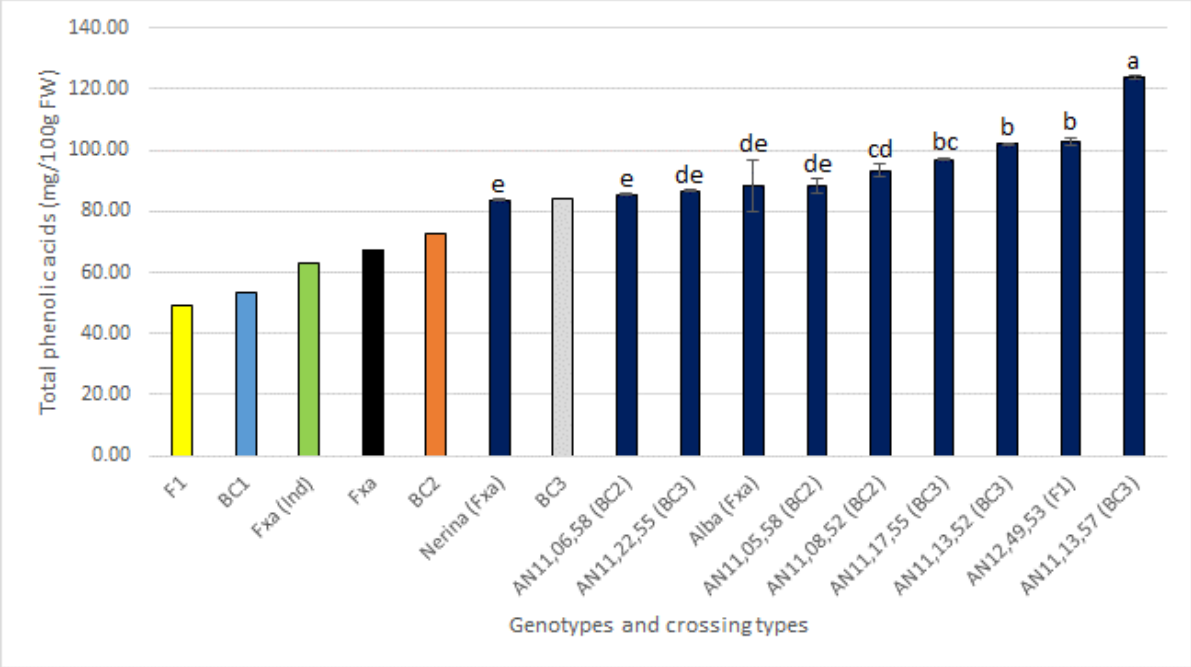

Figure 21 - Total Phenolic Acids content in different type of crossing and in the most interesting genotypes

Comparing genotypes with high Phenolic acids to those with high TAC and TPH content, it could be observed that there is a good correspondence between the phenolic acids content and the TAC, but also between phenolic acids and TPH content, which approves the importance of those phenolic compounds in contributing to health-promoting properties.

5.5.2 Vitamin C

The ANOVA test, considering the variable crossing type (Fxa, Fxa (Ind), F1, BC1, BC2 and BC3), showed statistically significant differences.

The SNK test with $p \leq 0.05$ carried out considering the different crossing types, showed the highest value for BC1, even if not statistically different from Fxa, F1, and BC3 (Table 27).

Type	Vitamin C (mg/100g)
Fxa	66.45 ± 17.23[a]
Fxa (Ind)	54.49 ± 8.92[b]
F1	62.26 ± 13.72[ab]
BC1	66.92 ± 13.59[a]
BC2	52.72 ± 7.69[b]
BC3	60.08 ± 7.69[ab]

Table 27 - Vitamin C for different types of crossing.

In this case, differences are very limited and almost always not statistically different; it is not possible to note a trend in the vitamin C content variation during the crossing program. This means that, being the vitamin C content high both in the wild germplasm (F1) and in the cultivated germplasm (Fxa), the variations are strictly related to the single parentals that are used for each crossing type. This crossing design tend to decrease the vitamin C moving from BC1 toward BC2; however, in BC3 it was possible to recover a good amount of vitamin C, higher than the mean values of BC2. This is probably due to the presence of Fxa genotypes that showed high amount of vitamin C and are suitable for crossing. To better understand this aspect, a statistical analysis was performed among the best strawberry genotypes detected in our study for the vitamin C content, and were compared with the mean values of the different crossing types in Figure 22.

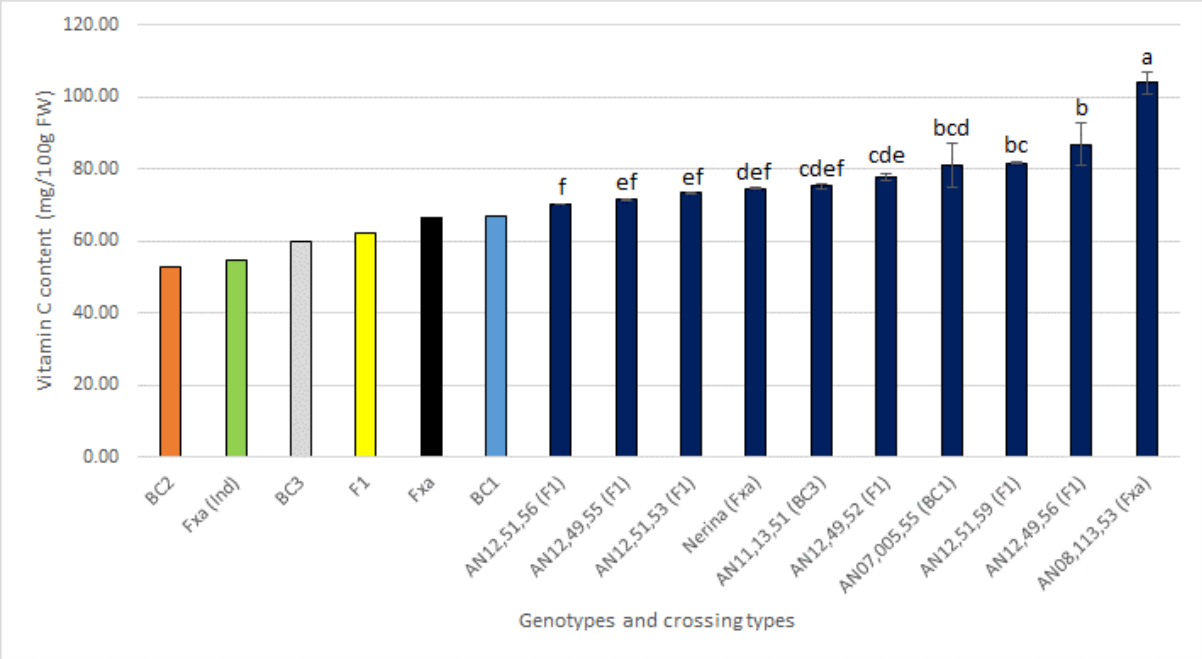

Figure 22 - Vitamin C content in different type of crossing and in the most interesting genotypes

As previously stated, the crossing type with the highest vitamin C content is BC1, in particular for the high content of vitamin C in the selection AN07,005,55 (80.91 mg/100g FW). The selection that showed the highest content of vitamin C was AN08,113,53 (103.88 mg/100g FW), which belong to the Fxa group. This data could explain the fact that the Fxa crossing type possess the second highest mean value among the evaluated crossing types. This selection, together with the Fxa cultivar Nerina (74.54 mg/100g FW), could represent a valuable choice for future breeding programs aimed to increase or maintain the vitamin C content.

Among the best selections for the vitamin C content, it is possible to find many F1 genotypes: AN12,49,56, AN12,51,59, AN12,49,52, AN12,51,53, AN12,49,55 and AN12,51,56, with 86.83, 81.70, 77.74, 73.34, 71.50 and 70.18 mg/100g FW, respectively. This is logical because they derived directly from a FVG parent, which is recognized to be a good source of vitamin C. The lower concentration of vitamin C were detected for selections belonging to Fxa (Ind) and BC2 selections, which did not present any selection with a vitamin C amount of at least 70 mg/100g FW. The final result of this breeding program is actually at the stage of BC3: the average value of vitamin C of this group reached a good value of about 60 mg/100g FW. However, this mean value is lower than the average value of Fxa, indicating that it is possible to continue the crossing process for the increase of this value in future progenies. In this regard,

the most interesting BC3 selection obtained in this study is AN11,13,51, with a vitamin C mean value of about 75 mg/100g FW.

Except Nerina, genotypes with a high vitamin C content are not necessarily among the genotypes with the highest TAC. This proofs that antioxidant properties originate not only in a high level of one compound, but in more complex interactions of various compounds.

5.5.3 PCA Analysis

5.5.3.1 PCA Analysis for Total Production, Average Fruit Weight, Vitamin C, Phenolic Acids and Anthocyanins.

Commercial parameters (Total Production and Average Fruit Weight) and nutritional parameters analyzed through HPLC (Vitamin C, Phenolic Acids and Anthocyanins content) were analyzed through PCA analysis. From Figure 23 it is possible to note that the parameters occupy all four quadrants generated by the two Cartesian axes (x, y) and that the percentage of population variability explained by the two Principal Factors is 59.48% (33.42% + 26.06%). Phenolic Acids and Anthocyanins content showed vectors close each other, even if they are located in two different quadrants. The same behavior was registered for Total Production and Average Fruit Weight, that are close each other as previously noted in the PCA of Part I of this study. Finally, vitamin C vector in located in the lower-right quadrant, far from all the other parameters and opposite to the vector representing the Phenolic Acids content. The closer is a selection to one or more of the vectors representing the parameters, the higher will be the amount of that parameter in the selection.

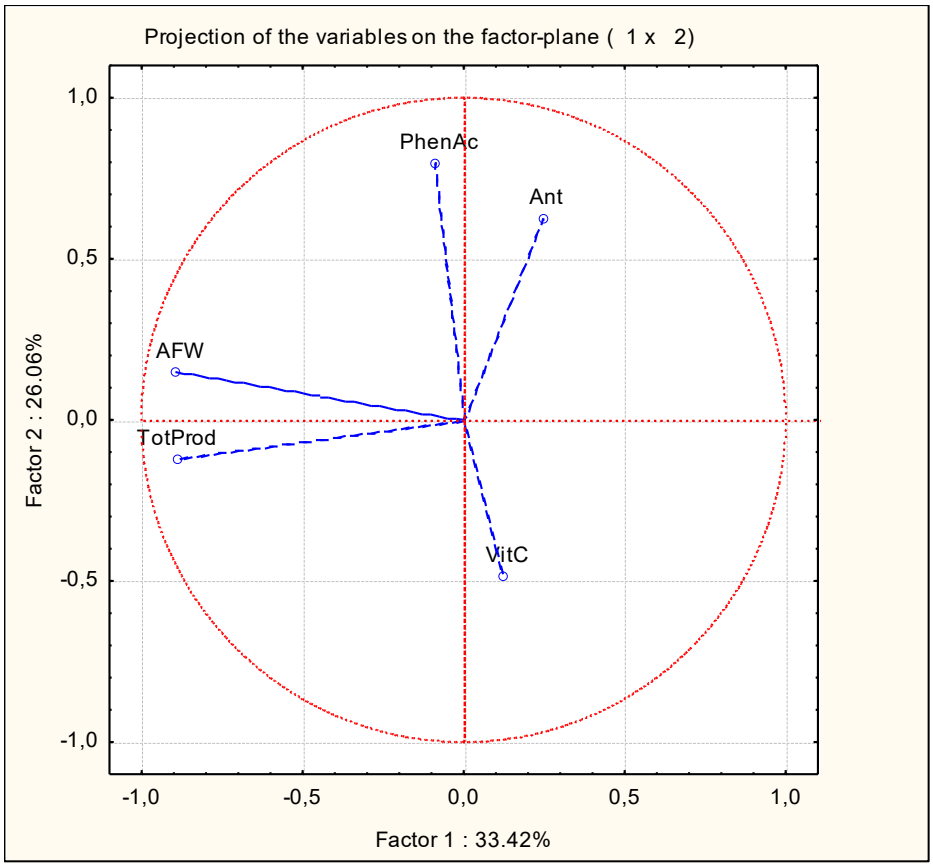

Figure 23 - PCA analysis of TotProd, AFW, VitC, PhenAc and Ant.

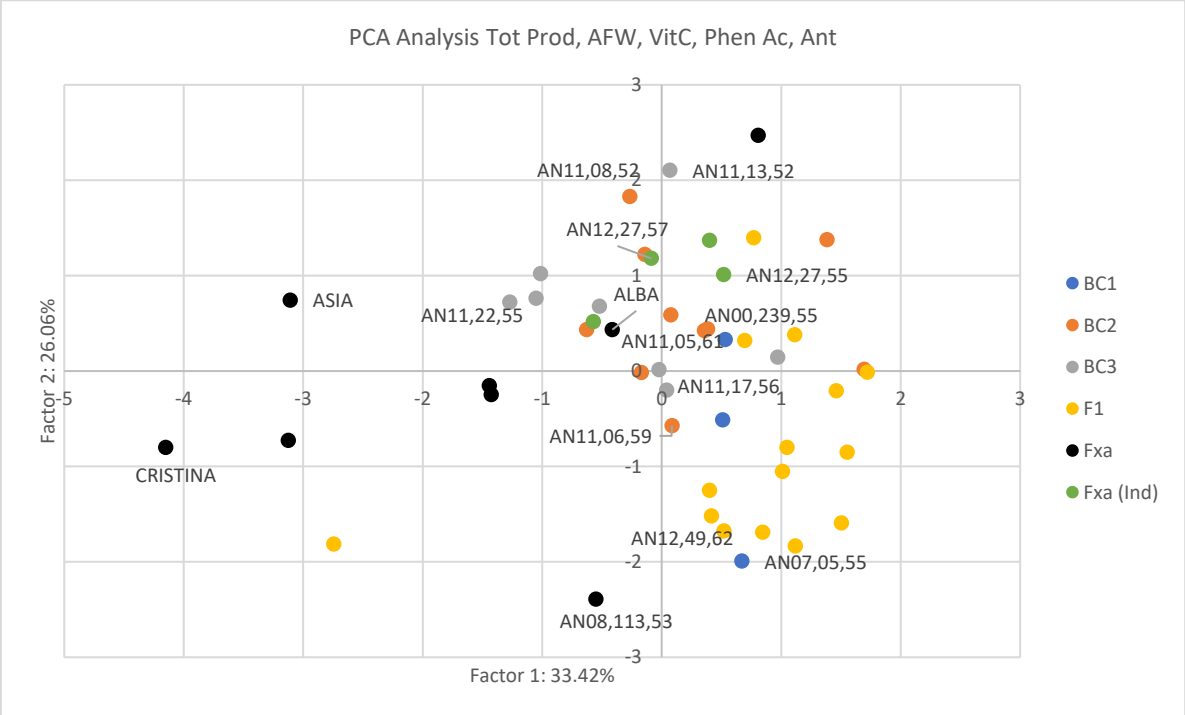

Figure 24 - PCA analysis for the observations according to Tot Prod, AFW, VitC, PhenAc, Ant with selections in evidence.

Fxa: the Fxa genotypes, as noted above, are positioned around the vector of Total Production and Average Fruit Weight. Among the varieties, stand out Cristina and Asia for these parameters. Closer to the vector of the phenolic acids it was placed the Alba variety, in line also with the anthocyanin vector. It is important to note also the presence of the selection AN08,113,53 very close to the vitamin C vector (Figure 24).

Fxa (Ind): as expected, and as previously observed, these selections are positioned around the anthocyanin vector such as AN12,27,55, but also around the vector of the phenolic acids such as AN12,27,57 (Figure 24).

F1: as previously underlined, many of these selections, which have so far been acidic, are placed around the vector corresponding to the Vitamin C content, such as AN12,49,62, while some other are more closer to the vector corresponding to the Anthocianins content (Figure 24).

BC1: the selections belonging to this cross-type are characterized by a good amount of vitamin C, such as AN07,05,55, and by the presence of anthocyanins, such as AN00,239,55 (Figure 24).

BC2: the mayor part of these selections is located in the upper part of the graph, in particular in correspondence of the Phenolic Acids vector (AN11,08,52) and Anthocyanin vector (AN11,05,61). One selection was also found quite near the Vitamin C vector (AN11,06,59).

BC3: the expected results from this last step of crossing were to obtain selections not only rich in antioxidant substances, but also in line with production parameters. From this point of view, taking a look to the Figure 24, it is clear how the crossing program, starting from the F1 selections, have ameliorated at each crossing step the presence of anthocyanins and phenolic compounds, also reaching better productive parameters, but losing some vitamin C moving toward BC3. An example of this process is the AN11,22,55 selection, which is near the vector of Phenolic Acids and, even if far from Fxa genotypes, it is approaching the vectors of Total Production and, in particular, of Average Fruit weight. For the content in phenolic acids and anthocyanins, the best BC3 obtained was the selection AN11,13,52 (Figure 24).

5.5.3.2 PCA Analysis for Soluble Solids, Titratable Acidity, Vitamin C, Phenolic Acids and Anthocyanins.

The last PCA analysis was performed comparing qualitative parameters (Soluble Solids and Titratable Acidity) and nutritional parameters (Vitamin C, Phenolic Acids and Anthocyanins content). From Figure 25 it can be noted that those parameters occupy only the two lower quadrants generated by the two Cartesian axes (x, y) and that the percentage of population

variability explained by the two Principal Factors was 57.04% (36.83% + 20.21%). Anthocyanins and Phenolic Acids vectors are located in the lower-left quadrant and, as in previous PCA, their presence in strawberries appear related. At the same time, Vitamin C, Soluble Solids and Titratable acidity vectors were in the lower-right quadrant, indicating a relation among these parameters. The closer is a selection to one or more of the vectors representing the parameters, the higher will be the amount of that parameter in the selection.

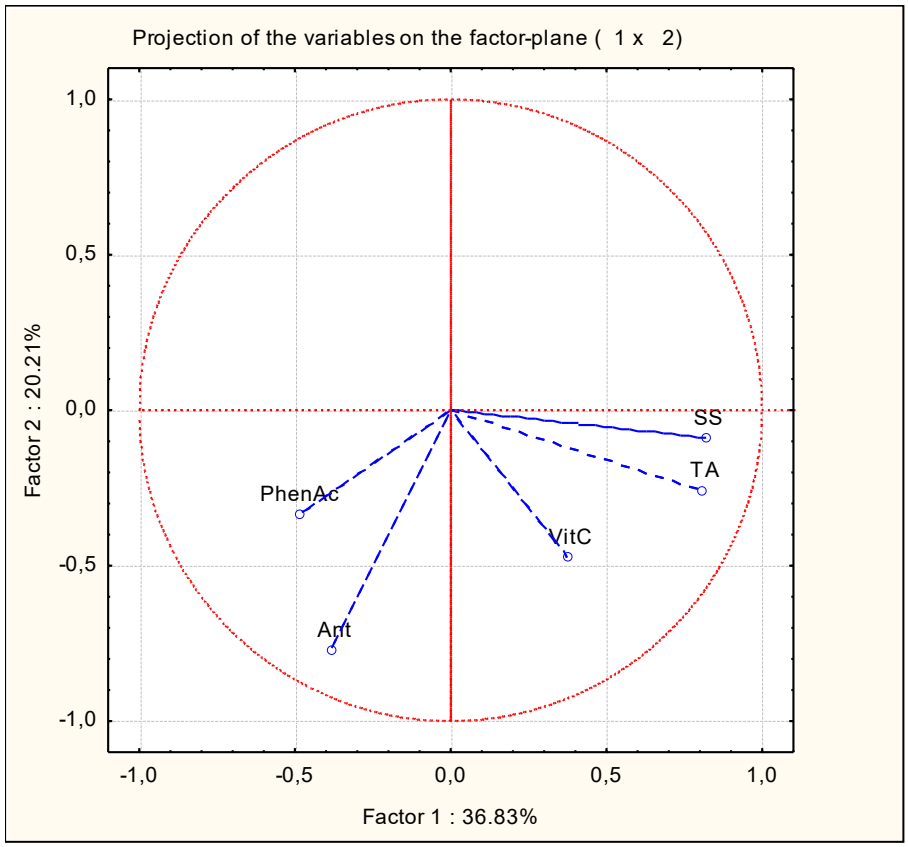

Figure 25 - PCA analysis of SS, TA, VitC, PhenAc, Ant.

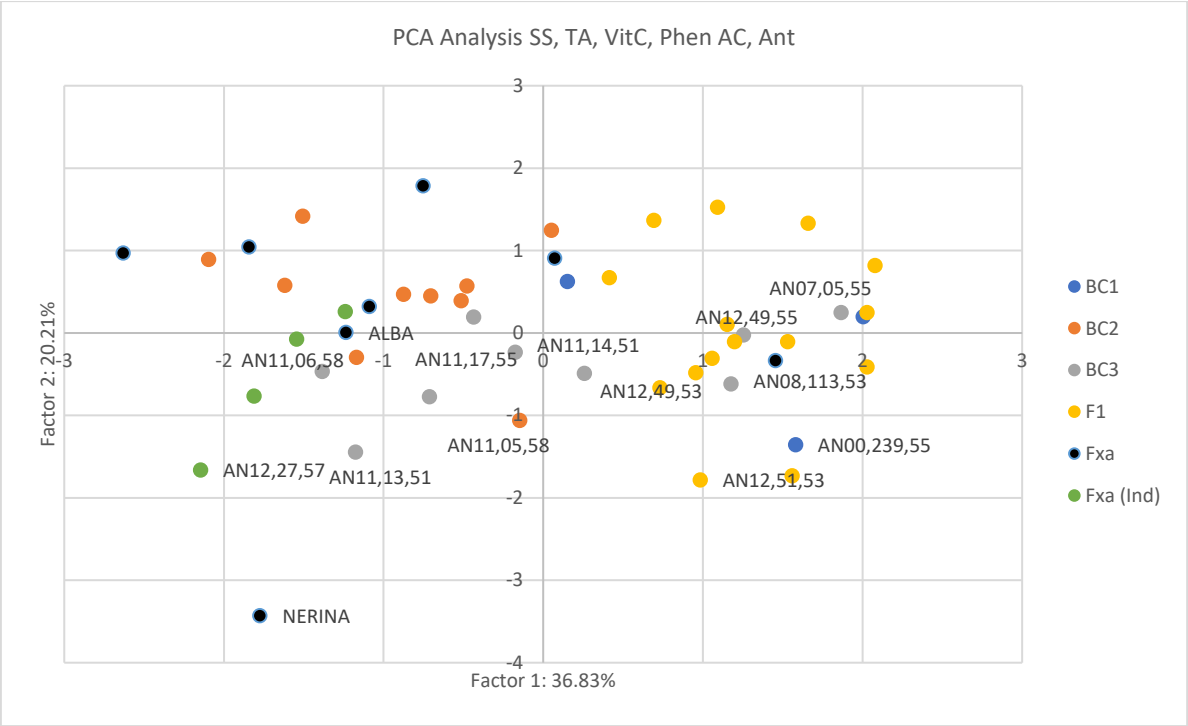

Figure 26 - PCA analysis for the observations according to SS, TA, Vit C, PhenAc, Ant with selections in evidence.

Fxa: Most Fxa selections are scattered across the four quadrants, but the mayor part rised in the upper-left quadrant, opposite to the vitamin C and to the qualitative parameters. Exception are the selection AN08,113,53, close to the vectors representing the qualitative parameters, and the variety Nerina, close to the vector of Anthocyanins (Figure 26).

Fxa (Ind): all those selections are in the left part of the graph, in particular in the lower-left quadrant, where vectors of Phenolic Acids and Anthocyanins are placed (Figure 26).

F1: these selections are all grouped in the right part of the graph, very close to the vectors of Soluble Solids and Titratable Acidity, and many of these cultivars are also next to the Vitamin C vector. The AN12,51,53 selection is closer to the vector of Vitamin C, AN12,49,43 to the Titratable Acidity and AN12,49,55 to the vector of soluble solids (Figure 26).

BC1: the selection AN00,239,55 is close to all the vectors present in the lower-right quadrant, as Vitamin C, Soluble Solids and Titratable acidity, while the AN07,05,55 selection is close to Titratable Acidity and, in particular, Soluble Solids vector (Figure 26).

BC2: these selections are located in the upper-left quadrant of Figure 26, opposite to Vitamin C and qualitative parameters vectors. However, the selection AN11,06,58 is close to the Phenolic Acids vector, while AN11,05,58 is positioned closer to the anthocyanin vector (Figure 26).

BC3: up to date, this type of crossing represents the actual last step of the breeding program of the study. The PCA graph demonstrated that the final result of the BC3 crossing it can be considered positive, giving that all the BC3 selections are distributed in the lower part of the graph. This result revealed that different kind of selections were obtained and can be used for further breeding programs, or for the creation of new varieties, according to the aims of the study. In particular, if the aim is to increase the nutritional quality, AN11,14,51 selection is close to the vector of Vitamin C, and AN11,13,51 selection is close to Anthocyanins and Phenolic Acids vectors.

5.6 Conclusions

This study was focused on the nutritional analyses performed in 2016 in samples deriving from our breeding program or utilized as parental in our crossings. This part was focused to study the variation of fruit total content of Anthocyanins, Phenolic Acids, and vitamin C, analyzed by HPLC, an accurate methodology to investigate the amount of specific compounds. The results confirm the capacity of a proper breeding program to produce new genetic material having fruits enriched with Anthocyanins and Phenolic Acids. In particular, the final selections of this study, BC3 selections, demonstrated a very high amount of phenolic acids, statistically higher than all the other crossing types. This result was obtained gradually along the back-crossing program, with the amount of phenolic acids increasing at each crossing generation until reach an average value higher than that of both the parents. In fact, both Fxa and BC2 genotypes resulted with a Phenolic acids content lower than BC3.

Regarding the anthocyanins analysis, as expected, the group of Fxa (Ind) showed a very high value of anthocyanins, almost double than all the other groups, that are statistically similar each other. As previously mentioned, Fxa (Ind) selections derive from a specific breeding program for the obtainment of dark red fruits, rich in anthocyanins, that can be able to resist to the industrial processing of transformation without suffering the browning process of the fruits, but giving a final product with a violaceous coloration appreciated by the consumer (Diamanti et al., 2016). In this contest, BC3 genotypes, even if without statistical differences, showed the highest ACY content among all the other crossing types studied in this work.

A final overview of BC3 results indicate that the crossing program was successful for the obtainment of a group of selections with increased nutritional qualities in terms of anthocyanins and phenolic acids content. In particular, the selections AN11,13,51 and AN11,13,52 resulted the more interesting for the high content of both these compounds. Their total production also result interesting according to the first part of this study (657 g/plant for AN11,13,51 and 452

g/plant for AN11,13,52), but their AWF must be absolutely improved (14.6 and 12.0 g, respectively). Also their qualitative parameters result of high interest from the part I of the study, with AN11,13,51 having 9.6 °Brix and 11.4 meqNaOH/100g juice, and AN11,13,52 having 9.9 °Brix and 12.9 meqNaOH/100g juice.

Regarding the vitamin C study, both the FVG and the Fxa genotypes were good sources of vitamin C for the development of a breeding program aimed to increase the vitamin C content. As a result, not big differences were detected among Fxa genotypes and the different stages of backcrossing program. However, it is interesting to note that, even if BC1 and Fxa genotypes showed the highest Vitamin C content, in BC2 the mean values slightly decreased. Fortunately, in BC3 generation the mean values of Vitamin C were increased, with the obtainment of very interesting genotypes, such as AN11,13,51 which represent the best BC3 selections for the vitamin C content.

Finally, PCAs were performed to evaluate the genotypes according to productive, qualitative and nutritional parameters according to HPLC analyses. Results were interesting, because it was clear that there is a trend during the evolution of the breeding program, with the progression of fruits from F1 generation, where they are more sweet, acid and with higher amounts of Vitamin C, toward fruits of BC3 generation that present less qualitative characteristics and Vitamin C content, but increased amounts of Anthocyanins and Phenolic Acids and better productive parameters, due to the increasing amount of Fxa genotype in their genetic.

As a summary of this part and the previous part of the study, the BC3 selections showed in general very good results after many years of crossing and genetic improvement. The best genotype obtained from this breeding program is actually the BC3 selection AN11,13,51 that, after optimal sensorial, nutritional and commercial (except AFW) values, it also demonstrated a very high amount of vitamin C. The only parameter that is limiting for the registration of this selection as a variety, according to the parameters analyzed in this study, is the AFW, but it is possible to try to increase this value through another back-crossing generation to obtain a selection with also this parameter improved.

6 CONCLUSIONS OF THE STUDY

PARAMETERS	F1	BC1	BC2	BC3	Fxa	Fxa (Ind)
SOLUBLE SOLIDS (°Brix)	AN12,49,53 (10.23)	AN00,239,55 (10.58)	AN11,05,57 (10.07)	AN11,13,55 (12.03)	AN08,108,56 (10.21)	AN12,27,55 (9.57)
TITRATABLE ACIDITY (meqNaOH/100g juice)	AN12,49,61 (12.00)	AN07,03,59 (12.44)	AN11,06,59 (9.58)	AN11,13,57 (10.28)	ROMINA (8.5)	AN12,27,57 (9.83)
TOTAL PRODUCTION (g)	AN12,49,61 (680)	AN07,03,59 (360)	AN11,05,58 (516)	AN11,13,52 (474)	AN06,164,52 (1159)	AN12,27,57 (575)
AVERAGE FRUIT WEIGHT (g)	AN12,49,61 (22.0)	AN07,05,55 (10.2)	AN11,05,53 (19.6)	AN11,13,51 (15.5)	CRISTINA (28.7)	AN12,27,56 (17.3)
TAC (mM Trolox eq/Kg fruit)	AN12,49,53 (31.2)	AN00,239,55 (32.4)	AN11,05,57 (29.1)	AN11,17,55 (34.2)	NERINA (38.6)	AN12,27,58 (29)
TPH (mg GA/Kg fruit)	AN12,49,53 (3415)	AN00,239,55 (3009)	AN11,05,56 (2804)	AN11,21,59 (3029)	NERINA (3035)	AN12,27,56 (2377)
ACY (mg PEL-3-GLU/Kg FW)	AN12,49,53 (534.5)	AN00,239,55 (465.6)	AN11,05,58 (458.7)	AN11,14,51 (614)	ROMINA (684.2)	AN12,27,57 (954.4)
PHENOLIC ACIDS (HPLC) (mg/100g FW)	AN12,49,53 (102.8)	AN00,239,55 (76.95)	AN11,08,52 (93.32)	AN11,13,57 (123.89)	ALBA (88.35)	AN12,27,55 (69.5)
ANTHOCYANINS (HPLC) (mg/100g FW)	AN12,51,53 (57.7)	AN00,239,55 (40.78)	AN11,05,58 (47.5)	AN11,13,51 (53.85)	NERINA (94.9)	AN12,27,57 (77.6)
VITAMIN C (HPLC) (mg/100g FW)	AN12,49,56 (86.83)	AN07,005,55 (80.91)	AN11,05,58 (61.97)	AN11,13,51 (75.35)	AN08,113,53 (103.88)	AN12,27,57 (64.67)

Table 28 – Summary of the best genotypes for each crossing group for the different parameters analysed during this study.

The results obtained in the two parts of the thesis have confirmed, first of all, that the harvesting year, the genotypes, and almost always their interaction too, affect the productive, sensorial, and nutritional parameters studied in a population of strawberry genotypes deriving from D3A-UNIVPM breeding program. The analysis of productive, sensorial and nutritional quality (in terms of TAC, TPH and ACY measured spectrophotometrically), was based on three years of study (2014, 2016 and 2017). Then, in the year 2016, a further evaluation of the nutritional quality regarding the fruit total content of Anthocyanins, Phenolic Acids and Vitamin C was performed by HPLC.

In the back-crossing program developed in this study, the choice of best parental genotypes for the various backcrossing generations (BC1, BC2 and BC3) allowed to obtain in the last generation, selections rich in antioxidant substances but also with a good recovery of productive characteristics, and ameliorating sensorial properties in respect to the previous crossing generations. At this point of the breeding process, it is possible to affirm that characters for the high amount of antioxidant substances were stabilized at BC3, even with a small increase in respect to both Fxa and F1 populations. The production in this group is not far from commercial requirements: with further increasing of the fruit weight by backcrossing with an F×a parent, some genotypes of BC3 have the potential to become commercial cultivars. In particular, from Table 28 it is possible to observe that, among the BC3 selections, the genotype AN11,13,51 resulted the most interesting for the good TAC (26.4 mM Trolox eq/Kg fruit), TPH (2208 mg GA/Kg fruit) and ACY (516 mg PEL-3-GLU/Kg fruit) values, confirmed also by the nutritional parameters measured through HPLC, in particular Vitamin C (75.35 mg/100g fruit), Total Anthocyanins (53.85 mg/100g fruit) and Phenolic Acids (81.13 mg/100g FW). Furthermore, also its qualitative parameters result of high interest, with a SS value of 9.6 °Brix and TA of 11.4 meqNaOH/100g juice. Its total production also result interesting (469 g/plant), but its AWF must be absolutely improved (15.5 g). The same consideration can be done also for other BC3 selections belonging to the same family (AN11,13 family), that possess very interesting characteristics for all the analyzed parameters, except the productive. Another BC3 selection, belonging to a different family, resulted of interest: AN11,17,55 in fact showed very high nutritional properties, in some cases also higher than the AN11,13,51 selection; however, its main defects are the low productive parameters (as all the BC3 selections, in particular for AFW) and a high fruit acidity, which is a very negative characteristic for the consumer.

For ameliorate these negative parameters of BC3 selections, another back-crossing generation (BC4) together with the best Fxa genotypes resulted from this study could be a valuable solution. For example, it could be very interesting to cross the BC3 selection AN11,13,51 with Romina or Nerina cultivar. The first one resulted of great interest in particular for its productive parameters (total production of 682 g/plant and AFW of 18.5 g/fruit) and the low fruit acidity (8.5 meqNaOH/100g juice); Nerina was very interesting for its very high TAC (38.6 mM Trolox eq/Kg fruit), anthocyanins content (94.90 mg/100g FW), and for the productive parameters (total production of 606 g/plant and AFW of 18 g/fruit).

This study could be considered important for these purposes:

- The obtainment of new genotypes with improved productive, sensorial and nutritional characteristics;

- The identification of cultivar and selections to utilize as parents in successive strawberry breeding programs, to reach the aim of the back-crossing program of this study that was the production of new improved varieties from the point of view of agronomic characters and of the overall quality of the fruit.

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