DEPARTMENT OF AGRICULTURAL, FOOD AND ENVIRONMENTAL SCIENCES

MASTER'S DEGREE COURSE: FOOD AND BEVERAGE INNOVATION AND MANAGEMENT

Yield and fruit quality variation in strawberry pre-breeding material and advanced selections.

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Chapter 1: Introduction

Nowadays lifestyle is changing from last decades. People are getting more aware of their health being and pay attention to the food that they introduce to their meals. That explains the growing interest of scientific research to invest more in foods with high health benefits, nutritional properties, and rich composition in functional components. The cultivated strawberry (*Fragaria* x *ananassa)* is one of most widely grown fruit crops in the world due to its flavor, appearance, sweetness, and its bioactive compounds composition, mainly anthocyanins, antioxidants, fiber and ellagic acid, which also brings the nutritional value for consumer health. This led to the increase of consumer demand during last years. In 2012, the world strawberry harvested area was 241000 ha, and in recent years its cultivation areas have increased significantly (FAOSTAT, 2014). In Europe, in 2015, the apparent consumption of strawberries was 1.2 million tons, with an average growth trend of 13% over the last 5 years. In 2018, approximately 1.3M tons of strawberries were produced in the European Union. The total output volume increased at an average annual rate of +1.6% over the period from 2007 to 2018. The general positive trend in terms of strawberry output was largely conditioned by a mild expansion of the harvested area and measured growth in yield figures (Global Trade Magazine, 2020). This global growth of the strawberry is determined by the increased consumer attraction towards this fruit and to the decrease in the purchasing price. Mainly strawberries are bought at retail outlets: the supermarket is at 36.4%, the superstore to 25.1% and 10.7% at discount stores. Traditional markets and the free service are declining.

The yield and quality of strawberries is highly related to different factors such as genotype, environment factors, cultivation techniques and adaptability to different biotic and abiotic conditions. In this study, we are going to evaluate the effect of the genotype on strawberry production and fruit quality, evaluating the effect of the introgression of wild germplasm in the breeding program of UNIVPM-D3A.

Chapter 2: Literature Review

1. Botanical classification of strawberry

Strawberry (family *Rosaceae*, genus *Fragaria*) is the edible fruit of more than twenty species of flowering plants.

The modern cultivated strawberry *(Fragaria x ananassa,* Duch.) is a hybrid of two largely dioecious, octoploid species*, Fragaria chilonesis* Duch *and Fragaria virginiana* Duch.

Strawberry is composed of five basic anatomical structures: leaf, root system, crown, stolon or runner, and daughter strawberry plant. The figure1 shows the different anatomic structure of strawberry.

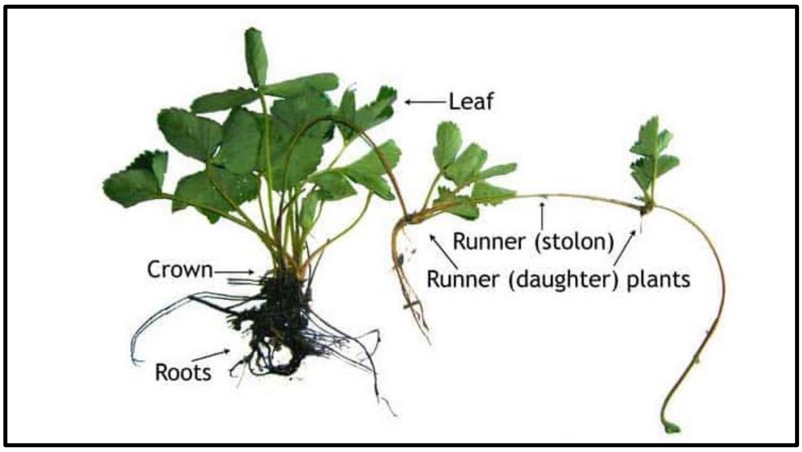

Figure 1: Strawberry plant anatomy.

First, the roots absorb water and nutrients from the soil to facilitate growth and reproduction, and are also involved in photosynthesis, together with the leaves. The productive engine of a strawberry plant is represented by the crown. It is where the runners and the inflorescences are produced. Containing the growth energy of a plant by clipping runners and early flower buds can cause crown multiplication, which will often result in more and higher quality plant in next years.

The leaves are subdivided in three folioles with sawed edges. The thickness varies according to the cultivar and their color is intense green.

The runners permit to maintain the daughter plants until their root bud meets soil and establishes an independent root system. At this level, the runner will dry, and eventually separate completely leaving a new and independent strawberry plant clone.

The flowers are grouped in inflorescences. They can be perfect with masculine and feminine organs or imperfect that means unisexual. Most of the commercial strawberries that are cultivated have perfect flowers. Each perfect flower has a calix with 5 sepals, a corolla bearing five white petals with variable shapes (rounded or oval). The receptacle contains the feminine organs or pistils. If the climatic conditions are not so favorable, a part of pistil may not be fertilized and therefore fruits obtained will be deformed. The male part of the flower is represented by the stamens which contain the pollen to fertilize the stamens. Then, the fecundation generates the strawberry fruit. The edible fruit is the false fruit formed by enlargement of receptacle tissue, while the real fruits correspond to the achenes.

The fruit shape can change according to the cultivar, and we distinguish the conic, long-conic, oblate, globose, globose-conic, necked, long-wedge and short wedge that are illustrated in figure 2.

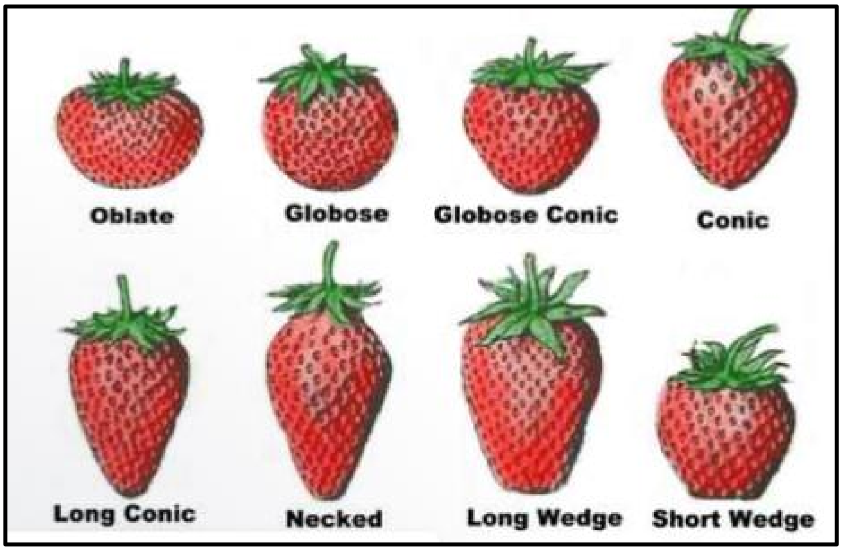

Figure 2: Different shapes of strawberry fruit.

2. Classification criteria and cultivars

The cultivated octoploid strawberries, *Fragaria* x *ananassa*, can be classified based on their flowering difference so the photoperiod in which they can initiate flowers. Different terms have been used to describe the flowering. We have short day, once flowering, seasonal flowering, single cropping plants, or June-bearing that have been used to describe plants which bloom in spring and produce one fruit crop per summer. Everbearing, day-neutral, perpetual flowering and long day plants have been used to describe plants which flower multiple times and produce multiple crops over summer. Seasonal flowering or single cropping or June bearing, and perpetual flowering are mostly chosen to describe strawberries according to their horticultural performance (Gaston et al., 2013).

Floral induction in mono-flowering is usually considered to be under short-day (SD) and low temperature control (Darrow and Waldo, 1934).

The flowering habit of strawberries is under qualitative or quantitative genetic control (Sonsteby and Heide, 2017). Moreover, under long-day conditions for more than 14 hours daylight, seasonal-flowering (SF) individuals behave as perpetual-flowering (PF) plants at low temperatures (Darrow, 1936; Darrow and Waldo, 1934).

Darrow and Waldo (1934) described ever bearers as long-day (LD) plants, since these cultivars initiate flowers during long days under favorable temperatures. Later, different publications can be found using the term “day-neutrals” (DN) for cultivars which can initiate flowers independently from photoperiod (Bringhurst and Voth, 1980; Nicol and Galetta, 1987).

The classification based on photoperiodic response of flower initiation is complicated since temperature also plays an important role. For June bearers, many research already demonstrated that the relation between photoperiod and temperature is important for flower initiation and may vary among cultivars (Ito et Saito, 1962; Heide, 1977). June bearers can initiate flowers independently from photoperiod when temperature is low enough (Guttridge, 1985). In fact, these studies demonstrated that all repeated flowering cultivars can respond as qualitative LD plants at high temperatures (>25°C), as quantitative LD plants at lower temperatures (10 to 25°C), and as DN plants at temperatures below 10°C (Heide et al., 2013).

3. Cultivation techniques

Strawberry cultivation is continuously increasing all over the world due to the varietal innovations obtained by numerous breeding and biotechnology projects and to the studies performed to discover more knowledge on plant’s physiology to develop innovative cultivation systems.

The strawberry plant is adaptable to different type of environment. It is cultivated in Europe, USA, China, and North Africa areas that have different geographical characteristics (Wang *et al.*, 2015).

For growing the strawberries, first criteria to be considered is the soil. For this type of fruit sandy or loam soils are favorable for a better growing, because they provide good drainage and warm up more during the day, which is important during cooler parts of the growing season.

Strawberry plant generally prefers sub-acid or acid soils with pH between 5.5 and 7. It is important to level the soil if it did not host any strawberry before since it facilitate the excess water flowing. Then, it is possible to apply an organic fertilizer for better development of the roots (figure 4).

Figure 3: Levelling the ground for strawberry cultivation.

When strawberry fields are prepared for planting, the soil should be fumigated using approved broad-spectrum pesticide to kill soil organisms that are harmful for the plant development and lead later to the decay of the plant. As an alternative, the rotation technique is also important to set up strawberry fields between different cultivation seasons, changing the cultivation site each year with a shift of 3-4 years, to avoid planting stress problems on the new plants. The rotation provides better soil quality and reduces the risk of introduction of pathogens into the plant. The rotation can be realized using pea and beans because they are able to improve the structure, enrich the soil nutritional components and reduce infestation. In some environment, in particular in the South, where the summer is particularly hot and the solar radiation high, the solarization could substitute the rotation. Solarization is a sterilization method of the soil where are used green or transparent polyethylene films to cover the soil for 4-8 weeks. This technique is efficient against pathogens.

The next step is to create the baulate field for plant cultivation at a height of 10 to 30 cm from the ground. This type of soil provides a protection from fungal infections and water stagnation (Muñoz, 2017). Beds have to be covered with plastic films which limit the fruit contact with the soil, preventing the infections of pathogens and the attack from harmful organisms, together with creating a favorable micro-environment for the root system and limiting the insurgence of weeds (Figure 4). Generally, opaque liners are used that provide different degrees of heat absorption.

Figure 4: Mulching plastic film for strawberry cultivation.

Water source for irrigation depends on the growing region and resources available to the farmer. Generally, water is delivered to the plants via drip irrigation system. These systems use rubber or plastic tubing buried in the raised beds so that water can be delivered to roots. This type of irrigation protects fruit from contact with irrigation water because it could transmit pathogens or can cause premature softening.

Usually, the fertilizers can be supplied to the plant through the irrigation water by injecting it to the water, this can be named as chemigation.

The plantation period always depends on the cultivation technique used and the geographical area. For northern regions, the plantation initiates from mid-June while for southern it begins from mid-August. Then the fruit harvest is a very crucial step and has to be well cared. Harvesting time is marked when the fruit reach a very red color in the different sides so in the overall surfaces. While harvesting, the fruits that are damaged because of sun or insect or fungal attack and the deformed fruits should be separated from the commercial fruits and have to be discarded/sold apart. Harvesting is generally done manually to avoid destruction of the fruit structure if it is destined to a fresh consumption, while they can be used mechanical methods if the fruit will be processed. The figure 5 shows the manual harvesting at the field.

Figure 5: Manual Harvesting of strawberry fruit.

4. Breeding Program and Cultivars

Biodiversity is a set of evolutionary processes and is defined as the whole of animal and vegetal species in the world. Biological diversity is necessary for human being. It is a complex resource indispensable for all survival organisms in the ecosystem. It is represented by the germplasm of cultivated plants that characterize the total genetic variability for a specific population (Mathey, 2017).

Strawberry germplasm is a more complex notion, since the commercial strawberry "*Fragaria x ananassa*" is not an old species. It was generated by chance in the mid-late-1700s from the accidental inter-specific cross among *Fragaria chiloensis* and *Fragaria virginiana*. Since the first decade of 1800s, breeding programs have begun to improve production yields and fruit quality. The strawberry breeding brought a huge variability among the commercial variety. In fact, new genotypes obtained by European and north American breeding programs in the nineteenth and twentieth century were cultivated all over the world until the beginning of twenty-first century, but a huge number of new genotypes developed were discarded and replaced with new genotypes showing better agronomic and qualitative characteristics. This approach induced a high reduction of strawberry genetic resources in the breeding pool, causing a huge loss of some characters, which could now have an important role for the development of new cultivars, with increased fruit quality and nutritional value (Mezzetti et al., 2018).

In Europe, there are many strawberry germplasm collections (Simpson, 2003). Germplasm collections are optimized in order to promote connections and exchanges among the germplasm collections already available. Thus, to create system that cover all information about pedigree, phenotypic data, regional adaptation, genotype, and breeding value (Mezzetti et al., 2018).

Due to the high costs of implementation and the high price of final product, it is crucial to choose the right cultivar for the strawberry production chain. It is crucial to choose the cultivar according to its adaptability to the different cultivation systems and market destination (Duarte Flho et al., 2007; Ruan et al., 2013). The selection of new stable strawberry cultivars for field condition offer the possibility to increase production efficiency, safety and quality for the consumer (Amarjeet et al., 2017).

The large variability among the species that compose the genetic base of *Fragaria x ananassa* allows a greater range of adaptation and quality of commercial strawberry cultivars (Passos, 1999; Santos & Medeiros, 2003).

The main objective of Genetic improvement programs is to obtain cultivars with plants that are easy to handle (small and erect), resistant to pests and diseases, high yielding with better quality, bearing big fruits with a good appearance and sweetness (Rios, 2007).

There are more than 40 strawberry breeding programs around the world, wherein considerable genetic variation in the available germplasm is of great economic interest (Chandler, Folta, & Dale, Whitaker, & Herrington, 2012).

The most marketed strawberry all around the world is *Fragaria* x *ananassa*, that's why main of the breeding programs were focusing on improving it to obtain better and stronger plants. Nowadays, breeding programs are giving better results because strawberry has a high ploidy value (eigh-ploidy) so it can interact with the surrounding environment and this leads to a wide phenotypic variability (Mezzetti *et al*., 2010).

In the last decade, a European project, financed by EU DG Agriculture, on berry genetic resources (Geneberry), was focusing on increasing the strawberry genetic material available in the different European collections to better characterize the available germplasm using advanced molecular tools (Bordeaux). The project has identified several research stations, located in different EU countries that will be responsible for strawberry germplasm collection and preservation (figure 6). The aim of this project was to bring clear and beneficial results through their application by breeders for the improvement of berry fruit taste, nutraceutical, and nutritional quality, meanwhile verifying the plant adaptation to different climatic conditions and cultivation systems (Mezzetti et al., 2018).

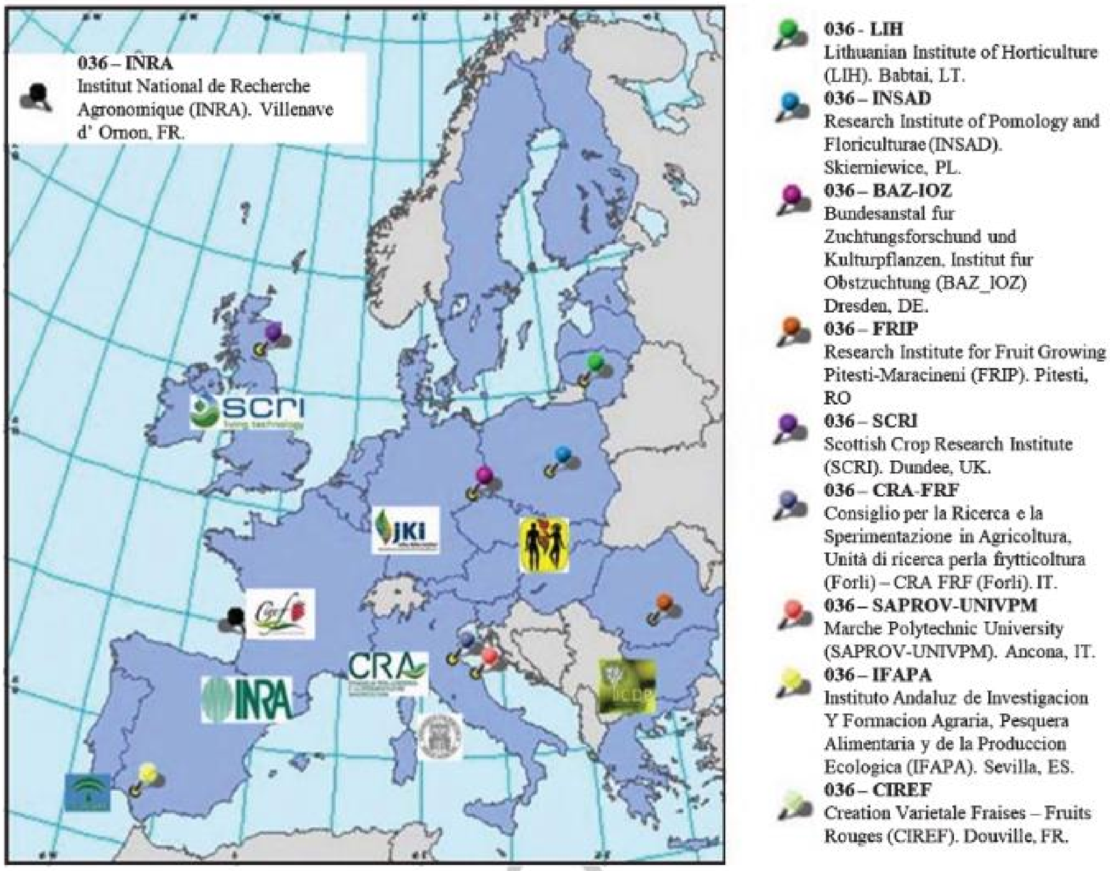

Figure 6: List of locations of the EU strawberry germplasm repository (Mezzeti et al., 2018).

Working on improvement strawberry genotypes lead to increase market position of this type of fruit by strengthening the quality traits including nutritional, nutraceutical and tasting characteristics.

Therefore, to develop a breeding program in order to obtain new genotypes with improved agronomic performance as well as fruit nutritional quality Diamanti et al. (2011) suggested that 10 criteria have to be considered for the acceptance of the new genotypes in the market. These criteria include:

1. crop productivity (i.e. yield) that must be maintained or increased to guarantee widespread farmer acceptance;

2. plant resistance/tolerance to pathogens that has to be increased so to reduce inputs requested for cultivation and fruit post-harvest lost.

3. plant adaptability to local cultivation conditions (soils and climates) and systems (open field, protected, soil-less);

4. plant habits that result in “easy picking”, it is preferred that fruits are brought away from center of foliage and easy to find and pick (in some areas are preferred cultivars which have single stems that stick out from the plant rather than trusses of fruit);

5. season wide production rather than a June bearer heavy producer (many areas are now picking the same plants for eight months in summer), now mostly achieved with the introduction of everbearing/day neutral plants so to expand plant ripening seasons;

6. high fruit sensorial quality, mainly due to increased content of soluble solids and aroma volatiles;

7. Uniform shaped, large fruit, in some areas even above 30 grams to reduce labour costs;

8. High fruit firmness to reduce damage during picking and long travel shipment;

9. Fruit color that is also critical and sometime influenced by local custom, although in intensive cultivation the reddish fruit color is preferred to the orange;

10. The micronutrient enrichment traits that must be relatively stable across various edaphic environments and climatic zones and must have significant impact on human health.

Breeding programs always ensure a continuous release of new cultivars that are tested in public and private research stations and centers located in different countries, by using the standard local cultivations systems (open field or protected), sometimes combined with advanced soilless technology. This induce a release of high number of cultivars even though the marketed is small or limited because in certain cultivation area and period it can still produce a good return and high yield in royalties to breeders (Mezzetti et al., 2018).

The D3A Department of Università Politecnica delle Marche (UNIVPM) has been part in strawberry breeding programs since many years in order to produce and release new genotypes with high yield capacity, nutritional and nutraceutical value and adaptability to resilient conditions. The breeding programs have been working on maintaining a continuous improvement and correlation between high production and high quality which is a complicated and hard work. Consumer conscience has increased last years about health issues and food benefits and importance. This led to investigate more in fruit quality, providing them with different varieties placed in the market that obey to their expectations and knowledge. For this reason, the D3A department has concentrated its efforts on breeding programs to improve more the concentration on nutritional and nutraceutical compounds present in strawberries such as ascorbic acid (vitamin C), folates (vitamin B9) and antioxidants. However, also the improvement of resistance to pests and pathogens are major objective of D3A breeding program. Since the D3A has been working on breeding for longer years, it was able to release eight new cultivars ("Adria", "Dina", Francesca", "Cristina", "Lauretta", "Romina", "Silvia", and "Sveva") and hundreds of new selections currently evaluated for their high commercial value.

5. Strawberry quality traits

To create a new cultivar, it is necessary to evaluate the yield and quality characters under certain conditions. They are the ultimate goal of any crop production and improvement of fruit quality (Rahman et al., 2015). In particular, sensorial and nutritional quality can be defined and measured through the analysis of many parameters, that will be reported in detail in the following chapters.

a. Sensorial quality

- **Total Soluble solids and titratable acidity**

Sensory attributes are important aspects of fruit quality. Color, texture, odor and the balance between sweetness and sourness have been demonstrated as very important determinants of overall quality of strawberry fruit (Shamaila et al., 1992). Thus, the flavor is one of the most important properties that gives a high commercial value to the fruits in general and consumer acceptability. Strawberry flavor is conditioned and defined as a balance between sugars and acids expressed in ripe fruit (Shaw, 1990). Brix is determined by analyzing pulped and filtered berry tissue and gives an indication of total soluble solids (TSS). Since time ago, sweetness has not become the primary concern during breeding and often TSS was adversely affected by increases in berry size or yield (Faedi et al., 2002). Consequently, commercial cultivars demonstrate large variations in their content.

Strawberry fruits have an initial phase of growth and enlargement followed by a maturation phase, during which the fruit starts to ripen. Ripening itself is known by a set of physicochemical changes characteristic of the fruit. Concentration of TSS and acidity changes with the ripening and stage development of the fruit.

Figure 7 shows the different stages of growing and ripening for strawberry fruit.

The stages below are characterized by the following: green (in growth phase, receptacle distinguishable from seeds), white (in ripening phase, achenes no longer tightly packed), turning (in ripening phase, fully expanded and 50% red) and ripe (in ripening phase, fully expanded and 100% red). The developmental phases are characterized by different biochemical processes and the development of strawberry fruits can be viewed as a discontinuous process (Montero et al., 1996). Then, the accumulation of TSS and acid increase with ripening.

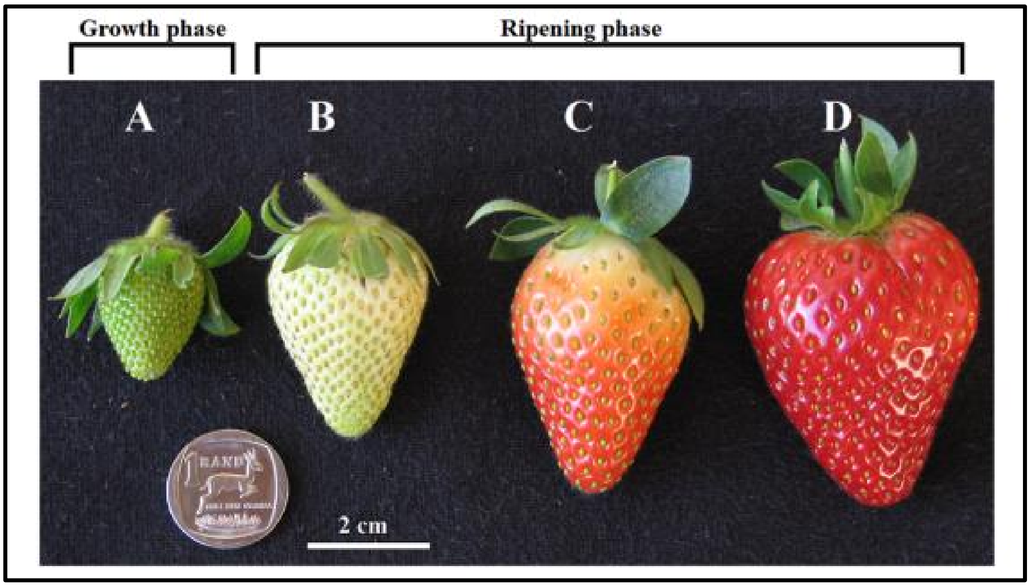

Figure 7: Developmental stages of fruit maturation A, green; B, white; C, turning; D, ripe.

Many research has demonstrated that fruits like strawberry accumulate sugars such as glucose, fructose and sucrose and organic acid mainly citric and malic acids (Montero et al., 1996; Serradilla et al., 2011). Strawberries accumulate sugars and organic acids, mainly citric and malic acid, during berry ripening (Montero et al., 1996; Ménager et al., 2004). However, there are some qualitative and quantitative variations concerning the composition of individual sugars and acids according to each genotype and maturation stages thus affecting the overall nutritive quality and consumer's acceptability of the fruit (Azondalou et al., 2003; Usenik et al., 2008).

For strawberry, sucrose, glucose, and fructose account for more than 99% of the total sugars in ripe fruit, with sorbitol, xylitol and xylose occurring in trace amounts (Maniken and Siiderling, 1980).

Kafkas et al. (2007) have proven that for most of the strawberry genotypes studied, fructose was the predominant sugar and its content increased with ripening. Few data are available on changes in sugar content during the ripening of strawberry. While for the citric acid, the change with the level of ripening varied with the genotype, instead for malic acid the concentration did not change during ripening. Another study of Basson et al., (2010) has showed that glucose was the predominant sugar, and it is consistent with the study of Kafkas et al. Its concentration increased with ripening.

Acids can affect flavor directly and are also considered as an important parameter in processing since they affect the formation of off-flavors and the gelling properties of pectin. In addition, acids regulate cellular pH and may influence the appearance of fruit pigments within the tissue. Few studies have been made on the changes in acid content in soft fruits during ripening. Studies have demonstrated that the main acids in strawberry are citric and malic acids; glycolic and shikimic acids are also present but in lesser quantities (Woodward, 1972). Also, this fruit constitute an excellent source of ascorbic acid (Lundergan and Moore, 1975). Previous studies on this fruit indicate that the total content of sugars increases rapidly during ripening. While, the total acidity decreases during ripening, but ascorbic acid increases (Avigdori-Avidov, 1986).

It is crucial to determine the best stage at which to harvest the fruit. Attempts have been made to assess the stage of ripeness of strawberry fruits in terms of titratable acidity or sugar/acid ratio.

b. Nutritional composition of strawberry

The consumption of fruit and vegetables is increasing more and is the way to increase the intake of antioxidants, in particular through the consumption of berries, which are rich sources of compounds with antioxidant activity (Prior et al., 1998, Scalzo et al., 2004). The fruit nutritional value is highly affected by the type of fruit, species, and variety within species, but it can be also affected by environmental conditions and cultivation techniques (Scalzo et al., 2005).

Berries, in particular strawberry fruit, are known for their important nutritional value and as a great source of flavonoids and other bioactive compounds. The levels of antioxidants and the corresponding Total Antioxidant Capacity (TAC) in strawberry extracts from whole fruits vary considerably among genotypes (Shiow et al., 2007; Scalzo et al., 2005; Wang et al., 2007).

Strawberries have also a high content of vitamin C which make it an interest source for dietary intake and for research.

Strawberry is considered among the fruits with higher folates (Vitamin B9) concentration. In fact, 250 g of strawberries (~60 µg of folate on average) can supply 30% of the daily European and U.S. folate recommended daily allowances. Moreover, the strawberry, although to a lesser extent, is a source of several other vitamins, such as thiamin, riboflavin, niacin, vitamin B6, vitamin K, vitamin A and vitamin E.

Table 1 Nutrient composition of fresh strawberries (US Department of Agriculture, 2010)

Type	Nutrient	Per 100 g
Proximates	Water (g)	90.95
	Energy (kcal)	32
	Protein (g)	0.67
	Ash (g)	0.40
	Total lipid (g)	0.30
	Carbohydrate (g)	7.68
	Dietary fiber (g)	2.0
	Sugars (g)	4.89
	Sucrose (g)	0.47
	Glucose (g)	1.99
	Fructose (g)	2.44
Minerals	Calcium (mg)	16
	Iron (mg)	0.41
	Magnesium (mg)	13
	Phosphorus (mg)	24
	Potassium (mg)	153
	Sodium (mg)	1
	Zinc (mg)	0.14
	Copper (mg)	0.048
	Manganese (mg)	0.386
	Selenium (μg)	0.4
Vitamins	Vitamin C (mg)	58.8
	Thiamin (mg)	0.024
	Riboflavin (mg)	0.022
	Niacin (mg)	0.386
	Pantothenic acid (mg)	0.125
	Vitamin B6 (mg)	0.047
	Folate (μg)	24
	Choline (mg)	5.7
	Betaine (mg)	0.2
	Vitamin B12 (μg)	0
	Vitamin A, RAE (μg)	1
	Lutein + zeaxanthin (μg)	26
	Vitamin E, α-tocopherol (mg)	0.29
	β-tocopherol (mg)	0.01
	γ-tocopherol (mg)	0.08
	δ-tocopherol (mg)	0.01
	Vitamin K, phylloquinone (μg)	2.2

The same amount of strawberries can provide about 5% of the adequate intake for potassium (Table 1) and has been qualified as a good source of iodine, magnesium, copper, iron, and phosphorus. Besides these nutritive compounds, strawberries contain another type of compounds which are non-nutritive components such as polyphenolic phytochemicals (flavonoids, phenolic acids, lignans, and tannins). A discussion of the overall range of compounds follows, with a specific focus on the most significant phytochemical compounds (on a quantitative basis), primarily anthocyanins and ellagitannins.

Strawberry phytochemicals are mainly represented by phenolic compounds that have many non-essential functions in plants and huge biological potentialities in humans (Hakkinnen and Torronen, 2000). They are mainly represented by the flavonoids (mainly anthocyanins, with flavanols and flavonols providing a minor contribution), followed by hydrolysable tannins (ellagitannins and gallotannins) and phenolic acids (hydroxybenzoic acids and hydroxycinnamic acids), with condensed tannins (proanthocyanins) that are a minor constituent (Kahkônen et al., 2001; Aaby et al., 2005).

- **Total polyphenols**

Polyphenols are among the beneficial compounds present in strawberries. They help to improve health properties and to prevent many diseases in human body (Balasundram et al. 2006; Giampieri et al. 2012; Tulipani et al. 2009b; Alvarez-Suarez et al. 2014). The polyphenols are the most important compounds in strawberries in terms of antioxidant properties. They are generally responsible of coloring, tastes and flavors and mainly act as radical scavengers. They are able to interact with proteins to protect the plant from excessive UV radiation. They may also help for protecting the plant from external dangers such as pathogens attacks and other biotic and abiotic stressing situations (Ozcan et al. 2014; Dixon and Paiva 1995; Bravo 1998).

The figure 8 presents the different classifications of polyphenols compounds. Polyphenols can be either flavonoid (anthocyanins), non-flavonoids (lignans, tannins) and phenolic acids (Tappi, 2018).

Other phenolic compounds, such as phenolic acids, occur in strawberries. These compounds are present in small quantities and they mainly derivate from hydroxycinnamic acid (caffeic acid) and hydroxybenzoic acid (gallic acid) (Giampieri et al., a2012; Giampieri et al., b2012; and Mattila et al., 2006). Ellagic acid in strawberry can occur both in the free form and esterified to glucose, the form in which they occur in hydrolysable ellagitannins (Tulipani et al., 2009) and has anticarcinogenic and antimutagenic activities (Okuda et al., 1989).

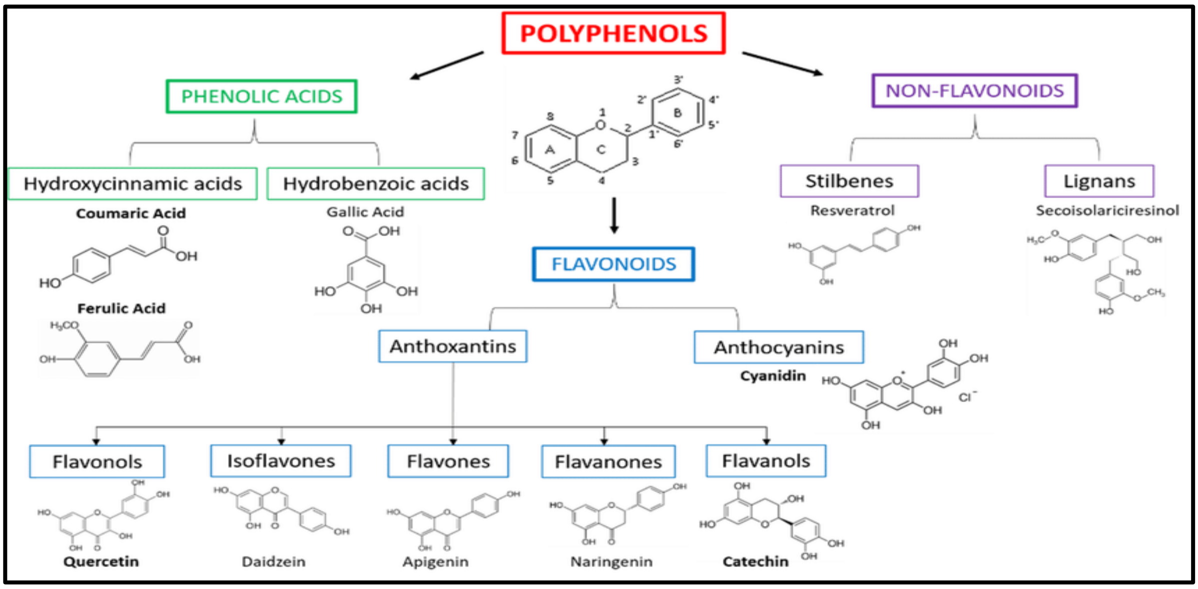

Figure 8: Different classes groups of polyphenols (Basheer and Kerem, 2015).

- **Anthocyanins**

Anthocyanins are among the most important compounds in fruits (Aaby et al., 2012). Different genotypes present different amounts of anthocyanins. In strawberry, more than 25 different anthocyanin pigments have been identified, and their distribution and amount differ among varieties and selections, (Lopez da silva et al., 2007) but the major anthocyanins in cultivated strawberry are pelargonidin-3-glucoside, pelargonidin-3-rutino-side, and cyanidin glucoside (Diamanti et al., 2014). According to the study of Mazza and Miniati (1993), strawberry anthocyanins collectively stem from pelargonidin (Pg) and cyanidin (Cy) aglycones. These anthocyanin compounds have radical scavenging activity and have been associated with a decrease in the incidence of cancer, heart disease, and other health beneficial effect (Wang et al., 2007). Moreover, the total antioxidant capacity (TAC), which measures the radical scavenging activity, is related to the amount of antioxidant compounds in strawberries, that indicates good fruit nutritional quality (Capocasa et al., 2016).

Chapter 3: Aim of the study

The purpose of the thesis was to evaluate strawberry wild genotypes, cultivars and advanced selections from the interspecific genetic improvement program developed at the Università Politecnica delle Marche, to characterize them according to production, qualitative and nutritional parameters, in order to establish which of these are most suitable for cultivation in conditions of non-brominated soil, tending to be clayey, with a high content of active limestone, typical of the Middle-Adriatic environment.

The selections under study, deriving from different level of hybridization (back crossings) between *F. x ananassa* and *F. virginiana*, were compared with the strawberry cultivars (*F. x ananassa*) already recommended for cultivation in the area of interest, and with some strawberry wild species (*F. virginiana*, *F. vesca* and *F. chiloensis*), considered source of sensorial and nutritional quality.

With this trial, therefore, it is possible to evaluate if the back crossing program of UNIVPM-D3A is producing selections with interesting parameters, intended as productive results similar to the cultivated *Fxa*, and increased sensorial and nutritional quality thank to the influence of the wild germplasm.

After the evaluation, the best selections can be patented, to be then marketed as new varieties and made available to farmers for cultivation in suitable environments, or be used as a breeding-parent in subsequent genetic improvement programs.

Chapter 4: Material and Methods

1. Plant Material

Strawberries samples used for analyses were harvested during April, May, and June 2021 in the field of Agugliano (AN).

The field is located on a flat plot with the standard soil conditions of the Marche region (mid-hills),s characterized by clayey texture (clay and sand), sub-alkaline pH, high level of limestone (12.1%), low content of organic matter (1.14%), high content of exchange bases (Magnesium and potassium) and high cation exchange capacity.

The climate of Agugliano is classified as humid temperate with very hot summer (cfa), and the average temperature in winter is considered between -3°C and +18°C, and for the hottest month is above 22°C and the rainfall is more abundant in spring and lesser in winter and summer (Strahler, 1993). The annual average of relative humidity is considered of 75.7% with a minimum of 71% in July and a maximum of 82% in November, (www.clima.meteoam.it/AtlanteClimatico/).

The analyzed parameters were plant productivity (fruit weight and commercial plant production), fruit sensorial (fruit firmness, solid soluble content, titratable acidity, and color), and nutritional parameters (total phenol content, and anthocyanin content).

2. Cultivation Technique

The cultivation technique used is the open field. It is based on the use of category A refrigerated plants with planting time on the third decade of July.

Planting of strawberries in our field is characterized by subsoiling to a depth of about 45cm, which has the purpose of preventing the stagnation of water, because it can induce proliferation of dangerous root fungi. Then manure is distributed to increase the supply of organic substance, to improve the chemical-physical characteristics of the soil, which was buried with plowing to a depth of about 30-35cm. This presents the thickness of the soil in which there is the greatest development of roots. Moreover, the refinement of the soil was carried out using harrows which make it more suitable for planting plantlets. To avoid using chemical products for soil fumigation, other alternatives have been used in our field such as long crop rotation and organic amendments.

Around one week before the planting, the black plastic film for mulching is laid on the ground and the double perforated hose for fertigation is spread (Figure 9).

The cold preserved plants were planted in the holes already prepared in twin rows on the black plastic film, the distance between the rows of each bin is 30cm while the distance between the plants along the row is 35cm. The distance between the rows was 1.3m.

Taking care of the crops is very crucial, by elimination of stolon produced in autumn and cleaning of plants in spring, by elimination of old foliage which can be a source of inoculation by pathogens. Indeed, in spring period the straw is placed between the rows to prevent the proliferation of weeds, prevent fruit from getting dirty by the ground (because of rain) and to make manual operations easier.

To manage strawberry fertilization and irrigation, nitrogen, phosphorus, potassium, or iron were supplied combined with water, through a volumetric injection system.

All the phytosanitary precautions have to be applied when the tolerance threshold is exceeded by protecting useful insects and preventing plants from fungal parasites, such as *Botrytis cinerea,* particularly in high humidity and rainy seasons.

Figure 9: strawberry cultivation using a black plastic mulch.

3. **Harvesting technique**

Harvesting was done manually, because strawberry fruit is a small fruit and easily destroyable and perishable, by cutting fruits from the peduncle, without removing the calix. Generally, we harvest when at least five fruits per parcel were fully reddish, full ripen, sound and not spoiled (Figure 10). Thus, the fruit samples of fully red berries (total surface) were harvested at the second, third, and fourth main pickings from April to end of June.

In the moment of harvesting, the strawberries should be divided into four groups: commercial (red color and big fruit without any damage), small fruits, misshaped fruits, and rotten fruits, and collected in different plastic box.

Figure 10: strawberry harvesting in the field dividing fruits into different boxes.

Among fruit sensorial parameters, color and firmness were determined on fresh fruit (on the same day of harvesting), while total soluble solids and titratable acidity were measured in frozen fruits. For the nutritional analyses, extracts were obtained from frozen fruits, and then analyzed.

The different crossing types are illustrated in the table with their correspondent genotypes: cultivars or selections. The crossing types belong to 10 groups including the wild genotypes: *Fragaria x ananassa* (Fxa), *Fragaria virginiana glauca* (FVG), first generation of Fxa and FVG crossing (F1), 4 generations of subsequent back crossings between Fxa and FVG (BC1, BC2, BC3, BC4), *Fragaria vesca* (F.vesca), *Fragaria chiloensis* (F.chiloensis), and first generation of crossing between *Fragaria chiloensis* and *Fragaria x ananassa* (Ch x Fxa). In table 2 are reported the names of the cultivars and the codes of the selections belonging to the different groups in order to determine which selections are the best and that have to be considered for establishing new cultivars or to be used as parents for the next breeding programs for better production and fruit quality improvement.

Table 2 The different crossing groups used for the breeding program of 2021 with their correspondent genotypes.

Fxa	FVG	F1	BC1	BC2	BC3	BC4	F. vesca	F.chiloensis	Ch x Fxa
BJ01-08-14	FVG1	AN94,414,52	AN16,22,53	AN11,05,53	AN00,239,5	AN17,12,51	AN01,188,53	AN12,56,53	AN13,43,51
BJ01-14-14	FVG2	AN94,472,51	AN16,22,56	AN11,05,58		AN17,12,52		AN12,56,60	AN13,44,52
BJ06-37-02	FVG3	AN12,48,54	AN16,28,51	AN15,04,54		AN17,12,53		AN12,56,61	
BJ06-37-07	FVG4	AN12,49,53	AN16,29,53	AN16,04,52		AN17,12,54		AN12,57,55	
BJ06-56-06	AN10,65,51	AN12,49,65	AN16,30,51	AN16,04,53		AN17,12,55			
H106		AN12,50,52	AN16,30,53	AN11,40,55		AN17,19,51			
H107		AN12,51,56	AN17,07,51	AN11,40,56		AN17,19,52			
H18		AN13,15,57	AN17,07,52	AN11,41,55		AN17,19,53			
SH2		AN13,20,52	AN17,07,53			AN17,19,54			
H33		AN13,20,58	AN17,07,54			AN17,19,55			
H38			AN17,07,55			AN17,19,56			
H71			AN16,27,52			AN17,19,57			
H81			AN16,27,53			AN16,37,51			
H97			AN16,27,54			AN16,37,52			
SVEVA			AN16,32,51			AN16,37,53			
AN14,12,58			AN16,32,53			AN16,37,57			
AN13,16,56			AN16,32,55			AN16,37,58			
AN14,22,51						AN16,37,60			
AN14,22,54									
AN16,15,53									
AN16,16,53									
AN16,42,54									
AN12,20,53									
AN15,07,51									
Francesca									
Lauretta									
Silvia									
Dina									
AN13,13,55									
AN12,13,58									
AN15,08,21									

4. Yield and quality analysis

4.1. Plant yield and fruit average weight

The yield of strawberry plant was evaluated for each single plot of all genotypes, by measuring total production, commercial production, second category production (misshaped and small fruits), waste production, and average fruit weight, evaluated on 20 commercial fruits from each harvest and expressed in (g)

The plant yield is determined collecting strawberries at each harvest day. For each parcel, at least five commercial fruits have to be harvested and put in the plastic box as described before. Then the box is weighed, and data are collected. The total fruit production is expressed as g/plant.

The average fruit weight is also an important parameter, together with plant yield, especially for commercial use and for market acceptance, but it also an indicator of breeding program results.

4.2 Evaluation of qualitative traits of strawberry plants

In this work, the characteristics that have been considered for describing the quality of the production obtained from different cultivars and selections are:

- color and firmness of fruits, that are important parameters to analyze the external appearance and the commercial acceptance of the product;
- acidity and soluble solids content, which are used to evaluate the organoleptic quality of the fruit.

Ten fruits were sampled for each plot and each day of harvest (I THINK TWICE A WEEK), among those classified as commercial fruits. The analyses started from the third harvest of the season, avoiding analyzing fruits from the first and second harvest, which consisted of mainly primary fruits having different characteristics from the commercial fruits obtained later in the season.
The samples are placed in plastic boxes with the identification code of the parcel and genotype. Each box contains ten fruits that are first analyzed with colorimeter with two measurements on two opposite sides of the fruit surface. Then for the same ten fruits, two-times measurements for each one were performed for measuring the firmness, using the penetrometer.
After finishing these two analyzes, the ten fruits of each sample are collected in transparent plastic bags to be frozen and be used later for soluble solids and acidity analyzes.

4.2.a. Strawberry sensorial parameters

- **Color analysis**

The color of the fruit was analyzed using a Chroma-Meters CR-400 automatic reflectance colorimeter (Konica Minolta, Japan). This tool, once applied to the surface of the fruit, can instantly provide us with three values: L *, a *, b *. The parameter a *, in the case of the strawberry, indicates the red hue, while b * in this case represents the yellow hue and together, these two values, provide us with the chromaticity coordinates, which describe the hue of the color of the fruit. Through a * and b * we can calculate the Chroma index according to the formula $[(a^*)^2 + (b^*)^2]^{1/2}$. The Chroma index (C) quantitatively indicates intensity of the color: high C values indicate colors with higher brilliance, while lower values are characteristic of dull. Different values of Chroma index can depend on many factors, but mainly on the variety and the state of ripeness: for example, overripe fruits have very low C values.
The third parameter provided by the colorimeter is L *, whose value is used to describe the brightness of the fruit color: high values of L * indicate light and bright colors, while lower values indicate darker colors.

Data were elaborated and expressed as chroma index. A high chroma index indicated pale fruit and a low chroma index represented dark fruit,

- **Firmness analysis**

The Firmness of the fruit was measured with the digital penetrometer (Model 53205 SW Turoni, Italy), an instrument equipped with a tip through which the epidermis and part of the pulp are pierced, which returns the measurement in g / cm^2, that represents the resistance of the fruit to perforation. The penetration is carried out for each fruit on two diametrically opposite points, free of lesions and evident rot, which could alter the measurement so should be avoided. This type of analysis is carried out by the operator himself. For the strawberry, the 6 mm diameter tip is used, with a star shape that contrasts the elasticity of the epidermis. The measurement scale ranges from 0 to 1000 g / cm^2. The values of this analysis depend on many factors such as genotype, climatic conditions, presence or absence of diseases, excessive handling of the fruit (can damage the fruit), which can weaken the surface and distort the result. Firmness is a crucial parameter because it demonstrates how the fruit structure is able to resist to crushing factors and excessive handling, so to increase fruit shelf life after harvesting and during storage.

- **Total Soluble solids analysis**

The measurement of the soluble solids content is carried out using the manual refractometer with automatic temperature compensation (Atago N-1 E, Japan).

The refractometer is based on the physical principle of light refraction, according to which as the density of a substance increases (for example a sugary substance is dissolved in water), its refractive index increases proportionally. The refraction of the light is then projected, thanks to a sophisticated lens system, to the reading scale of the refractometer, on which the measurement result can be read directly and registered.

To carry out this analysis, the juice is extracted by crushing the thawed fruit from the samples that had been frozen after color and firmness analyzes. From the filtered juice, extracted in a beaker, two drops are taken, placed on the surface of the refractometer prism and the value is read in degree Brix.

- **Titratable acidity analysis**

Titratable acidity allows the quantification of free acids present in strawberry juice, mainly ascorbic, malic, succinic, and citric acids. For this type of analysis, the chemical principle of titration is applied, which is based on the neutralization of dissolved acids thanks using a strong base, in our case sodium hydroxide.

For the determination of the neutrality point, an indicator is used which, when the neutral pH value is reached, induce change of the solution color that turns to blue.

To carry out this analysis, 10 g of juice are taken from the previously extracted one (used for soluble solids), to which 10 g of distilled water are added, obtaining a total of 20 g of solution. Bromothymol Blue is then added as an indicator of the color change of the solution (color change at pH 7), and titration is carried out with a 0.1 N NaOH solution.

The titratable acidity is measured in milliequivalents of NaOH on 100 g of juice (meqNaOH / 100g) and this is given by the milliliters of 0.1 N NaOH solution used for the titration.

4.2.b. Strawberry Nutritional analysis

- **Fruit sampling**

After have being stored in plastic bags at -18°C in laboratory freezes, five strawberries are chosen from each bag and each fruit has to be cut in 2 pieces from the opposite surfaces of the fruit to avoid bias related to sunlight effect during cultivation. Then, strawberry pieces were chopped and weighted (10g) in order to extract the active compounds using methanol (Figure 11). Extraction is suitable for the analyses of antioxidants activity, anthocyanins, polyphenols, and phenolic acids.

Figure 11: strawberry fruit sampling.

- **Anthocyanin analyses (ACY)**

❖ **Methanolic extraction**

The strawberry samples were subjected to a methanolic extraction to obtain the polyphenolic extract (particularly anthocyanins and phenolic acids).

10 g of fruits pieces, taken from the bags previously mentioned, were weighed, and collected into a falcon tube where pieces of strawberries have to be taken from different fruits, to have a representative

sample. The extraction was done using a specific type of alcohol which is the methanol, because it allows the stability of antioxidant compounds during extraction and during sample storage. Methanol was added to the 10 g of strawberries in 1:4 ratio (1 part of fruits and 4 parts of methanol) in two steps by following double methanol extraction. During the first extraction, the procedure was quite simple: the tube containing the 10 g of strawberries, was filled with 20 ml of methanol. The whole material, strawberries, and methanol was homogenized with the ultraturrax-T 25 (Janke & Kunkel, IKA-Labortechnik) for 20/30 seconds. Then, the tube with the methanolic suspension was closed and placed in a rotary shaker for 30 minutes, protected from light. Subsequently, after the shaking time is reached, the suspension was centrifuged at 4000 rpm for 10 minutes, at 4°C. At the end of the centrifugation, 1 ml of the supernatant homogenate was taken and placed in amber vials (six vials have been used for each sample). These vials were stored in freezing conditions (-20° C). The residual pellet, obtained after the centrifugation from the same tube, was re-filled with other 20 ml of methanol (second methanol extraction), and placed in a rotary shaker for 30 minutes again, protected from light. Then, the tube was centrifugated at 4000 rpm for 10 minutes, at 4°C. At the end of the second centrifugation, 1 ml of supernatant was withdrawn and added to the prior vials. That extract was then conserved at freezing temperature (-20° C) (Figure 12). The whole procedure was repeated for each strawberry sample.

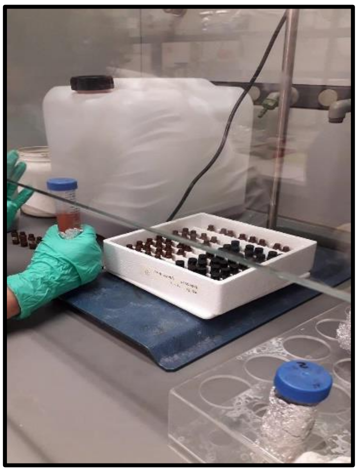

Figure 12: Double methanolic extraction of ACY.

- **Spectrophotometry (pH shift method)**

Anthocyanin pigments change hue and intensity at different pH values: at pH 1.0, anthocyanins exist in the colored oxonium or flavylium form, and at pH 4.5 they are predominantly in the colorless carbinol form. An aliquot of the methanolic anthocyanin solution is added to pH 1.0 solution, and another aliquot to pH 4.5 solution. The difference in absorbance is proportional to the anthocyanin content. Determination of anthocyanin content is based on Lambert-Beer's Law using the spectrophotometer (Figure 13). Molar absorbance values for purified pigments taken from literature are used, making it unnecessary to determine them.

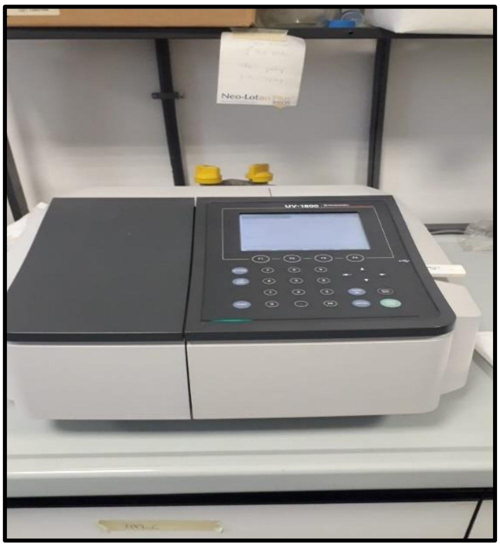

Figure 13: Spectrophotometer.

Pelargonidin-3-glucoside is the major anthocyanin in strawberry, so the total anthocyanin content is calculated and expressed as reference to the pelargonidin-3-glucoside concentration. The concentration of anthocyanins is given as Pel-3-gl [mg/kg FW] fruit, and it is expressed by the formula:

$$\frac{mg\ Pel-3-glu}{kg\ FW}=\frac{[(A\lambda \mathrm{max}-\mathrm{A700})\mathrm{pH4,5}]\ \mathrm{x\ MW\ x\ F\ x\ 1000}}{\varepsilon\ x\ d\ x\ E}$$

Where:

A= absorbance [-]

MW= molecular weight of pelargonidin-3-glucoside=433.2 [g/mol]

F= dilution factor [-] = 10

d= cell pathlengths [cm]

ε= molar absorbance of Pel-3-glu= 15600 [$\frac{L}{mol\ x\ cm}$]

E= sample weight [kg/L extracting agent]

1000= Factor for mg.

- **Total Polyphenol analysis**
  - ❖ **Methanolic extraction**

The extraction is the same protocol described previously for anthocyanins.

- **Spectrophotometry (Folin Ciocalteu)**

The determination of total phenolics has been carried out through the Folin-Ciocalteu assay.

Folin–Ciocalteu (F–C) is an assay regularly used for the prediction of the total phenolics in strawberry as well as in a variety of other fruits and vegetables (Prior et al., 2005). The total phenolics assay does not only determine phenolics but also reducing agents like ascorbic acid, because the basic mechanism is an oxidation/reduction reaction. The exact chemical nature is not known but is believed to contain heteropolphosphotunstates molybdates. Molybdenum is easily reduced in the complex. An electron-transfer reaction occurs between reductants and Mo (VI) under alkaline conditions which results in a blue color with a maximum absorbance of 760 nm. The total phenolic contents (TP) was calculated based on the calibration curve of gallic acid and expressed as gallic acid equivalents (GAE), in milligrams per kg of the sample. The calibration is calculated by linear regression ($\Delta A = ac + b$, c = concentration of Gallic Acid mg/L, ΔA=absorbance, a = slope, b= intercept):

$$\frac{mg\ gallic\ Acid\ eq}{kg\ Fruit} = \frac{(\Delta A - b)\ \times\ F}{a\ \times e}$$

ΔA = A sample/standard (sample)
a = slope (calibration line)
b = intercept (calibration line)
F = Dilution factor (20)
E = sample weight [kg/L extracting agent]

5. Statistical Analysis

Data from the above analyses were analyzed statistically using “Statistica 7” (Stasoft, Tibco Software, Palo Alto, California, USA). One-way analysis of variance (ANOVA) for mean comparison and intergenotype significant differences (calculated according to LSD test) was used. Differences at $p \leq 0.05$ were considered statistically significant.

Chapter 5: Results and Discussion

1. Plant Yield

Regarding the plant yield for 2021, Fxa was the crossing type with the highest yield (1254,8 g/plant), followed by BC4 with 1023,8 g/plant then BC3 693,6 g/plant. While the wild genotypes FVG and the crossing types BC1 and ChxFxa had the lowest production for 2021. The yield has improved by each crossing genotype from BC1 to BC4. It increased from 27.8 g/fruit to 1023,8 g/fruit. Thus, in the last crossing type we reached the average production of the commercial strawberry with more than 1000 g/plant, that is close to the average value of Fxa group and that obey to the productive standards required by the market (Mezzetti et al., 2021).

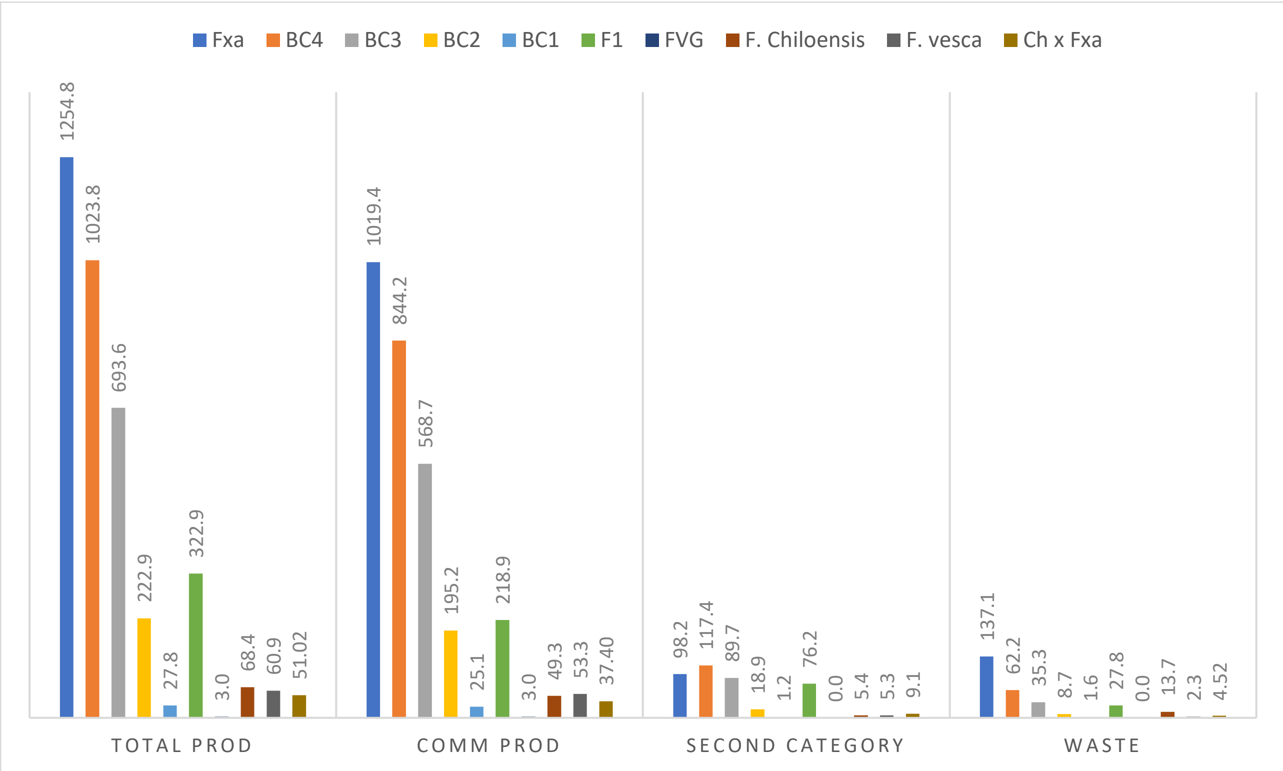

Figure 13: Average fruit weight of populations from different type of cross combinations, the data are expressed as mean ± standard error. Fxa=F. x ananassa, BC1=back crossing 1, BC2=back crossing 2, BC3=back crossing 3, BC4=back crossing 4, Ch x Fxa= F. chiloensis x F. x ananassa, FVG=F. virginiana glauca, F1=F. x ananassa x FVG

2. Average fruit weight

The average fruit weight of different cultivars and selections present a variation from high values to lower values between 19,42 and 1,04 g/fruit (Figure 14). **<u>Fxa</u>** is the group with the highest average fruit weight followed by BC2, BC3, BC1, BC4, and Ch x Fxa, presenting a close average weight between 16 and 17 g/fruit. The Figure 14 shows a strong variation of average fruit weight between Fxa and F1 which had 7.17 g/fruit. Moreover, FVG and *F.vesca* species present the lowest values so they have small size. These results are in accordance with the study of Mezzetti et al. (2021) that demonstrated that Fxa, which is the commercial genotype, has the higher fruit weight, while the wild genotype FVG and the crossing type F1 present the lowest average weight. Moreover, the back crossing has participated to increase average fruit particularly in the selections of BC2 and BC3. While a slight decrease, but not significant, is observed for the selections of BC4. These results are in accordance with the ones found by Mezzetti et al. (2021) for the strawberries harvested in 2020. The only difference is that for the selections of BC4, the average fruit weight was slightly higher than the one of BC3.

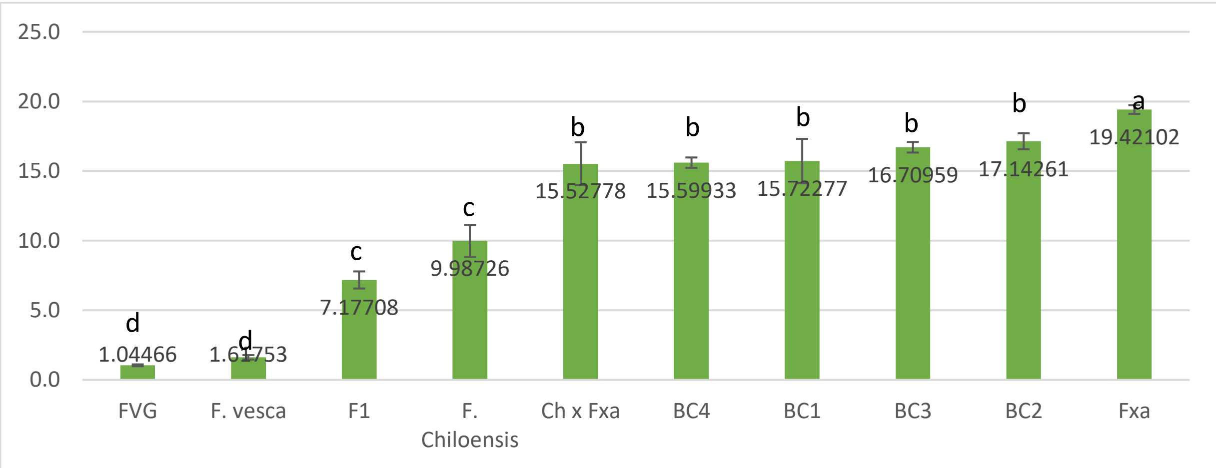

Figure 14: Average fruit weight of populations from different type of cross combinations, the data are expressed as mean ± standard error. Fxa=F. x ananassa, BC1=back crossing 1, BC2=back crossing 2, BC3=back crossing 3, BC4=back crossing 4, Ch x Fxa= F. chiloensis x F. x ananassa, FVG=F. virginiana glauca, F1=F. x ananassa x FVG

3. Sensorial analysis

a. Firmness and color analyses

For firmness, the figure 15 shows a significant variation of measures among populations of the different cross combinations. In fact, the back crossing generation **BC4** showed the firmest fruit with a measure of about 525g, followed by Fxa, BC3, and BC1, that they have almost the same firmness around 450g. Then BC2 and F1 with lower firmness. Finally, the species *F. chiloensis* and *F.vesca* present the lower results. Thus, the firmness of fruits is increasing during back crossing generations from BC1 to BC4. It is possible that other factors (such as cell wall degrading enzymes) are also responsible for fruit firmness. Firmer fruits have a higher potential to be better handled in transport and are less likely to decay (Esmel et al., 2008). Fruit firmness is affected by plant genetics and growing conditions (Dunn and Able, 2006; Prange and DeEll, 1997). But it is also affected by fruits size, stage of maturity (Ourecky and Bourne, 1969; Schmitz and Lenz, 1985) and harvest day (Puchalski et al., 1994). In addition, firmer fruits such as BC4 in this case, tend to be less susceptible to fruit rot caused by *Botrytis cinerea* than softer fruits (Hancock et al., 1990; Maas, 1978). Fruit firmness is assuming increasing importance for modern strawberry genotypes because it keeps the fruits quality for longer time and tolerate different kind of transport or handling (Terrettaz and Carron, 1991).

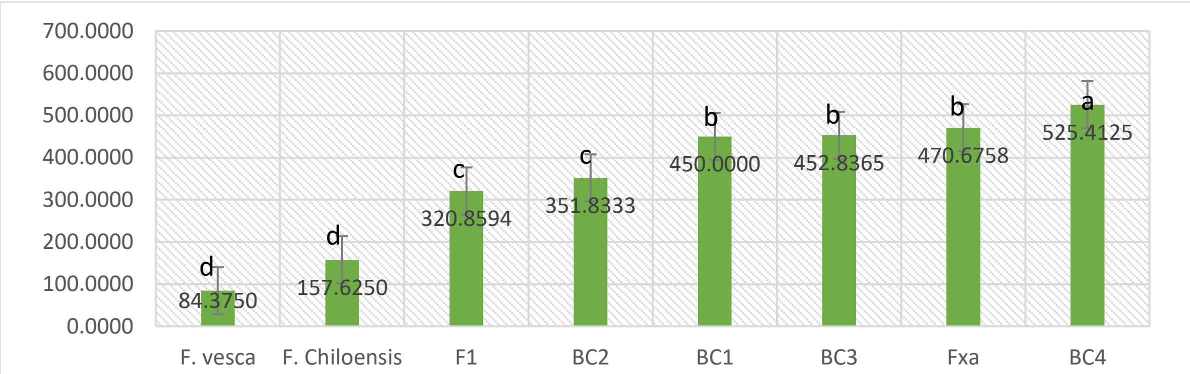

Figure 15: Average fruit firmness of selection populations from different cross combinations, the data are expressed as mean ± standard error. Fxa=F. x ananassa, BC1=back crossing 1, BC2=back crossing 2, BC3=back crossing 3, BC4=back crossing 4, F1=F. x ananassa x FVG

For the fruit color presented in figure 16, which is expressed as a chroma index, ***F. chiloensis*** present the highest value with 51.47 following by F1, BC3, that have approximatively similar chroma index 49.28 and 48. Then F*a, BC1, BC2 and *F. vesca* present similar results with 44,41 chroma index as an average. The increasing of the red color degree among the back crossing generations from BC1 to BC3, indicates that the back crossing is contributing to better stabilize the color among the varieties to be more commercially appreciable and acceptable. Finally, the color analysis did not show a significant variation between the generations: F*a and the back crossing groups BC1, BC2, BC3, and BC4.

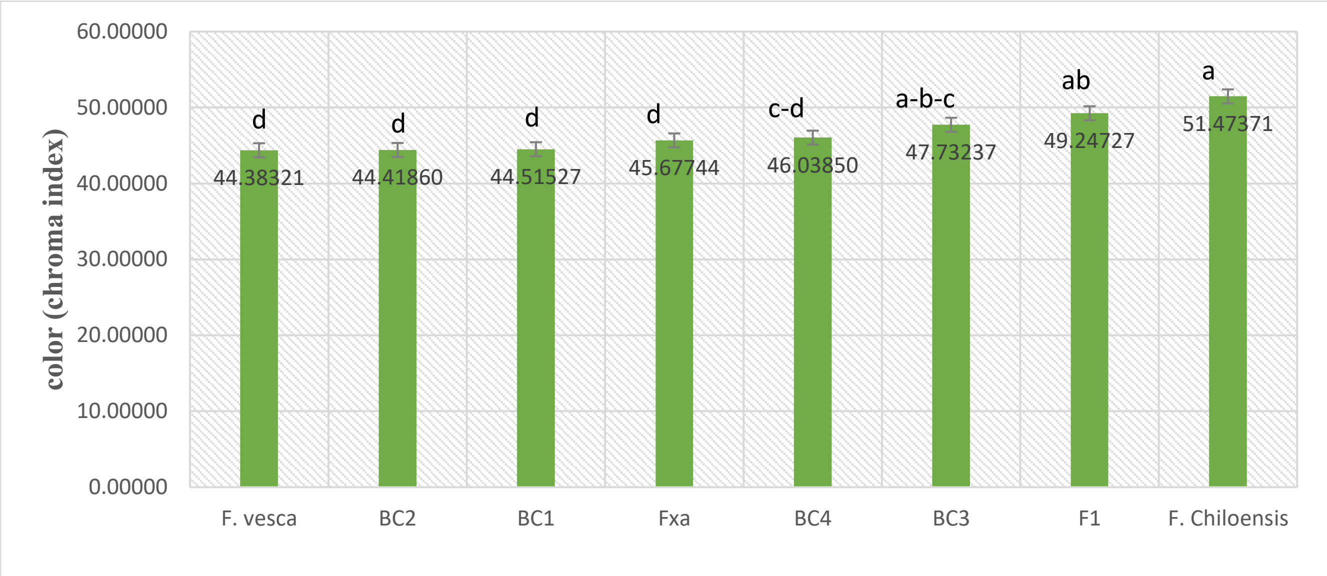

Figure 16: Chroma index of selection populations derived from different cross combinations, the data are expressed as mean ± standard error. Fxa=F. x ananassa, BC1=back crossing 1, BC2=back crossing 2, BC3=back crossing 3, BC4=back crossing 4, F1=F. x ananassa x FVG

b. Sugar and acidity

Sugar and acidity can be interpreted together since they represent the sensorial properties of strawberries fruits. In fact, the results in figure 17 and 18 demonstrated that ***F.vesca*, BC1 and F1** are the populations having fruits with the highest values of fruit soluble solid and acidity. Lower value, even if not statistically different have been detected also form fruit harvested from the BC4 population, but differing for lower acidity. While Fxa population follows the first group for the higher values of fruit acidity but with soluble solid content similar to fruit of BC3 selections. Moreover, the back crossing generations BC3 and BC2 have similar levels of acidity and soluble solid content as BC4. Finally, fruits of FVG and *F. chiloensis* present the lowest values for both parameters. These results differ from what observed by Mezzetti et al. (2021) in which FVG lines differing for the highest soluble solid content and the lowest acidity in comparison with other Fxa populations. This could be explained also by environmental factors that have affected at different extent the sensorial quality of the wild genotypes. In general, we have obtained a positive result because the latest back crossing generations BC3 and BC4 presented similar results to the commercial fruit Fxa, which is always appreciated by the consumers in the market.

The average amount of fruit soluble solids content of BC3 and BC4 is 9.5 Brix° which is higher than the results found for them in 2020, as illustrated by Mezzetti et al. (2021), that had a sugar content of 8.8 Brix°, and is higher than the brix value generally present in the market. This could be explained by the fact that the environmental conditions are changing year by year, affecting the fruit sugar content of different genotypes.

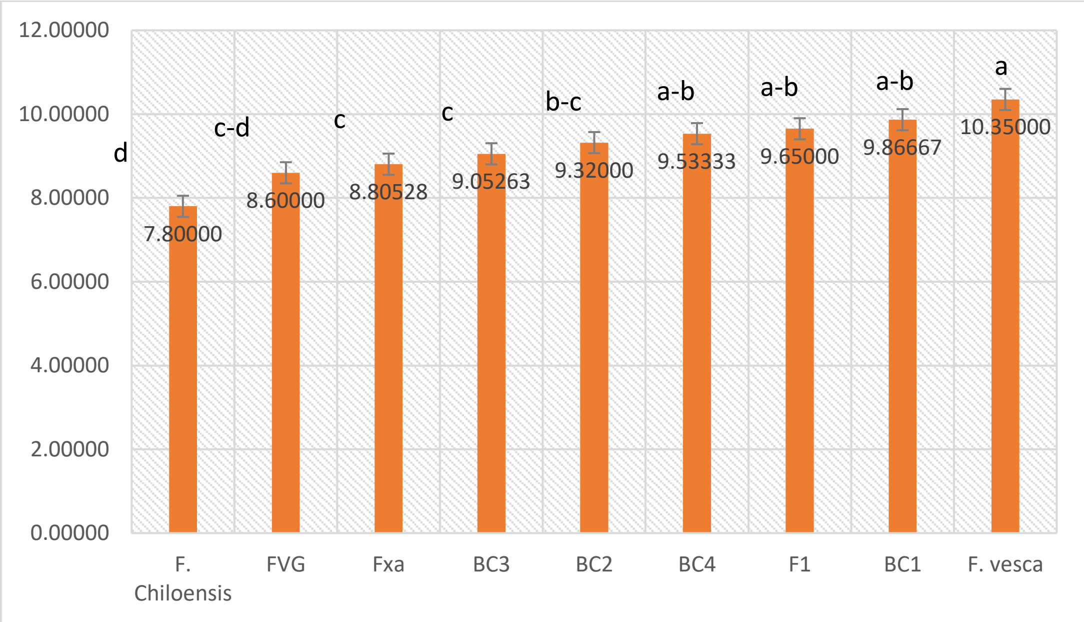

Figure 17: Average values of fruit total soluble solids content of populations derived from different cross combination. Data are expressed as mean ± standard error. Fxa=F. x ananassa, BC1=back crossing 1, BC2=back crossing 2, BC3=back crossing 3, BC4=back crossing 4, FVG=F. virginiana glauca, F1=F. x ananassa x FVG.

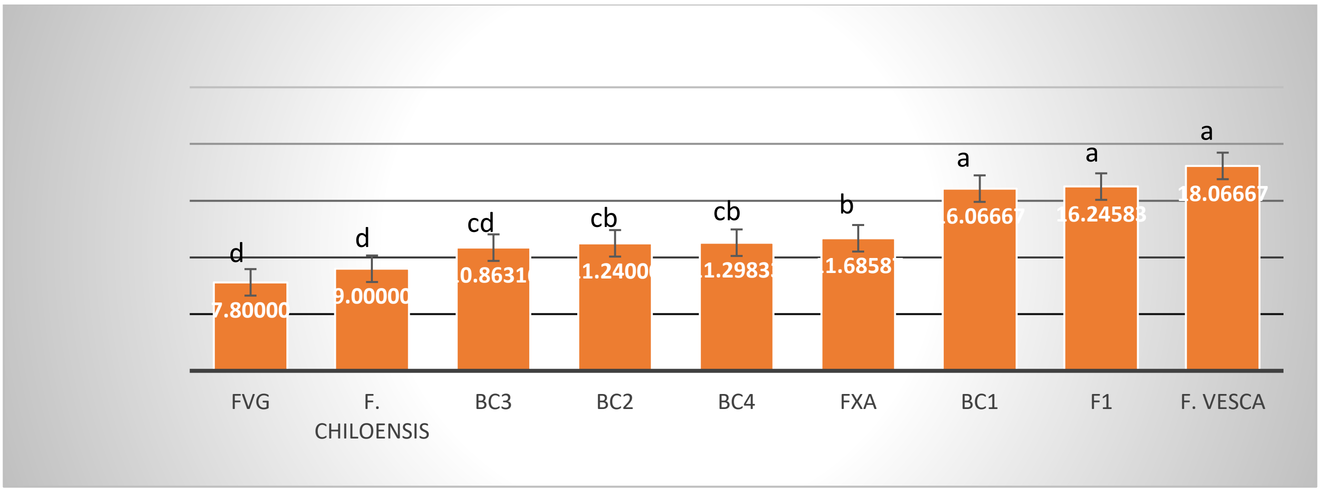

Figure 18: Average values of fruit titratable acidity of populations from different cross combination. Data are expressed as mean ± standard error. Fxa=F. x ananassa, BC1=back crossing 1, BC2=back crossing 2, BC3=back crossing 3, BC4=back crossing 4, FVG=F. virginiana glauca, F1=F. x ananassa x FVG.

4. Nutritional analysis

a. Total phenols

The highest mean value of the total polyphenols content was detected in fruit of **FVG,** that has been also used as a parent to obtain the F1 generation (figure 19). This explains the fact that **F1** showed high phenols content too, with 4089 mg/100g FW. While BC1 and BC2 (2832 mg/100g FW) present lower content than FVG and F1, but higher than Fxa (1536 mg/100g FW) and the two other back crossing generations BC3 and BC4 (1481 mg/100g FW and 1576 mg/100g FW, respectively). The fruit phenolic acid content is decreasing during the back crossing generations, because of the influence of Fxa genotype, which presents low content of TPH. Mezzetti et al. (2020) have found that F1 presented the highest concentration of TPH. However, this research has found that BC1 and BC3 presented similar concentrations to F1 which is not the case for the fruits of 2021. Thus, the fruit TPH content is decreasing in the back crossing generations, with fruit of BC1 and BC2 populations presenting higher values than BC4 and BC3. This could be explained by the impact of some environmental factors or, in particular, by *F.* x *ananassa* genotypes used as parenst in the different backcrossing combinations. The positive finding is that the total phenols content of BC4 fruits is slightly higher than Fxa fruits, demonstrating an ameliorative trend of the back crossing program in respect to cultivated genotypes.

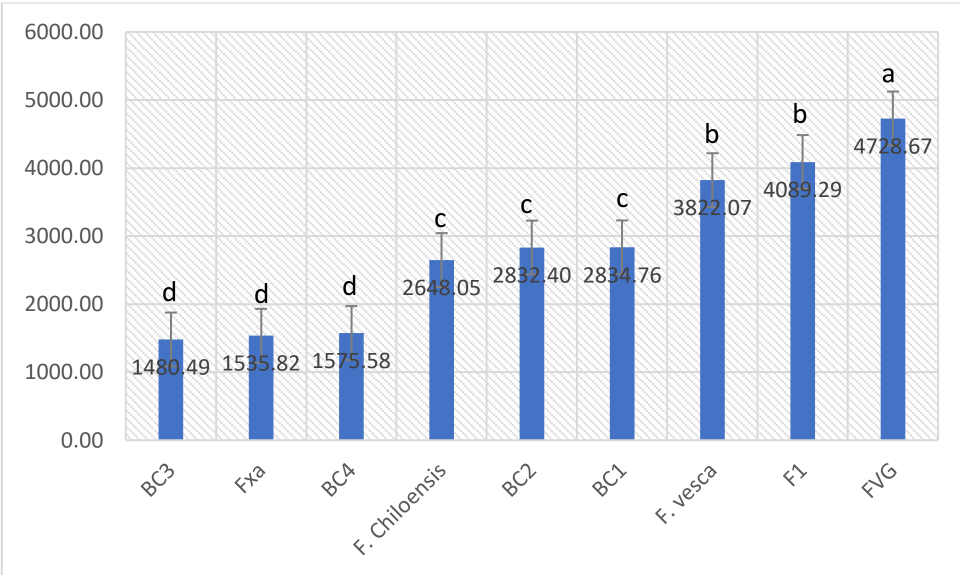

Figure 19: Average fruit total phenols content of populations from different cross combinations. Data are expressed as mean ± standard error. Fxa=F. x ananassa, BC1=back crossing 1, BC2=back crossing 2, BC3=back crossing 3, BC4=back crossing 4, FVG=F. virginiana glauca, F1=F. x ananassa x FVG.

b. **Total anthocyanins content**

Higher values of fruit total anthocyanins content (figure 20) have been detected the population of **Fxa** selections, with 348.71 mg pel-3glu/kg FW, followed by F1. While fruits from populations of the back cross populations (BC4, BC3, BC2) present lower ACY content, with the lowest value from BC1 population. Mezzetti et al. (2020) have also found, using spectrophotometry, a high fruit anthocyanins content in Fxa cultivars and selections analyzed in 2014 and 2016, and HPLC analyses performed 2017 confirmed high ACY fruit accumulation in Fxa genotypes. The lower amounts were detected in fruits of the back crossing generations BC4 and BC3, with the following concentrations: 218.56 and 197.48 mg pel-3glu/kg FW. Then the BC1 and BC2 crossing types, with respectively 182.18 and 171.20 mg pel-3glu/kg FW. These lower concentrations could be explained by the fact that one of the parents (FVG) does not present fruits with a high concentration of ACY. The high concentration in Fxa signifies the high reddish color detected in the color analysis (sensorial analysis). This as an indication that the breeding programs aimed to increase commercial appearance of the fruit have also contributed to maintain high contents of ACY. The ACY parameter affects positively the color stability (Diamanti et al., 2016). The crossing types with fruits with high ACY concentration (Fxa and F1) will be considered of high nutritional value and positive health effects upon consumption. In fact, the ACY helps to mediate different biological effects such as antioxidant activity, anti-inflammatory and other health benefits (Williamson, 2017). Even if the BC4 average value is quite far from Fxa, the back crossing program is moving on the right direction, given that the last two crossing generations (BC3 and BC4) present fruits with higher ACY value than BC1 and BC2.

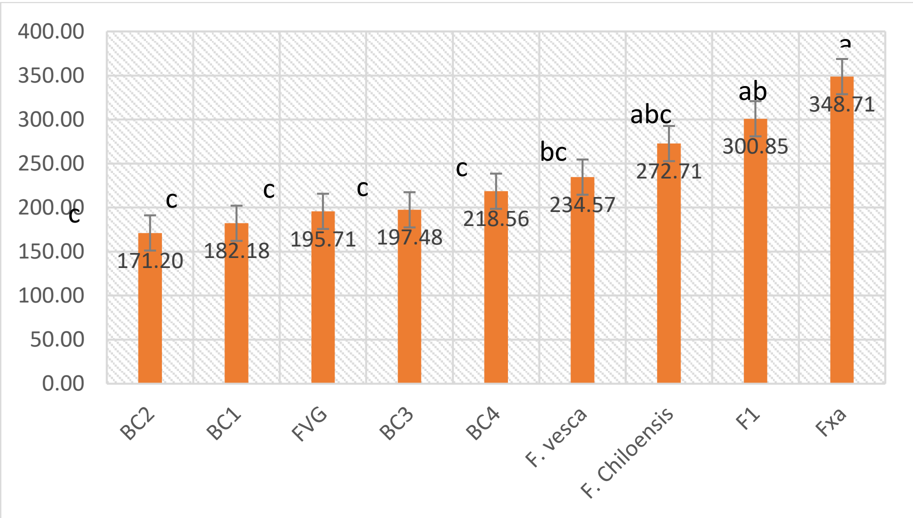

Figure 20: Average values of frui anthocyanins content of populations from different cross combinations. Data are expressed as mean ± standard error. Fxa=F. x ananassa, BC1=back crossing 1, BC2=back crossing 2, BC3=back crossing 3, BC4=back crossing 4, FVG=F. virginiana glauca, F1=F. x ananassa x FVG.

Discussion

The table 3 shows that, for the plant yield Fxa, BC4 and BC3 had the best average production yield. The best genotype from the crossing type Fxa is Sibilla with the highest yield in this group: 4258 kg/plant. For the group BC4, the best selection is AN17,19,54 where it had a production yield around 574g/plant. While in the back crossing generation BC3, the selection with the highest production yield was AN17,07,51 around 972g/plant.

Concerning the average fruit weight, the highest values belong to fruits from Fxa population of cultivars and advanced selections, where a significant weight is observed for the selections AN15,08,51 and AN12,20,53, followed by the back crossing generations BC2 where the best selections are AN16,04,53 and AN11,05,53, and for BC3, BC1 and BC4 respectively, with AN11,04,51, AN00,239,55 and AN16,37,57 as best selections for each group (Table 4). A slight decrease in the fruit average size is observed from BC1 to BC4 but the most important finding is that from BC1 onward, the average fruit weight reached similar values to Fxa, which is commercially appreciated. The different crossing generation allowed to increase the average fruit weight from the FVG and F1 generations (Mezzetti et al., 2021).

Concerning the sensorial parameters which are illustrated in table 5, starting with the firmness, the back crossing group with highest average value was BC4 where the selections observed with the highest interest are AN16,34,55 and AN17,12,52. For the color, sugar, and acidity, the F1 is always among the best generations. The best selection for the color parameter among the F1 group is AN12, 48,52 while the best selections for the sugar analysis are AN12,51, 56 and AN13,20,52. Finally for the acidity analysis, AN12,49,65 identified with high acidity content. Moreover, *F.vesca* was identified with high sugar and acidity. An excessively high acidity and/or sugar are not very appreciable by the consumer and the market. This result is in accordance with Mezzetti et al. (2021), where the wild genotype has higher SS content than the Fxa commercial genotype. In addition, in the more recent backcrossing genotypes (BC3 and BC4), we obtained almost similar amount of total soluble solids and titratable acidity to commercial genotype (Fxa), which is a good result because demonstrated a similar sensorial profile between the back crossing generations and the commercial genotypes (Mezzetti et al., 2021).

Generally, a balanced sugar/acidity ratio is more appreciated where the sugar is slightly higher than the acidity, which gives an appreciated flavor of strawberry fruit. However, the selection AN00,239,55 in BC1 group_had the highest sugar and acidity in this crossing group. Thus, it is obvious that the sensorial parameters were improved along the back crossing generations, where BC4 identified with an intense red color, best firmness value, and increased SS mean values combined with not too high TA (Total acidity), therefore maintaining an optimal sugar/acid ratio but with an increased sensorial perception for the consumer.

However, the best genotypes identified for the nutritional parameters were different and identified in table 6. FVG is the best group, with the highest TPH content. While Fxa and F1 had the highest ACY content. Janiss is the cultivar among Fxa group that had the best ACY content. F1 possess high content in ACY and TPH, deriving from the fact that was generated by crossing FVG and Fxa as parents. The best selections related to the high TPH and ACY content among the F1 crossing type group are respectively: AN13,20,58 and AN12,50,52.

The high content of anthocyanins can justify the higher red color of the fruits belonging to F1. However, the low ACY content of BC4 group justify the light color obtained for this crossing type. The back crossing generation BC3 had an intense color but combined with low ACY content. However, this is not in accordance with results found by Mazzoni et al. (2019), where BC3 fruits obtained similar ACY content as F1. Thus, a decrease in ACY content among the latest back crossing generations has occurred the last years, although the sensorial parameters were improved along the back crossing generation, where BC4 was identified with an acceptable fruit color and high firmness. This could be explained by the fact that a genotypic variation has been occurred among the selections of the back crossing generations, mainly by the selection of the best performing parents, and by environmental factors.

The fruit anthocyanins content is important for the contribution to the color of fresh fruit, a valuable quality attribute for the consumer appreciation, and for color stability in processed fruit (Diamanti et al., 2016). In addition, high content of ACY contributes to an increase in antioxidant capacity of the fruits and therefore their nutritional value.

Table 3 Strawberry genotypes for each crossing type group with the highest production yield

Crossing type	Genotype	Plant yield value
Fxa	**Sibilla**	4KG/plant
BC4	**AN17,19,54**	852g/plant
BC3	**AN17,07,51**	972g/plant

Table 4 Strawberry genotypes with the highest fruit average weight.

Crossing type	Genotype (cultivar/selection)	Fruit average weight (g/fruit)
F*a	AN15,08,51	250
	AN12,20,53	220
BC2	AN16,04,53	27.5
	AN11,05,53	27
BC3	AN11,04,51	28.18
BC1	AN00,239,55	26.2
BC4	AN16,37,57	35

Table 5 Strawberry genotypes with the highest sensorial quality (according to the classification of the statical program).

Sensorial parameter	Crossing type	Genotype	Measure
Firmness (g/cm^2)	BC4	AN16,34,55	937,5
		AN17,12, 52	811,25
Color (chroma index)	F.chi	F.chi	53, 74
	F1	AN12,48,52	56,03
	BC 3	AN16,19,51	53,45
		AN16,32,55	53,26
		AN17,07,51	53,05
	BC4	AN17,19,53	54,34
Sugar (Brix°)	F.vesca (wild genotype)	F.vesca	10,35
	BC1	(AN00,239,55)	10.5
	F1	(AN12,51,56)	10,6
		AN13,20,52	10,8
	BC4	AN17,12,53	12,4
		AN16,37,57	12
Acidity (1meqNaOH/100g F)	F.vesca (wild genotype)		
	F1	AN12,49,65	21,2
	BC1	AN00,239,55	16,06

Table 6 Strawberry genotypes with the highest nutritional quality (according to the classification of the statical program).

Nutritional parameter	Crossing type	Genotype	Measure
Total phenols (mg/100g FW)	FVG F1	FVG AN13,20,58	4728.26 5871,61
Anthocyanins (pel-3glu/1kgFW	F*a F1 F.chi (wild genotype)	Janiss AN12,50,52 *F.chiloensis Rosa*	888,61 785,92 234,07

Chapter 6: Conclusions

The results of the breeding program developed at UNIVPM-D3A showed a variation among the different crossing types, and among genotypes belonging to the different crossing types. The last back crossing generation BC4, which is the most recent of the program, developed many improved characters in respect to both wild (FVG) and cultivated (Fxa) parents. A high production yield was observed in the Fxa group where particularly the selection ‘Sibilla’ possess the most interesting production yield 4258kg/plant for 2021 following by the selections ‘AN17,19,54’ from BC4 group. However, it was important to observe that at each back crossing generation, the productive values were ameliorated, reaching acceptable marketable values at BC4. Regarding the average fruit weight, BC4 had an acceptable value and, even if could be potentially ameliorated, it was statistically similar to Fxa value. The selection ‘AN15,08,51’ and ‘AN12,20,53’ among the Fxa group proved to have a superior fruit size.

Concerning the sensorial quality, BC4 group had the best selections ‘AN17,49,65’ and ‘AN16,37,57’ having the most interesting SS around 12 Brix° content with low level of acidity. This balanced ratio is a fundamental criterion to be a fruit commercially appreciated. Fruits from BC4 were identified with the highest firmness particularly the selections ‘AN16,34,55’ and ‘AN17,12,52’ with almost 900g/cm2, which is crucial for commercialization and to heal handling and transport. The firmer the fruit, the lesser losses occur. Regarding the color detected for BC4 fruits, expressed as Chroma, is slightly lower than F1, but higher (and similar) to Fxa that could be related to the anthocyanins content, which was lower than Fxa, but similar to FVG. The most interesting genotype with the best color identification is ‘AN12,48,52’ from F1 group, with a chroma value of 56,03. According to the results founded related to sensorial analysis, the different selections AN17,49,65’, ‘AN16,37,57’, ‘AN16,34,55’, ‘AN17,12,52’ and ‘AN12,48,52’ were the most interesting selections that have to be considered in further breeding program and to be used even as parents for enhancing better sensorial quality in the new fruits.

For the TPH value, BC4 fruits showed among the lower levels, even if they slightly increased this value in respect to the commercial parent (Fxa), while the best genotype with high performing TPH content is ‘AN13,20,58’ with almost 5900mg/100g FW from F1 group. FVG, the wild germplasm which resulted high in phenolic acid fruit content, is confirmed as an important genetic source for increasing fruit PA content (Diamanti *et al*., 2012) This selection could be of interest as parent for the improvement of the nutritional quality. Finally, the cultivar Janiss from Fxa group proved to have superior quality in terms of ACY content with 890 pel-3glu/1kgFW followed by the selection ‘AN12,50,52’ from F1 group. The cultivar Janiss and the selections ‘AN13,20,58’, ‘AN12,50,52’ are the best performing genotypes for nutritional quality that have to be considered for improving ACY and TPH content for the next fruit generations of the breeding programs.

In conclusion, it is possible to affirm that with four backcrossing generations it is possible to reach the productive and sensorial levels of the commercial genotypes and fruit marketing requirements, with some increased values also for the nutritional quality.

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Bibliography

Andrianjaka-Camps, ZN., Heritier, J., Anc¸ay, A., Andlauer, W., and Carlen, C. (2017). 'Evolution of the taste-related and bioactive compound profiles of the external and internal tissues of strawberry fruits (Fragaria x ananassa) cv, 'Clery' during ripening'. *Journal of Berry Research*,7:11-22.

Aaby, K., Mazur, S., Nes, A., Skrede, G. (2012). 'Phenolic compounds in strawberry (Fragaria × ananassa Duch.) fruits: Composition in 27 cultivars and changes during ripening.' *Journal of Food Chemistry*, 132: 86–97.

Amarjeet K., Rajandeep S., and Harmeet Singh. (2017). Evaluation of strawbeery cultivars for growth and yield characteristics in sub-tropical region of Punjab. *International journal of advanced research*, 5(3), 257-26.

Aaby, K., Skrede, G., and Wrolstad, R.E. (2005). 'Phenolic composition and antioxidant activities in flesh and achenes of strawberries (Fragaria ananassa)'. *Journal of Agriculture and Food Chemistry*, 53:4032–40.

Alvarez-Suarez, J. M., Mazzoni, L., Forbes-Hernandez, T. Y., Gasparrini, M., Sabbadini, S.Giampieri, F. (2014). 'The effects of pre-harvest and post-harvest factors on the nutritional quality of strawberry fruits: A review.' *Journal of Berry Research*, 4(1): 1–10.

Avigdori-Avidov, H., (1986). 'Strawberry. In: S.P. Monselise (Editor), Handbook of Fruit Set and Development'. *CRC Press*, Boca Raton, pp. 419-448.

Azodanlou, R., Darbellay, C., luisier, J., Villettaz, J. and Amado, R. (2003). 'Quality Assessment of Strawberry (Fragaria Species), ` *Journal of Agricultre and Food Chemistry*, 51, 715–721.

Azodanlou, R., Darbellay, C., Luisier, J.L., Villettaz, J.C., and Amadò, R. (2003). Development of a model for quality assessment of tomatoes and apricots. *Lebensm. Wiss. Technol,* 36:223–233.

Bravo, L. (1998). 'Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance.' *Journal of Nutrition Reviews*, 56(11): 317–333.

Balasundram, N., Sundram, K., Samman, S. (2006). 'Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses.' *Journal of Food Chemistry*, 99(1): 191–203.

Basson, C.E., Groenewald, J.H., Kossmann, J., Cronje, C., and Bauer, R. (2010). 'Sugar and acid-related quality attributes and enzyme activities in strawberry fruits: Invertase is the main sucrose hydrolysing enzyme'. *Food Chemistry*, 121:1156–1162.

Battino, M., Beekwilder, J., Denoyes-Rothan, B., Laimer, M., McDougall, G.J., and Mezzetti, B. (2009). 'Bioactivities of berries relevant to human health'. *Journal of Nutrition Reviews*, 67:145-50.

Bravo, L. (1998). 'Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance.' *Journal of Nutrition Reviews*, 56(11): 317–333.

Bringhurst, R.S, and Voth, V. (1980). Six new strawberry varieties released. *California Agriculture department*, 34:1215.

Burger, A. L. (2000). 'Proving more versatile than just 'strawberries and cream?' The use of strawberries in the genetic manipulation of grapevine fruit metabolism. Wynboer. Available from http://www.wynboer.co.za/recentarticles/ 200505strawberries.php3.

Capocasaa, F., Diamantia J.,Tulipanib, S.,Battinob M. and B. Mezzetti. (2008). 'Breeding strawberry (Fragaria X ananassa Duch) to increase fruit nutritional quality'. 'Joural of International Union of Biochemistry and Molecular Biology.

Capocasa, F., Diamanti, J., Mezzetti, B., Tulipani, S., and Battino, M. (2008). 'Breeding strawberry (Fragaria × ananassa Duch) to increase fruit nutritional quality.' *International Union of Biochemistry and Molecular Biology, Biofactors*, 34 (1): 67–72.

Chandler, C. K., Folta, K., Dale, A., Whitaker, V. M., & Herrington, M. (2012). Breeding new improved clones for strawberry production in Brazil. *Acta Scientiarum. Agronomy*. *39*(2), 149-155.

Cordenunsi, B. R., Genovese, M. I., do Nascimento, J. R. O., Hassimotto, N. M. A., dos Santos, R. J. and Lajolo, F. M. (2005). 'Effects of temperature on the chemical composition and antioxidant activity of three strawberry cultivars.' *Food chemistry*, 91(1): 113-121.

Darrow, G.M., and Waldo, G.F. 'Responses of strawberry varieties and species to the duration of the daily light period'. *Bulletin of the US D*. 453,1934.

Darbellay, C., Carlen, C., Azodanlou, R., and Villettaz, J. C. (2002). 'Measurement of the organoleptic quality of strawberries'. Acta Horticulturae, 567, 819–822.

Diamanti J., Battino, M., and Mezzetti, B. (2011). 'Breeding for fruit nutritional and nutraceutical quality'. Book Chapter, in: Breeding for FruitQuality. *Pharmaceutical Journal*, 61-79.

Diamanti, J., Balducci, F., Di Vittori, L., Capocasa, F., Berdini, C., Bacchi, A., Giampieri, F., Battino, M., Mezzetti, B., 2016. Erratum: Corrigendum to "Physico-chemical characteristics of thermally processed purée from different strawberry genotypes'. *Journal of Food Composition and Analysis,* 43C:106–118.

Dixon, R. A., Paiva, N. L. (1995). 'Stress-induced phenylpropanoid metabolism.' *Journal of The Plant Cell*, 7(7): 1085.

Dunn, J.L. and Able, A.J. (2006). 'Pre-harvest calcium effects on sensory quality and calcium mobility in strawberry fruit'. *Acta Horticulturae*, 708:307–311.

Duarte-Filho, J., Antunes, L.E.C., and Pádua, J.G. (2007). ' Productive potential of strawberry cultivars'. *Informe Agropecuário, Belo Horizonte*, 28 (236), 20–23.

Esmel, C.E., Duval, J.R.,Santosi, B.M., SARGENT, S.A., AND Simonne, E.H. (2008). 'Is Strawberry Fruit Firmness Associated with Tissue Ca Concentration'? *Proceedings of the Florida State Horticultural Society* 121:281–284.

Faedi, W., Mourgues, F., and Rosati, C. (2002). 'Strawberry breeding and varieties: Situation and Perspectives'. *Acta Horticulturae*, 567, 51–59.

Fan, Z., Hasing, T., Johnson, T.S., Garner, D.M., Barbey, C.R., Colquhoun, T.A., Sims, C.A., Resende, M.F.R. and Whitaker, V.M. (2021). 'Strawberry sweetness and consumer preference are enhanced by specific volatile compounds'. *Horticultural Sciences Department, University of Florida, IFAS Gulf Coast Research and Education Center*, 8-66.

FAOSTAT. (2014). Agricultural Data; http://www.faostat.fao.org.

Folta, K. M., and Dhingra, A. (2006). 'Transformation of strawberry: The basis for translational genomics in Rosaceae'. *In Vitro Cellular and Developmental BiologyPlant Journal*, 42, 482–490.

Giampieri, F., Tulipani, S., Alvarez-Suarez, J.M., Quiles, J.L., Mezzetti, B., and Battino, M. (2012). 'The strawberry: Composition, nutritional quality, and impact on human health'. Nutrition *Journal*, 8:9-19.

Giampieri, F.; Alvarez-Suarez, J. M.; Mazzoni, L.; Romandini, S.;Bompadre, S.; Diamanti, J.; Capocasa, F.; Mezzetti, B.; Quiles, J. L.; Ferreiro, M. S.; Tulipani, S.; Battino, M. (2012). 'The potential impact of strawberry on human health'. *Natural Product Research*.

Guttridge, C.G. (1985). Fragaria×ananassa, In: A.H. Halevy (ed.). *CRC handbook of flowering*, 3 pp. 16-33.

Hakkinen, S.H., and Torronen, A.R. (2000). 'Content of flavonols and selected phenolic acids in strawberries and Vaccinium species: influence of cultivar, cultivation site and technique'. *Food Research International Journal*, 33:517–24.

Hancock, J.F., J.L. Maas, C.H. Shanks, P.J. Breen, and J.J. Luby. (1990). 'Genetic resources of temperate fruit and nut crops; Strawberries (Fragaria)'. *International society of Horticulture science Journal*, p. 489–546.

Heide, O.M. (1977). 'Photoperiod and temperature interactions in growth and flowering of strawberry'. *Journal of plant physiology*, 40:21-26.

Heide O.M, Stavang, J.A., and Sønsteby, (2013). 'A. Physiology and genetics of flowering in cultivated and wild strawberries–a reviews.' *Horticulture science and Biotechnology Journal,* 88(1):1-18.

Ito, H., and Saito, T. (1962). 'Studies on the flower formation in the strawberry plants: I. Effects of temperature and photoperiod on the flower formation'. *Journal of Agricultural Research*, 13:191-203.

Kafkas, E., Kosar, M., Paydas, S., Kafkas, S., and Baser, K.H.C. (2007). 'Quality characteristics of strawberry genotypes at different maturation stages'. *Food Chemistry*, 100:1229–1236.

Kähkönen. M.P., Hopia, A.I., and Heinonen, M. (2001). 'Berry phenolics and their antioxidant activity'. *Journal of Agricultural and Food Chemistry*, 49:4076–82.

Lopes da Silva, F.; Escribano-Bailon, M. T., Perez Alonso, J. J., Rivas-Gonzalo, J., and Santos-Buelga, C. (2007). 'Anthocyanin pigments in strawberry'. *Journal of Food Science and Technology,* 40, 374−382.

Lundergan, A.C. and Moore, J.N., (1975). 'Inheritance of ascorbic acid content and color intensity in fruits of strawberry (Fragaria anunassa Duch.)'. *Journal of the American Society for Horticultual Science* (6): 633-635.

Mathey, M.M., Mookerjee, S., Mahoney, L.L., G ̈unduz, K., Rosyara, U., Hancock, J.F., Stewart, P.J., Whitaker, V.M., Bassil, N.V., Davis, T.M., and Finn, C.E. (2017). 'Genotype by environment interactions and combining ability for strawberry families grown in diverse environments'. *Euphytica*,213:112.

Mattila, P., Hellstrom, J., and Törrönen, R. (2006). 'Phenolic acids in berries, fruits, and beverages. *Journal of Agricultural and food Chemistry*, 54, 7193−7199.

Maas, J.L. 1978. Screening for resistance to fruit rot in strawberries and red raspberries: *Journal of Horticultural science*, 13:423–426.

Mazzoni, L., Di Vittori, L., Balducci, F., Forbes-Hernandez, T.Y, Giamperi, F., Battino, M., Mezzeti, B., and Capocasa, F. (2019). 'Sensorial and nutritional quality of inter and intra—Specific strawberry genotypes selected in resilient conditions'. *Journal of Scientia Horticulturae.*

Mezzetti, B., Giampieri, F., Zhang, Y.T. and Zhong, C.F. (2018). 'Status of strawberry breeding programs and cultivation systems in Europe and the rest of the world'. *Journal of Berry Research, 205-221.*

Mezzetti, B., Faedi, W., Maltoni, ML, Denoyes, B., Chartier, P., Petit, A. and Sguigna, V. (2010). 'European Small Berries Genetic Resources, GENBERRY: Testing a Protocol for Detecting Fruit Nutritional Quality in EU Strawberry Germplasm Collections.' In: *XXVIII International Horticultural Congress on Science and Horticulture for People (IHC2010): International Symposium, 926*. pp. 33-37.

Mezzetti, B., Mazzoni,L., Qaderi, R., Balducci, F., Marcellini, M., and Capocasa, F. (2021). 'Generating novel strawberry pre-breeding material from a Fragaria × ananassa backcrossing program with F. virginiana subsp. glauca inter-specific hybrids'. *International Strawberry Symposium,* 1309.

Ménager, I., Jost, M., & Aubert, C. (2004). 'Changes in physicochemical characteristics and volatile constituents of strawberry (cv. Cigaline) during maturation'. *Journal of Agricultural and Food Chemistry*, 52, 1248–1254.

Michalska, A., Carlen, C., Heritier, J., and Andlauer, W. (2017). 'Profiles of bioactive compounds in fruits and leaves of strawberry cultivars'. *Journal of Berry Research*, 7:71-84.

Montero, T. M., Mollá, E. M., Esteban, R. M., and López-Andréu, F. J. (1996).’ Quality attributes of strawberry during ripening’. *Journal of Horticultural Science*, 65, 239–250.

Muñoz, K., Buchmann, C., Meyer, M., Schmidt-Heydt, M., Steinmetz, Z., Diehl, D. and Schaumann, G. E. (2017). ‘Physicochemical and microbial soil quality indicators as affected by the agricultural management system in strawberry cultivation using straw or black polyethylene mulching.’ *Journal of Applied Soil Ecology*, 113: 36-44.

Nielsen, T. H., Skjærbæk, H. C., and Karlsen, P. (1991). ‘Carbohydrate metabolism during fruit development in sweet pepper (Capsicum annuum) plants’. Physiologia Plantarum, 82, 311–319.

Nicoll, M.F., and Galletta, G.J. (1987). ‘Variation in growth and flowering habits of June bearing and everbearing strawberries’. *Journal of the American society for horticulture science*, 112:872-80.

Okuda, T.; Yoshida, T., and Hatano, T. (1989). Ellagitannins as active constituents of medical plants. *Plant Medicine*, 55, 117−122.

Ozcan, T., Akpinar-Bayizit, A., Yilmaz-Ersan, L. and Delikanli, B. (2014). ‘Phenolics in human health.’ *International Journal of Chemical Engineering and Applications*, 5(5): 393–396.

Ourecky, D.K., and Bourne, M.C. (1968). ‘Measurement of strawberry texture with an Instron machine’. *Journal of the American Society for Horticultural Science*, 93:317– 325.

Prior, R.L., Lazarus, S.A., Cao, G., Muccitelli, H. and Hammerstone, J.F. (2001). ‘Identification of procyanidins and anthocyanins in blueberries and cranberries (Vaccinium Spp) using high performance liquid chromatography/mass spectrometry’. *Journal of Agricultural food and Chemistry,* 49 , 1270–1276.

Prior, R.L., Cao, G., Martin, A., Sofic, E., McEwen, J., Brien, C.O., Lischner, N., Ehlenfeldt, M., Kalt, W., Krewer G., and Mainland, C.M. (1998). ‘Antioxidant Capacity as Influenced by Total Phenolic and Anthocyanin Content, Maturity, and variety of Vaccinium Species’. *Journal of Agricultural food and Chemistry,*46, 2686–2693.

Puchalski, C., J. Gorzelany, and Goracy, Z. (1994). ‘The effect of maturity and harvest date on firmness of strawberry fruit’. Zemedelska Technika 40:33–43

Rahman, M.M., Hossain, M.M., Khaleque Mian, M .A. and Khaliq, Q.A. (2015). FIELD PERFORMANCE AND FRUIT QUALITY OF STRAWBERRY GENOTYPES UNDER SUBTROPICAL CLIMATE. *'Bangladesh Journal of Agriculture Center.*

Ruan, J., Lee, Y.H., and Yeoung, Y.R. (2013). 'Flowering and fruiting of day-neutral and ever-bearing strawberry cultivars in high-elevation for summer and autumn fruit production in Korea'. *Journal of Horticultural and Environmental Biotechnology*, 54 (2), 109–120.

Scalzo, J., Capocasa, F., Palandrani, A., M. Battino and Mezzetti, B. (2004). 'Quality and Nutritional Value in Strawberry Breeding and Variety Evaluation'. *Euro berry Symposium, Acta Hort, (ISHS) 649, 61–64.*

Scalzo, J., Politi, A., Mezzeti, B., and Battino, M. (2005). 'Plant genotype affects total antioxidant capacity and phenolic contents in fruit'. *Nutrition Journal,* 21, 207–213.

Scalzo, J., Battino, M., Costantini, E. and Mezzeti, B. (2005). 'Breeding and biotechnology for improving berry nutritional quality'. *Biofactors Journal,* 213-220.

Schmitz, F., and Lenz, F. (1985). 'Einfaches Gerät zur messung der Festigkeit von Erdbeerfrüchten'. *Gartenbauwissenschaft,* 50:261–264.

Serradilla, M.J., Lozano, M., Bernalte, M.J., Ayuso, M.C., López-Corrales, M., and González-Gómez, D. (2011). 'Physicochemical and bioactive properties evolution during ripening of Ambrunés : sweet cherry cultivar'. *Journal of Food Science and Technology.* 44:199–205.

Shamaila, M., Baumann, T.E., Eaton, G.W., Powrie, W.D. and Skura, B.J.(1992). 'Quality attributes of strawberry cultivars grown in British Columbia'. *Journal of Food Science*., 57(3): 6%-699.

Shaw, D.V. (1990). 'Response to selection and associated changes in genetic variance for soluble solids and titratable acids contents in strawberries'. *American Journal of Horticultural Science,* 115(5): 839-843.

Shiow, Y., Wang, L., and Kim, S. (2007). 'Antioxidant capacity and flavonoid content in wild strawberries'. *American Journal of Society and Horticultural Science*, 132: 629–637.

Simpson, D.W. (2003). 'How should strawberry germplasmm collections be evaluated to maximize their value to plant breeders?' *Proceedings of the EuroBerry Symposium COST836 Final Workshop, Acta Hortic*, 649:45-48.

Terrettaz, R., and Carron, R. (1991). 'Essai de varietes de fraisiers en montagne'. *Revue suisse de viticulture a'arboriculture et d'horticulture*. 23:249–251.

Tulipani, S., Mezzetti, B., Capocasa, F., Bompadre, S., Beekwilder, J., Ric de Vos, CH., et al. (2008). 'Antioxidants, phenolic compounds and nutritional quality in different strawberry genotypes'. Journal of Agricultural and food Chemisty, 56:696-704.

Tulipani, S., Mezzetti, B., Battino, M. (2009). Impact of strawberries on human health: Insight into marginally discussed bioactive compounds for the Mediterranean diet. *Public Health Nutrition* 12(9A):1656–1662.

Usenik V., Fabcic, J., and Stampar, F. (2008). 'Sugars, organic acids, phenolic composition and antioxidant activity of sweet cherry (*Prunus avium* L.)'. *Journal of Food Chemistry,* 107:185–192.

USDA. (2005). USDA National Nutrient Database for Standard Reference, Release 18, Washington DC, US Department of Agriculture, Agriculture Research Service.

US Department of Agriculture, Agriculture Research Service. (2010). USDA national nutrient for standard references, release 23. Fruits and fruit juices, pp. 785–7.

Wang, S.Y., Lewers, and K.S. (2007). 'Antioxidant capacity and flavonoid content in wild strawberries'. *Journal of the American society and Horticultural science*, 132: 629–637.

Woodward, J.R., (1972). 'Physical and chemical changes in development strawberry fruits'. J. Sci. Food Agric., 23: 465-473.

Wang, S. Y.; Lewers, K. S. (2007). 'Antioxidant capacity and flavonoid content in wild strawberries'. *Journal of the American society and Horticultural science*, 132, 629−637.