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# DETERMINATION OF THE QUALITY AND PHYTOCHEMICAL CONTENT OF F1 STRAWBERRY GENOTYPES SELECTED IN BILECIK PROVINCE ECOLOGICAL CONDITIONS

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## ABSTRACT

The production of strawberry cultivation in wide ecological regions, its aroma, rich mineral content and taste affect the preference of consumers positively. The high amount of aroma of local cultivars enables them to be used as parents in breeding studies. In this study, it was aimed to determine the morphological and pomological characteristics and phytochemical contents of the superior F1 strawberry genotypes selected after hybridization with the local Osmanli strawberry under the ecological conditions of Bilecik province. Morphological and pomological analyzes were made and the best ten genotypes were selected according to the weighted grading method. According to this, the titratable acidity amount of the selected genotypes was determined between 0.28–0.51, the total soluble solid amount was determined between 5–10.5%, and the pH amount was determined between 3.3–3.78. By looking at the average fruit weight, fruit width and fruit length values, genotypes that can compete with commercial cultivars were selected and these were determined as table genotypes. While the total phenolic content of the ten genotypes selected by phytochemical analyzes was found between 2675.56–3983.89 µg GAE/g TA, the total amount of anthocyanin was determined between 156.19–39.12 µg Plg-3-glu/gta. The antioxidant amount was 18.95 µmol TE/ g ta-7.54 µmol TE/ g in the FRAP method.

**Key words:** anthocyanin, antioxidant, breeding, *Fragaria ananassa*, Osmanli strawberry

## INTRODUCTION

Strawberry, which belongs to the *Rosales* order, *Rosaceae* family and *Fragaria* genus and is in the berry group, has started to be cultivated in Europe in the 1300s [Hancock and Luby 1993]. Strawberry production in the world has increased rapidly in the last fifty years. The most important reasons for this are the good taste and aroma, positive effects on human health, high adaptability and new cultivars developed as a result of breeding [Akbas et al. 2021].

While strawberry production in Turkey was 9,700 tons in the 1970s, it reached 546 thousand tons in 2020. With this production, Turkey ranks fourth in world strawberry production. Strawberry cultivation is carried out all over the country and it is focused in the Mediterranean, Marmara and Aegean regions.

Among the fruit species, especially berry fruits contain high amounts of bioactive substances that are of great importance for health due to their anticarcinogenic and antioxidant properties. Antioxidants neutralize molecules called free radicals, which arise after metabolism products in the human body. It is known that if free radicals are produced in large quantities, there

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is damage at the cell nucleus level and cancer tumors occur as a result of the activation of some enzymes [Ozgen et al. 2006]. Thus, it is thought that these risks can be reduced by the consumption of foods with high antioxidant content [Sandra 2004]. With the ellagic acid, minerals, oils, vitamins A, B, C, calcium, iron, amino acids, rich fiber and phenolic substance content in a 100 g strawberry, consumption and importance of strawberries increase both in terms of cellulose content and anti-cancer properties [Atasay and Turemis 2008]. In studies conducted in recent years, it has been determined that strawberries, which are consumed as processed or fresh, have a protective effect on humans against oxidative stress, inflammation, type 2 diabetes, cardiovascular diseases, blood pressure, esophageal cancer and other cancers [Terefe et al. 2013].

With the research done in the world and the increase in strawberry cultivation, breeding studies have also increased. The primary objectives in strawberry breeding studies can be listed as yield increase, size, resistance to road time, aroma, resistance to diseases and pests, adaptation to different soil conditions, flowering period, ripening time, neutral or short-day conditions. In addition to these features, it is known that its contribution to health is also included in breeding studies [Akbas et al. 2021].

The nutritional value of fruits is affected by the structure of the fruit, its species and diversity within species. However, it is known that growing conditions have an effect on nutritional value. It has been reported that the quality and antioxidant properties of strawberries differ between genotypes. In the results of the study on the total antioxidant capacity of the plant genotype and its effect on the phenolic content, it was stated that wild strawberries had more antioxidant content than cultured strawberries [Ozgen et al. 2007, Gunduz 2010]. The factors affecting the antioxidant capacity of fruits are genetic differences, shelf life and ripening times [Connar et al. 2002].

Although the local varieties like Osmanli cultivar in Turkey have demanding superior aroma properties, due to low yield, small fruits and short shelf life with soft fruit flesh, these genotypes cannot compete with commercial cultivars in the markets. Therefore, breeding studies were carried out by several researchers in Turkey in order to transfer the superior aroma characteristics to commercial varieties [Konarlı et al. 1984, Ustun and Paydas 1995, Yasa 1997, Erenoglu et al. 1998, Caglar and Paydas 2002, Serce 2006, Serce and Ozgen 2014, Ozturk Erdem and Cekic 2017]. In our preminally studies, many cross breedings were made using three local genoypes (Osmanli, Karacilek and Tuylu) and three commercial cultivars (Sweet Charlie, Sweet Ann and Kabarla). In all crosses, Osmanlı local cultivar was used as female parent due to it's morphologically male infertile character. Among the thousands of F1 plants, some F1s were first selected according to some fruit characters as well as aroma properties. The selected F1s were compared with each other and with parents in the first step of the study in Tokat ecological condition of Turkey [Ozturk Erdem and Cekic 2020]. In the current study, the 53 F1s obtained by first selection were propagated in Bilecik ecological conditions of Turkey, compaired with each other and then, the fruit properties of ten superior F1s obtained by the second selection according to the weighted grading criteria were analysed compairing with sweet charlie cultivar, one of parent used in first crosses.

## MATERIAL AND METHOD

Among the 1600 F1 strawberry genotypes obtained by hybridization in the 'Osmanli Strawberry Breeding-I' thesis study between 2013–2018, the best 53 F1 strawberry genotypes were selected according to the weighted grading criteria [Erdem 2018]. After that, these genotypes were evaluated by weighted grading in Bilecik ecological conditions and ten genotypes came to the fore [Ozturk Erdem and Cekic 2020]. These ten selected genotypes and standard Sweet Charlie commercial strawberry cultivar were used as the material of this study.

F1 strawberry genotypes were brought from Tokat Gaziosmanpaşa University Application Center to Bilecik Şeyh Edebali University Agricultural Application and Research Center in November 2018 and the plants were propagated under controlled conditions. In July 2019, the beds with a width of 70 cm and a spacing of 25 cm were covered with a black greenhouse ground cover. Five plants from each genotype were planted on the bed with a triangular planting system at 30 × 30 cm intervals. In order for the plants to develop more vigorously in the first planting year, the flowers and stolons formed after planting were

plucked throughout the vegetation, moreover, cultivation applications and fertilization were made.

During the vegetation period of 2020, morphological and pomological analyzes were made in all genotypes and sorted according to the weighted grading method. The features used in the weighted rating method, variable score and relative scoring were made according to the previous study. Besides, the morphological observation findings determined according to the UPOV Description of the ten best genotypes selected in Bilecik ecological conditions were not included in this article because they were found to be similar to the findings in Tokat ecological conditions [Ozturk Erdem 2018, Ozturk Erdem and Cekic 2020].

**Average fruit weight.** Fruit weight was calculated by dividing the total fruit weight in each harvest by the number of fruits.

**Fruit width and length.** The width and length of the fruits were determined with the help of a digital caliper.

**Total acidity.** The acidity of the fruits was determined in terms of citric acid using the titration acidity method and the values were expressed as "%" [Cemeroglu 2007].

**pH.** The pureed fruits in the homogenizer were measured with a pH meter by directly immersing the glass electrode [Cemeroglu 2007].

**Total soluble solid (TSS).** After 10 fruits of each genotype were homogenized, they were passed through coarse filter paper and the first drops were taken on a hand refractometer (0-53 scale, Refractometer PAL-1) calibrated according to pure water, and the results were expressed as "%" [Cemeroglu 2007].

**Total antioxidant capacity.** The antioxidant capacities of strawberries were determined using two different methods, FRAP and TEAC, which are frequently used for plant materials [Ozgen et al. 2006].

**FRAP analysis (iron reduction antioxidant capacity).** For FRAP analysis [Benzie and Strain 1996], a buffer was prepared by mixing 0.1 mol/L acetate (pH 3.6), 10 mmol/L TPTZ, and 20 mmol/L ferric chloride solutions (10/1/1). Finally, 2.97 mL of the prepared buffer solution was added to 30 µL of extract and mixed, and the absorbance was measured at 593 nm wavelength in the spectrophotometer after 30 minutes.

**TEAC analysis (trolox equivalent antioxidant capacity).** For TEAC analysis [Özgen et al. 2006], 7 mM ABTS – 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) – was mixed with 2.45 mM potassium bisulfate and kept in the dark for 12–16 h. Then, this solution was simplified with 20 mM sodium acetate (pH 4.5) buffer solution to 0.700 ±0.01 absorbance at 734 nm wavelength in the spectrophotometer. Finally, 30 µL of the extract was mixed with 2.97 mL of prepared copper, and the absorbance was measured after 10 minutes at a wavelength of 734 nm in the spectrophotometer.

**Determination of total phenol.** Total phenol amount determination was made according to the method of Singleton and Rossi [1965] using Folin-Ciocalteu's reagent. For this purpose, acetic acid, water and acetone (0.5/29.5/70) solution was added for the homogenized mash extraction process and kept for one day. Folin-Ciocalteu's reagent and distilled water were mixed and left for eight minutes, and then 7% sodium carbonate was added. The absorbance of the solution, which turned bluish after two hours of incubation, was measured in a spectrophotometer at a wavelength of 750 nm. The results were calculated as fresh fruit in terms of Gallic acid µg GAE/g.

**Determination of total anthocyanin.** Total anthocyanin was determined by the pH difference method according to Giusti and Wrolstad [2005]. Samples were extracted by preparing pH 1.0 and 4.5 solutions, and readings were made at 520 and 700 nm wavelengths. The total anthocyanin content was calculated based on pelargonidin 3-glucoside (molar absorption coefficient = 31600) and the absorbances were calculated with the formula [(A520–A700) pH 1.0-(A520–A700) pH 4.5], and the values determined as µg anthocyanin/g dry matter.

## RESULTS AND DISCUSSION

The sum of the scores obtained by multiplying the variable score and the relative scores of each characteristic gave the weighted grading total value score of the cultivars, and ten of the genotypes with the highest total value score were selected (Tab. 1). According to the weighted grading method, 53 genotypes used in the study were scored as 25% with their fruit aroma, 20% with morphologically male organ fertility (the present of male organs), 15% with everbearing character of plants, fruit weight and fruit firmness, 10% with fruit color as a total of 100 points. Then, the superior

10 F1s were reevaluated in terms of fruit largeness, measuring the fruit width and height, which are demanding in marketing.

**Table 1.** F1 individuals and scores selected as a result of the weighted grading

| F1 Code | Score | F1 Code | Score |
|---|---|---|---|
| DB48 | 755 | DA88 | 700 |
| DB35 | 750 | DK1-24 | 680 |
| DA1 | 745 | DT2-23 | 680 |
| CC48 | 705 | CC60 | 675 |
| DA9 | 700 | DB119 | 665 |

The numbers after local cultivar names show where the genotypes were collected

The genotypes, which are in the first ten of the present study in Bilecik province ecological conditions, are among the first twenty genotypes of the preminaly study in Tokat province ecological conditions. It is known that quality components such as aroma, fruit firmness, fruit weight and color, which are among the scaled grading characteristics, are affected by genetic and especially environmental factors [Sistrunk and Morris 1985].

The average fruit weight, fruit width and fruit length of the genotypes are given in Table 2. DA-88 genotype was determined as the best genotype in terms of fruit width and weight. DA-88 genotype was followed by DB-119, DB-48 and CC-60 genotypes, respectively. DA-88 and DB-119 genotypes were found to be better in weight than the Sweet Charlie cultivar.

TSS amounts obtained as a result of analyzes in genotypes are given in Table 3. The amount of TSS is important in terms of quality criteria, it determines the amount of sugar in the fruit, and it is known that fruits with high sugar content are demanded by the consumer. TSS values in the study varied between 5% and 10.5%. DB119, DA9, DA88 and DT2-23 genotypes exceeded the acceptable flavor limit in strawberries.

In the studies of determining the quality components of strawberry cultivars, TSS values varied between 7.3% and 9.5% in Erzurum province [Ozbahceli and Aslantas 2015], 8.70% and 9.91% in Amasya province [Gecer et al. 2018], and 6.4% and 9.9% in Bursa province [Ozok 2021].

In the study of Shaw [1988], it was reported that fruit structures are affected by genetic factors, but the effect of the interaction of genetic factors and environmental conditions on fruit structure was not stated. In that research, it was indicated that acid contents, which are affected by genetic factors, remain stable during the production cycle, but TSS rates may change with environmental conditions. It is thought that the differences in the amount of TSS determined in Tokat province conditions of the genotypes used in the present study and the differences determined in Bilecik province are due to environmental conditions.

The pH values of the genotypes were found to be 3.78 in the DT2-23 hybrid as the highest and 3.3 in the DB119 hybrid as the lowest (Tab. 3).

Esitken and Alan [2016] determined that the pH values between 3.54 and 3.63 in the study they conducted on Sweet Ann, Kabarla, Crystal, Fern and Red-

**Table 2.** Average fruit weight (g), fruit width-length (mm) of genotypes

| Genotypes code | Weight (g) | Width (mm) | Length (mm) | Genotypes code | Weight (g) | Width (mm) | Length (mm) |
|---|---|---|---|---|---|---|---|
| DA88 | 15.93 | 32.63 | 35.15 | DB35 | 9.00 | 27.17 | 27.1 |
| DB119 | 12.33 | 31.10 | 35.29 | DT2-23 | 8.95 | 28.10 | 27.04 |
| DB48 | 11.77 | 30.26 | 30.1 | DK1-24 | 7.63 | 23.68 | 29.38 |
| CC60 | 11.63 | 30.59 | 31.76 | CC48 | 3.92 | 21.12 | 20.61 |
| DA9 | 10.82 | 27.90 | 33.93 | Sweet Charlie | 12.23 | 30.45 | 32.13 |
| DA1 | 10.13 | 27.22 | 33.78 | | | | |

**Table 3.** Amounts of genotypes, total soluble solid (TSS, %), pH, titreable acidity (TA, %)

| Genotypes code | TSS (%) | pH | TA (%) |
|---|---|---|---|
| DB119 | 10.5 | 3.3 | 0.51 |
| DA9 | 9.1 | 3.44 | 0.47 |
| DA88 | 8.5 | 3.36 | 0.38 |
| DT2-23 | 7 | 3.78 | 0.3 |
| CC48 | 6 | 3.49 | 0.28 |
| Sweet Charlie | 5.9 | 3.31 | 0.36 |
| DB35 | 5.8 | 3.42 | 0.43 |
| CC60 | 5.5 | 3.55 | 0.4 |
| DA1 | 5.1 | 3.5 | 0.48 |
| DB48 | 5.1 | 3.45 | 0.33 |
| DK1-24 | 5 | 3.47 | 0.29 |

lans Hope strawberry cultivars grown in Kayseri province ecological conditions. Oguz et al. [2017] found the pH values between 3.61 and 3.85 in the cultivars (San Andreas, Monterey, Portola, Kabarla, Albion) they examined in their study in the Nevşehir province, while Gunduz and Bayazit [2017] found pH values between 3.25 and 3.90 in 42 strawberry cultivars developed as a result of breeding programs in America, Europe and Turkey. Akbas et al. [2021] reported that pH values varied between 3.43 and 3.58 in the study they conducted with Fortuna, Rubygem, Festival cultivars and the genotypes that came to the fore in the breeding study conducted by Cukurova University in Adana province.

Although the results obtained in our study are similar to other studies, it is thought that the differences in some cultivars occur due to environmental conditions such as light, nutrition, and also genetic factors [Sistrunk and Morris 1985].

As seen in Table 3, the titratable acidity values of the genotypes were found to be 0.51% in the DB-119 genotype as the highest and 0.28% in the CC-48 genotype as the lowest.

Ozbahcali and Aslantas [2015] found the titratable acid values of the cultivars between 0.3% and 0.5% in their study in Erzurum province; Celebioglu et al. [2018] found titratable acidity to be 1.08% in Osmanli cultivar, 0.82% in Sweet cultivar, and 1.26% in Tüylü cultivar in Tokat province; Gunduz and Bayazit [2017] found the titratable acidity rates of the cultivars they used in their studies to be between 0.44% and 1.72%, while another researcher reported that this rate ranged from 0.53% to 0.91% among the cultivars used in Bursa province ecological conditions [Ozok 2021]. Titratable acid content ratios, which we examined in our study and included in the taste criteria, were found to be compatible with other studies.

The total phenolic content of the genotypes ranged from 2675.56 to 3983.89 µg GAE/g TA (Tab. 4). When the genotypes were examined, the highest total phenolic substance content was found in DT2-23, and the lowest total phenolic substance content was found in the DA-88 genotype [Ozturk Erdem 2018]. As a result of the research carried out with the same genotypes in Tokat province conditions in 2016, the amount of phenolic substance varied between 1807.72 and 3116.31 µg GAE/g TA and it was determined that the DA88 genotype had the lowest phenolic substance. At the same time, when the two studies were compared, the increase in total phenolic substance was observed in all genotypes in Bilecik province ecology. It is thought that factors such as direct exposure to light, harvest time, day length, ecological differences are effective in the production of phenolic compounds [Ozgen et al. 2007, Gunduz 2010].

In the study conducted by Copetti et al. [2012], the total phenolic content of different strawberry cultivars was determined as 998.0–1177.5 µmol TE/100 g, moreover, in a study investigating fruit quality characteristics in four different harvest periods in Ordu province ecological conditions by Karakaya et al. [2015], the lowest and highest total phenolic contents were

**Table 4.** Total phenolic content (TPC), total anthocyanin (TA), total antioxidant capacity (TEAC-FRAP) values of the genotypes

| Genotypes code | TPC (µg GAE/g ta) | TA (µg Plg-3-glu/gta) | TEAC (µmol TE/ g ta) | FRAP (µmol TE/ g ta) |
|---|---|---|---|---|
| CC60 | 3435.56 | 61.6 | 12.65 | 9.89 |
| CC48 | 2730.56 | 62.6 | 16.44 | 12.32 |
| DT2-23 | 3983.89 | 39.12 | 12.78 | 9.03 |
| DA-1 | 3207.23 | 57.76 | 12.63 | 9.14 |
| DA-88 | 2675.56 | 72.66 | 11.46 | 7.54 |
| DA-9 | 3005.56 | 40.58 | 15.51 | 11.02 |
| DK1-24 | 3853.89 | 60.87 | 18.95 | 14.23 |
| DB-35 | 3623.89 | 109.12 | 17.85 | 11.62 |
| DB-48 | 3085.56 | 65.53 | 13.69 | 9.71 |
| DB-119 | 3218.89 | 156.19 | 15.1 | 10.99 |
| Sweet Charlie | 3324.12 | 63.41 | 15.48 | 10.3 |

found to be 545.27 mg/kg – 1069.15 mg/kg in the second harvest period. Besides, Oz and Eker [2016] stated that they found the total phenolic content to be 3053 mg/kg in the Osmanli cultivar and 3774 mg/kg in the Rubygem cultivar.

Strawberry is an important source of phenolic compounds with natural antioxidant properties such as flavonoids, anthocyanins, minerals and vitamins [Lopes da Silva et al. 2007]. It is known that color formation, which is an important criterion in strawberry fruit, is caused by anthocyanins, which constitute 44% of phenolic compounds. These anthocyanins are pelargonidin 3-glucoside and cyanidin 3-glucoside. It is known that the amount of total anthocyanin in strawberries varies between 150–300 µg/g [Clifford 2000]. In the study, the lowest total anthocyanin amount among the genotypes was determined as 39.12 µg Plg-3-glu/gta, and the highest was 156.19 µg Plg-3-glu/gta. Furthermore, the highest total anthocyanin content was seen in the DB-119 genotype (Tab. 4).

The data obtained by the TEAC and FRAP methods used to determine the antioxidant capacities of strawberry genotypes in the study are given in Table 4. The data obtained as a result of using the FRAP method were found to be similar to the results obtained in the TEAC method. The highest antioxidant amount in the DK1-24 genotype was found to be 18.95 µmol TE/g ta with the TEAC method and 14.23 µmol TE/g ta with the FRAP method. Besides, the lowest was found to be 11.46 µmol TE/g ta with the TEAC method and 7.54 µmol TE/g ta with the FRAP method in the DA-88 genotype.

In a study using 16 strawberry cultivars and 4 selected hybrid individuals by Capocasa et al. [2008], the antioxidant capacities of the cultivars were determined between 11.2 and 18.4 µmol TE/g with the TEAC method. Moreover, in a study conducted by Gunduz [2010] with 13 strawberry cultivars, it was stated that the antioxidant capacities of the cultivars varied between 3.92 and 14.36 mmol TE/kg in 2007–2008 and 6.56–9.05 mmol TE/kg in 2008–2009. Saracoglu and Ozgen [2015] reported that they obtained similar results from the FRAP and TEAC methods in their study. When the previous studies were examined, it was observed that the results were similar to the results in our study.

## CONCLUSIONS

The production of strawberry cultivation in wide ecological regions, its aroma, rich mineral content and taste affect the preference of consumers positively. This situation makes the strawberry the most cultivated fruit among the berry fruits. The main purpose of strawberry cultivation is to have a say in domestic and foreign markets by growing high quality and early cultivars. In addition to the superior aroma characteristics of our local cultivars, low yield, small fruit size and soft fruit flesh do not allow profitable cultivation.

In this study, the adaptation of the selected promising F1 strawberry genotypes in Bilecik ecology was determined and the best ten genotypes were selected. Among the selected genotypes, those that will compete with the commercial cultivars in terms of average fruit weight, fruit width and fruit length were determined and these were evaluated for table consumption, while DB-35, DT2-23, DK1-24 genotypes with smaller fruit sizes were evaluated as industrial consumption. Some of F1s have also higher total soluble contents than commercial cultivar used in Bilecik and the other all commercial parents in Tokat region [Ozturk Erdem 2018]. In practice, TSS is one of the indexes for judging the maturity and sugar content, so that sweetness, of fruits. Phenolic substances in the composition of strawberries and anthocyanins, which are responsible for the formation of red color, increase the antioxidant capacity of strawberries and provide important contributions to human health. In breeding studies, the priority is the aroma, large size, resistance to transportation, resistance to diseases and pests, hard fruit flesh, ripening time, compatibility with different soil conditions, and recently the high amount of antioxidants. The chemical properties of the genotypes that formed the material of the study, which started as Osmanli Strawberry Breeding-1, were examined both in Tokat and Bilecik provinces. In the present and preminally studies [Ozturk Erdem 2018] showed that phytochemical contents of the ten best performing genotypes are higher values of those both local and commercial parents used for F1s contributed to the ongoing breeding works.

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