Investigation of Yield and Pomological Attributes of a Moroccan *ex-situ* Collection of Apple Varieties (*Malus domestica* Borkh.)

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Summary

Several studies reported that apple adaptation was influenced by many factors related to environmental conditions and the genotype. In the present research, the impact of the cultivar factor was assessed within the specific context of Moroccan conditions. Therefore, we conducted tests on twenty-six different apple cultivars to evaluate their productive potential, vegetative growth and pomological characteristics of their fruits. The findings revealed a substantial variation among apple cultivars across all measured characteristics. The yield of the fruits as well as their weight (a range of 19.64 – 67.92 kg tree^{-1}) and their size (128.16 – 265.61 g and 60.02 – 81.86 mm) were within the corresponding limits. However, chemical analysis indicated that the concentration of total soluble solids in the apple fruit ranged from 13.46 °Brix to 18.29 °Brix, while the titratable acidity varied between 0.044% and 0.143% of citric acid. Biochemically, remarkable differences were found in the apple varieties tested. The total sugar content ranged from 113.96 to 142.57 g GE L^{-1}, while the total phenols content ranged from 1.56 to 23.29 g GAE L^{-1}. Additionally, the antioxidant activity varied between 19.13% and 90.53% respectively. The data analyses further indicated that the variety 'Gala Royal-2' exhibited the highest annual shoots growth, while 'Po55' displayed the highest leaf area. In conclusion, the finding revealed that the cultivar had a significant impact on the fruit yield, as well as the physical, biochemical and vegetative characteristics of apples. Thus, these results contribute to enhancing our knowledge of the diverse apple cultivars and their potential applications in various fields, such as breeding, cultivation and the food industry.

Key words

Malus domestica L., cultivar, productive potential, vegetative growth, biochemical traits

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Introduction

Apple trees are one of the most widely cultivated fruit trees globally, belonging to the *Rosaceae* family and the *Malus* genus (Cornille et al., 2019). The origin of apple trees can be traced back to Central Asia, particularly the region covering present-day Kazakhstan, Tajikistan, and Kyrgyzstan. From there, they spread to neighboring regions such as China and Europe (Bondonno et al., 2014). The distribution of apple trees expanded over time due to human cultivation and trade. Today, they are grown in various parts of the world, including North America, South America, Europe and Asia (Harris et al., 2002).

Apart from their delightful taste and culinary uses, apple trees also possess various medical and nutritional properties (Hussain et al., 2014). Apples are a rich source of dietary fiber, vitamins (particularly vitamin C), and minerals such as potassium and antioxidants (Salehi, 2020). Consuming apples regularly has been associated with several health benefits, including improved digestion, reduced risk of heart disease and lowered cholesterol levels (Boyer and Liu, 2004; Jensen et al., 2009). Apples also contain phytochemicals, such as flavonoids and polyphenols, which have anti-inflammatory and antioxidant properties (Tu et al., 2017; Ferrario et al., 2022). These compounds may help in preventing chronic diseases, such as cancer, Alzheimer's disease and diabetes (Tripathi and Mazumder, 2020). Additionally, the dietary fiber in apples promotes a healthy gut by aiding digestion and preventing constipation (De Lima et al., 2014).

The popularity and adaptability of apple trees have led to the development of numerous cultivars, resulting in a wide range of apple varieties with distinct flavors, textures and colors (Dan et al., 2015). However, other research reported that the diversity of apple trees was not only evident in their fruits but also in their growth habits, disease resistance and adaptation to different climates and soil conditions (Veteto and Carlson, 2014). This diversity has been nurtured and expanded through centuries of selective breeding and natural selection, resulting in a wide range of apple varieties that can thrive in various regions worldwide.

In Morocco, apple trees are an important part of the country's agricultural landscape (Moinina et al., 2019). Thus, the country is known for its diverse apple orchards that produce different varieties of apples, catering to both domestic and international markets. Morocco's apple production is mainly concentrated in the Middle Atlas and High Atlas regions, where the climate and soil conditions are suitable for apple cultivation (Razouk et al., 2018). These areas provide the necessary cold winters which fulfill the chilling requirements for apple trees and cool summers, which are required for the optimal growth of apple trees and promote better fruit development (Razouk et al., 2018; Moinina et al., 2019). According to the MAPMDREF, (2022), the total apple production in Morocco reached 889736 tons in 2021, making it one of the leading fruit crops in the country.

In spite of various *Malus domestica* genotypes grown in different Moroccan areas, few published studies on the diversity of these cultivars are available in the literature and to the best of our knowledge, all research reported in this sense was based only on the fruit morphometric attribute. Therefore, the aim of the present investigation was to analyze and compare the yield, vegetative growth, fruit physical, chemical and biochemical attributes of an apple collection of twenty-six genotypes widely cultivated in Moroccan regions with the objectives of evaluating the importance of cultivar factor in the adaptation of the apple crops under the semi-arid conditions.

Materials and Methods

Plant Material and Experimental Design

The plant material comprised 26 apple cultivars, each twelve years old, planted with a spacing of 5 × 3 m and pruned to form a goblet canopy shape, following a randomized complete block (RCB) design, with six trees allocated for each cultivar. Uniform fertilization was administered to all trees (100 kg of N, 60 kg of P_2O_5, and 120 kg of K_2O per hectare). The trees irrigated at 3500 m^3 per year supplied from April to October. Pest control followed local commercial practices, and effective weed control measures were implemented.

The experiment was carried out at Annoceur experimental station of the National Institute for Agricultural Research (INRA) (33°42'E; 4°49'N; Alt. 1350 m). The soil possesses a sandy-clay texture, conforming to the internationally recognized standards as stated by FAO (2006). Additionally, the soil contains 1.03% organic matter, and its P_2O_5 and K_2O contents measured were 29 and 361.4 ppm respectively. Moreover, at a soil depth of 0-35 cm, the electrical conductivity of the soil saturation extract was recorded as 0.27 mS cm^{-1}.

The meteorological data during the experimental season reported that the site had a wide range of temperatures throughout the year (Fig. 1). During the summer months (June to August), temperatures typically range from 26 °C to 41 °C, with occasional heat waves pushing the mercury even higher. Winters (December to February) are relatively mild, with temperatures averaging around -7 °C to 15 °C. Spring and autumn have transitional temperatures between these extremes. The site receives an average annual precipitation of approximately 408.6 mm. Rainfall is in larger amounts during the spring and autumn months. Summer tends to be drier, with occasional thunderstorms providing localized downpours. Winter precipitation primarily occurs in the form of rain, although occasionally some snow may occur.

Yield and Fruit Traits

At harvest, a total of twenty fruits were gathered from each experimental tree and promptly transferred to the laboratory for analysis. To ensure a comprehensive assessment, the fruits were selected from various levels of the trees, aiming for representativeness. The average weight of the fruits was determined using a digital balance of precision of at least 0.001 g. The fruit yield for each cultivar was calculated by multiplying the weight of the fruits by the count of fruits per tree. Furthermore, the equatorial fruit diameter was measured utilizing an electronic digital slide gauge (Mitutoyo, China), with an accuracy of 0.01 mm.

Chemical and Biochemical Traits

Juice chemical characterization was assessed by measuring the total soluble solids (TSS), titratable acidity (TA) and maturity index (MI). Indeed, the fruit TSS content was measured using a digital refractometer (PR-101 ATAGO, Norfolk, VA, USA), and the values were expressed as °Brix. The TA of the apple fruits was evaluated by measuring the proportion of citric acid in the prepared extract, employing a titration method that involved titrating 2.5 mL of juice skillfully mixed with 50 mL of water with 0.1 M of sodium hydroxide. The maturity index was determined as a ratio between the TSS and TA of the apple fruit.

The study also examined the biochemical analyses of apple fruits, including the assessment of total sugars (TS), total phenols (TP), total flavonoids (TF), anthocyanins content (AC) and antioxidant activity (AA). The SSC was determined using the method described by Dubois et al. (1956), with slight modifications. To begin, 100 µL of apple fruit extract combined with 500 µL of phenol and 2.5 mL of sulfuric acid solutions gave the mixture which was vortexed and allowed to stand for 10 minutes, after which it was placed in a water bath at 30 °C for 20 minutes. The absorbance of both the samples and the control was measured at 480 nm. The results were expressed as grams of glucose equivalent (g GE) per kg.

The determination of the total phenolic content (TPC) was carried out spectrophotometrically using the Folin-Ciocalteu reagent, following the procedure outlined by Khanizadeh et al. (2008) with slight modifications. In brief, 100 µL of phenol extract combined with 100 µL of FC reagent was left at room temperature for 4 minutes. Subsequently, 800 µL of 5% Na_2CO_3 solution was added to the mixture. After 20 minutes at 40 °C, the absorbance at 750 nm was measured. The results were reported as gallic acid equivalents (GAE), representing the amount of gallic acid in milligrams per kg of fruit fresh weight.

The determination of total flavonoid content (TFC) followed the method outlined by D'Abrosca et al. (2007), with minor modifications. 1 mL of the prepared extract at various concentrations was combined with 0.3 mL of 5% (m/v) $NaNO_2$. After 5 minutes, 0.3 mL of 10% (m/v) $AlCl_3$ was added. Following a 6-minute interval, 2 mL of 1 M NaOH were introduced. The absorbance was measured at 510 nm, and the quantification of flavonoids was performed using a standard curve of quercetin (10–160 µg/mL). The results were expressed as milligrams of quercetin equivalents (QE) per 100 g of fresh material.

The total anthocyanin content (TAC) was determined following the pH differential method described by Giusti MM (1999). To quantify TAC, a diluted extract sample (1 mL) was mixed with separate 9 ml portions of pH 1.0 and pH 4.5 buffers, in triplicate. The absorbance of these mixtures was measured at 520 and 700 nm using a UV–vis spectrophotometer. TAC was expressed as mg of cyanidin 3-glucoside equivalent (with a molar extinction coefficient of 26,900 and a molecular weight of 449.2) per 100 ml of fresh weight (mg of $C_3gE/100$ g).

Figure 1. The meteorological data (maximum, minimum and mean temperature (°C), dew point (°C), vapor pressure deficit (KPa), relative humidity (%) and precipitation (mm) of the experimental station

Table 1. Physical and chemical properties of the soil in the experimental station

Soil depth	Clay (%)	Silt (%)	Sand (%)	Organic matter (%)	P_2O_5 (ppm)	K_2O (ppm)	pH	EC (mS cm^{-1})
0-35 cm	29.5	31.9	38.6	1.03	28.7	361.4	8.36	0.27
35-70 cm	22.9	34.7	42.4	1.24	15.8	146.3	8.38	0.13

The measurement of antioxidant activity (AA) in apple fruits to eliminate the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was conducted following the procedure described by Siano et al. (2016). In this experiment, 0.25 mL of the phenolic fraction of the fruit extract was combined with 0.5 mL of a methanolic solution containing DPPH radicals (6×10^{-6} M). The resulting mixture was vigorously vortexed and incubated for 30 minutes in darkness at room temperature. Subsequently, the absorbance at 517 nm was determined using a spectrophotometer. To establish a baseline, the absorbance of the DPPH in 80% methanol was measured as the negative control. The DPPH scavenging effect was quantified as the percentage of DPPH discoloration, employing the following equation (Eq. 1):

$$\text{Percentage scavenging effect} = [(Abs_{control} - Abs_{test}) / Abs_{test}] \times 100 \quad (1)$$

Vegetative Properties

In June, the measurements of shoot length, number of leaves per 10 cm of shoot, and leaf area were conducted for all trees of each cultivar. The determination of shoot length involved adding up the lengths of all shoots growing on 12 branches (two-year-old) per cultivar, which were marked from the four cardinal directions (East, West, North, and South) of the trees. To minimize variability resulting from branch vigor, the sum of shoot lengths within each branch was transformed to achieve an average shoot length in cm.m^{-1}. Foliage density on the selected shoots was determined by counting the number of leaves per 10 cm of shoot. Additionally, leaf area was measured on the ten most developed leaves per tree, which were selected from shoots of similar lengths. This measurement was performed using a leaf area meter (adc, bioscientific Ltd).

Statistical Analysis

The data analysis was conducted with SPSS v22, employing analysis of variance (ANOVA). The significance of differences among means was assessed at $P < 0.05$ using Tukey's HSD test. Thus, the correlation coefficients were determined using Pearson correlation on mean values to identify the relationships between all measured traits. The hierarchical clustering of cultivars was carried out utilizing the unweighted pair-group method using arithmetic average (UPGMA).

Results and Discussion

Yield and Fruit Morphological Properties

The fruit yield, fruit weight, fruit diameter and fruits stem length of twenty six apple cultivars are shown in Table 2. Statistically significant differences ($P < 0.05$) were observed in all measured parameters. Indeed, the fruit yield of twenty six apple cultivars analyzed varied between 19.64 and 67.92 kg $tree^{-1}$. The highest fruit yield was harvested in 'Starking Delicious' (67.92 kg/tree) followed by 'Washington Spur' (63.28 kg $tree^{-1}$) and 'Fuji Zen' (62.65 kg $tree^{-1}$) and the lowest was detected in 'Burki Gala' and 'Po55' cultivars by an average of 20.48 kg/tree. Similar fruit yield values were reported by Raada et al. (2019) at apple growers in the Ifrane province of the Middle Atlas of Morocco.

In the same way, Glenn et al. (2001) reported that the fruit yield of the apple cultivars growing in Chile varied between 21.9 and 30.1 kg $tree^{-1}$. On the other hand, the obtained results of all analyzed apple cultivars were higher than the values reported in the Brazilian (Amarante et al., 2008), Slovenian (Tajnko and Čmelik, 2005) and Ithacan (Merwin et al., 1994) apple cultivars. The variations in the obtained results across the analyzed apple cultivars can be primarily linked to the diverse environmental factors influencing apple cultivation. Different cultivar types exhibit distinct responses to varying climate and soil conditions, leading to fluctuations in yield and fruit quality. Additionally, regional disparities in climatic nuances, such as temperature, precipitation and sunlight exposure, can significantly impact physiological processes in apple trees. Furthermore, variations in soil types, including nutrient composition and drainage capabilities, contribute to the observed differences.

The morphological traits measured in apple fruits hold significant economic importance, not only due to their impact on consumer acceptance or rejection but also because of their influence on the fruits industrial manipulation. In the same way, the fruit weight of all apple cultivars varied greatly among genotypes, from a minimum of 128.16 g ('Jeromine') to a maximum of 265.61 g ('Washington Spur'). However, the highest values of the fruit size were detected in the cultivar 'Jeromine C2' (81.86 mm), while the lowest values were observed in 'Elstar' (61.52 mm), 'Po52' (63.87 mm), 'Po53' (63.49 mm), 'Gold Rush' (63.49 mm), 'Jeromine' (61.53 mm) and 'Gala Royal-2' (60.02 mm). On the other hand, the greater fruit stem length was revealed in 'Golden Delicious' followed by 'Gala Royal-1' by an average 38.64 mm, and the least fruit stem length was observed in 'Red-Delicious', 'Po45', 'Gala 47', 'Po55' and 'Fuji Zen' by an average value of 15.58 mm.

Our values are very similar to the fruit mean weight reported for some French (Le Bourvellec et al., 2015), Chilean (Glenn et al., 2001) and Czech (Blažek and Hlušičková, 2007) apple genotypes, whereas these values were generally higher than those of various Brazilian (Amarante et al., 2008) and Israeli apple cultivars (Naor et al., 2008). Several factors contribute to explaining the difference in the weight and size of apple fruits such as environmental conditions, soil properties, nutrient requirements, water irrigation quantity and management practices (Wang et al., 2016; Mašán et al., 2018). In the same way, Pardo and Borges, (2020) report that flowering and pollination are critical stages in the apple production process and successful pollination leads to a proper fruit set, higher yield, better fruit quality and genetic diversity in apple orchards. Thus, adequate management of pollination, including ensuring the presence of pollinators or using compatible apple varieties for cross-pollination can have a substantial positive impact on apple production (Ramírez and Davenport, 2013).

These findings indicate that the twenty six apple cultivars examined have exhibited distinct variations in both yield and physical attributes of the fruit. Notably, 'Washington Spur', among others, displayed the most promising combination of high fruit yield and size, rendering it a valuable genotype for further cultivar breeding endeavors.

Table 2. Mean values of the principal morphological fruit parameters of the twenty six apple cultivars

Cultivar	Yield kg tree^{-1}	Fruit weight (g)	Fruit size (mm)	Fruit stem length (mm)
Golden Delicious	46.06 ^{de}	172.73 ^{efghi}	67.43 ^{cd}	43.44 ^{a}
Po41	58.78 ^{bc}	168.39 ^{fghi}	67.45 ^{cd}	31.44 ^{abc}
Starking Delicious	67.92 ^{a}	190.96 ^{defgh}	66.65 ^{cd}	20.45 ^{bcd}
Red-Delicious	58.70 ^{bc}	227.15 ^{abcd}	76.91 ^{ab}	15.83 ^{d}
Red Chief	45.52 ^{cd}	211.62 ^{bcdef}	76.28 ^{ab}	20.29 ^{bcd}
Po 45	47.09 ^{de}	242.51 ^{abc}	77.38 ^{ab}	15.95 ^{d}
Po 46	35.57 ^{f}	256.20 ^{ab}	77.49 ^{ab}	20.74 ^{bcd}
Gala 47	32.12 ^{fg}	244.20 ^{abc}	78.97 ^{ab}	14.14 ^{d}
Gala Royal	24.71 ^{gh}	186.03 ^{defgh}	67.88 ^{cd}	33.85 ^{ab}
Gala Galaxy	48.86 ^{de}	158.23 ^{ghi}	64.46 ^{cd}	20.46 ^{bcd}
Starkrimson	51.88 ^{d}	174.04 ^{efghi}	66.31 ^{cd}	26.34 ^{bcd}
Elstar	45.65 ^{de}	155.92 ^{ghi}	61.52 ^{d}	27.80 ^{bcd}
Po 52	32.87 ^{fg}	163.81 ^{fghi}	63.87 ^{d}	20.47 ^{bcd}
Po 53	44.9 ^{de}	155.77 ^{ghi}	63.49 ^{d}	27.51 ^{bcd}
Gold Rush	34.87 ^{f}	155.77 ^{ghi}	63.49 ^{d}	27.51 ^{bcd}
Po55	21.32 ^{h}	203.18 ^{cdefg}	79.59 ^{ab}	15.16 ^{d}
Er Rad	29.05 ^{fgh}	179.00 ^{efgh}	72.59 ^{bc}	26.13 ^{bcd}
Golden Reinders	36.66 ^{f}	149.90 ^{hi}	68.24 ^{cd}	23.89 ^{bcd}
Jéromine	41.72 ^{e}	128.16 ^{i}	61.53 ^{d}	27.63 ^{bcd}
P59	34.19 ^{f}	217.38 ^{bcde}	78.75 ^{ab}	24.67 ^{bcd}
Burki Gala	19.64 ^{i}	167.87 ^{fghi}	66.31 ^{cd}	18.46 ^{cd}
Gala Royal1	29.61 ^{fgh}	144.60 ^{hi}	60.02 ^{d}	24.75 ^{bcd}
Washington Spur	63.28 ^{ab}	265.61 ^{a}	77.63 ^{ab}	22.33 ^{bcd}
Jéromine C2	40.23 ^{e}	247.83 ^{abc}	81.86 ^{a}	31.50 ^{abc}
Gala-Val	59.42 ^{bc}	170.62 ^{efghi}	67.63 ^{cd}	21.30 ^{bcd}
Fuji Zen	62.65 ^{ab}	176.01 ^{efghi}	65.09 ^{cd}	16.82 ^{d}

Note: The values followed by the same letter show no statistically significant differences ($P < 0.05$)

This morphometric characterization of the collection offers also a vital insight into the apple industry, enabling the identification of genotypes best suited for growth in Moroccan environmental conditions, with the most suitable characteristic to food processing and transforming.

Chemical and Biochemical Traits

The chemical characteristics of the assessed apple cultivars exhibited significant differences ($P < 0.05$) as shown in Table 3.

Table 3. Chemical traits of the twenty-six apple cultivars

Cultivar	TSS	TA (g CA 100 mL^{-1})	Maturity index
Golden Delicious	14.63 ^{defg}	0.14338 ^{a}	33.160 ^{h}
Po41	15.94 ^{abcdefg}	0.10318 ^{bc}	44.266 ^{gh}
Starking Delicious	15.36 ^{bcdefg}	0.07102 ^{hi}	51.542 ^{f}
Red-Delicious	14.36 ^{defg}	0.06432 ^{j}	58.266 ^{de}
Red Chief	13.50 ^{g}	0.04422 ^{l}	53.444 ^{ef}
Po 45	14.10 ^{efg}	0.07102 ^{hi}	47.341 ^{fgh}
Po 46	13.46 ^{g}	0.06968 ^{i}	49.828 ^{fg}
Gala 47	13.60 ^{fg}	0.05628 ^{jk}	55.454 ^{def}
Gala Royal	14.16 ^{efg}	0.09112 ^{de}	60.312 ^{d}
Gala Galaxy	18.29 ^{a}	0.09648 ^{cd}	43.691 ^{gh}
Starkrimson	16.10 ^{abcdef}	0.06968 ^{i}	31.296 ^{i}
Elstar	16.76 ^{abcd}	0.07906 ^{fgh}	43.421 ^{gh}
Po 52	14.16 ^{efg}	0.05762 ^{jk}	55.185 ^{def}
Po 53	14.35 d^{efg}	0.08576 ^{ef}	58.750 ^{de}
Gold Rush	13.43 ^{g}	0.05494 ^{jk}	76.364 ^{a}
Po 55	14.20 ^{efg}	0.05092 ^{jkl}	60.000 ^{d}
Er Rad	14.16 ^{efg}	0.0804 ^{fg}	56.538 ^{de}
Golden Reinders	14.25 ^{efg}	0.10586 ^{b}	70.455 ^{bc}
Jéromine	16.20 ^{abcde}	0.07504 ^{ghi}	73.171 ^{ab}
P59	17.73 ^{ab}	0.06164 ^{j}	59.820 ^{d}
Burki Gala	17.16 ^{abc}	0.07236 ^{hi}	69.427 ^{bc}
Gala Royal1	16.43 ^{abcde}	0.07637 ^{ghi}	74.976 ^{ab}
Washington Spur	14.43 ^{defg}	0.06566 ^{ij}	66.298 ^{cd}
Jéromine C2	15.19 ^{cdefg}	0.06432 ^{ij}	72.288 ^{abc}
Gala-Val	16.50 ^{abcde}	0.06164 ^{j}	60.119 ^{d}
Fuji Zen	16.76 ^{abcd}	0.06566 ^{ij}	70.039 ^{bc}

Note: The values followed by the same letter show no statistically significant differences ($P < 0.05$)

These parameters play an important role in fruit maturity and when deciding about harvest time, and they are also important for consumer acceptance of apples. Indeed, the soluble solid content (TSS) varied between 13.43 and 18.29 °Brix, with the highest concentration observed in the 'Gala Galaxy' and 'P59' cultivars. These results are consistent with other studies realized on different apple cultivars under different countries' conditions. Blažek et al. (2007) reported that the total soluble solids of fifty apple cultivars growing in the Czech Republic ranged between 11.0 and 15.1 °Brix. Hoehn et al. (2003) reported a TSS variability (12.2 and 13.7 °Brix) between three apple cultivars growing under Switzerland's environmental conditions. Moreover, Wu et al. (2007) found that the TSS in the eight Chinese apple varieties ranged between 10.48 and 14.68 °Brix. On the other hand, Jakobek et al. (2020) reported that the mean value of twenty-five Croatian apple accessions was 15 °Brix, which is higher than this reported in our studied cultivars.

The highest titratable acidity was observed in 'Golden Delicious' (0.14338 g CA/100 ml) and the lowest was detected in the 'Red Chief' (0.04422 g CA 100 mL^{-1}) cultivar. The values of titratable acidity reported in the bibliography consulted are found between 0.095 and 0.771 g CA/100 ml (Violeta et al., 2010), 0.20 and 0.36 g CA 100 mL^{-1} (Vieira et al., 2009) and between 0.3 and 1.1 g CA 100 mL^{-1} (Jakobek et al., 2020).

On the other hand, the maturity index is a crucial tool for studying the variation in taste and flavor of apple cultivars, especially in the context of agriculture and horticulture. It provides valuable information about the developmental stage and readiness for the harvest of different cultivars. Understanding the importance of the maturity index involves considering several key points such as the external color of the fruit, assessing the fruit firmness and size, as a well-developed sweetness and fully developed seeds contribute to the overall quality of the fruit (Wu et al., 2007). However, Vieira et al. (2009) report that apple cultivars with sugar/acid ratios lower than 20 are sharp and appropriate for processing and cider production, while cultivars with sugar/acid ratios higher than this value are sweet and good for direct consumption. As can be seen from Table 3, all cultivars studied had a maturity index higher than 20, being classified as sweet cultivars. Similar results were induced in other research works (Nogueira et al., 2006; Vieira et al., 2009; Jakobek et al., 2020).

The biochemical fruit attributes of the twenty-six apple cultivars tested in the present study varied greatly among genotypes (Table 4). Notably, the content of total sugars (SSC) in the tested fruit ranged from a minimum of 113.96 g GE per kg of FW (as observed in the 'Gala-Val' cultivar) to a maximum of 139.8 g GE per kg of FW (on average, in the 'Red-Delicious' and 'Er Rad' cultivars). Our data are very similar to recent total sugar values (128.2-191.55 g kg^{-1}) reported in nine Slovenian apple cultivars (Petkovsek et al., 2007) and in six Brazilian cultivars (Paganini et al., 2004) with SSC values varying between 118 and 148 g kg^{-1}. On the other hand, Petkovsek et al. (2007) indicated that seed diameter was ranging between 128 and 191 g kg^{-1} in a collection of nine apple accessions. Therefore, the last results are higher than the ones presented by the apple cultivars in this work.

Beyond the aggregate sugar content, apple fruits encompass an additional vital biochemical component pivotal to their flavor, hue, fragrance, and myriad health advantages, namely, the polyphenols (Kschonsek et al., 2018). As presented in Table 4, the 'Red Chief' cultivar demonstrated the highest total phenol content value (23.29 mg GAE per kg of FW) among the apple collection, followed by 'Po 55' (21.32 mg GAE per kg of FW). On the other hand, the lowest total phenol value (1.56 mg GAE per kg of FW) was detected in the 'Burki Gala' cultivar. Research conducted on apple cultivars grown in Ukraine (Shevchuk et al., 2021), Canada (Khanizadeh et al., 2008), Hungary (Ficzek et al., 2013) and Germany (Kschonsek et al., 2018) revealed total phenolic compound values ranging from 1.27 to 2.62 mg kg^{-1}, 0.99 to 4.51 mg kg^{-1}, 1.94 to 4.79 mg kg^{-1}, and 0.99 to 4.65 mg kg^{-1}, respectively.

Table 4. Biochemical traits of the twenty-six apple cultivars

Cultivar	SSC	TPC	FTC	TAC	AA
Golden Delicious	126.48 ^{efghi}	10.46 ^{i}	3.29 ^{eghij}	0.33 ^{h}	43.01 ^{lm}
Po41	130.59 ^{cdef}	8.12 ^{jk}	1.49 ^{jkl}	2.75 ^{bcdefg}	25.26 ^{o}
Starking Delicious	123.58 ^{hi}	13.04 ^{gh}	4.37 ^{de}	1.669 ^{cdefgh}	72.90 ^{gh}
Red-Delicious	142.54 ^{a}	18.55 ^{cd}	7.30 ^{b}	2.170 ^{bcdefgh}	82.36 ^{bc}
Red Chief	133.27 ^{bcd}	23.29 ^{a}	9.72 ^{a}	2.421 ^{bcdefgh}	90.53 ^{a}
Po45	134.98 ^{bc}	15.42 ^{efg}	5.96 ^{c}	3.673 ^{bc}	79.03 ^{de}
Po46	123.01 ^{hi}	16.13 ^{e}	4.06 ^{def}	7.347 ^{a}	77.74 ^{ef}
Gala 47	128.02 ^{defgh}	14.72 ^{efg}	3.85 ^{efgh}	2.588 ^{bcdefg}	75.69 ^{fg}
Gala Royal-1	131.80 ^{bcde}	16.95 ^{de}	5.29 ^{cd}	4.174 ^{b}	85.05 ^{b}
Gala Galaxy	116.00 ^{jk}	14.60 ^{efg}	2.67 ^{ghijk}	3.089 ^{bcde}	61.50 ^{j}
Starkrimson	130.28 ^{cdefg}	15.58 ^{ef}	3.81 ^{efgh}	00.667 ^{gh}	72.04 ^{h}
Elstar	130.99 ^{bcdef}	6.52 ^{k}	4.41 ^{de}	0.834 ^{fgh}	19.13 ^{p}
Po52	130.99 ^{bcdef}	16.64 ^{de}	3.85 ^{efgh}	3.005 ^{bcdef}	50.96 ^{k}
Po53	124.10 ^{ghi}	9.92 ^{ij}	0.63 ^{l}	1.836 ^{cdefgh}	31.72 ^{n}
Gold Rush	125.58 ^{efghi}	11.13 ^{hi}	1.61 ^{jkl}	4.091 ^{b}	43.76 ^{l}
Po 55	121.40 ^{ij}	21.32 ^{ab}	7.79 ^{b}	2.755 ^{bcdefg}	81.82 ^{cd}
Er Rad	137.07 ^{b}	13.24 ^{fgh}	2.90 ^{ghijk}	2.838 ^{bcdefg}	70.75 ^{hi}
Golden Reinders	125.41 ^{efghi}	14.84 ^{efg}	1.86 ^{ijkl}	1.502 ^{cdefgh}	40.16 ^{m}
Jeromine	125.10 ^{fghi}	15.58 ^{ef}	2.55 ^{hijk}	2.254 ^{bcdefgh}	71.50 ^{h}
P59	134.84 ^{bc}	11.67 ^{hi}	2.32 ^{ijkl}	1.168 ^{efgh}	68.27 ^{i}
Burki Gala	120.61 ^{ij}	1.56 ^{l}	1.08 ^{kl}	0.667 ^{gh}	43.22 ^{lm}
Gala Royal-2	133.87 ^{bcd}	12.92 ^{gh}	2.75 ^{ghijk}	0.751 ^{gh}	52.90 ^{k}
Washington Spur	126.55 ^{efghi}	16.67 ^{de}	1.97 ^{hijkl}	3.423 ^{bcd}	43.33 ^{lm}
Jeromine C2	133.51 ^{bcd}	15.27 ^{efg}	3.35 ^{efghi}	1.419 ^{defgh}	62.25 ^{j}
Gala-Val	113.96 ^{k}	13.24 ^{fgh}	2.40 ^{hijkl}	1.586 ^{cdefgh}	40.32 ^{m}
Fuji Zen	122.44 ^{hi}	20.35 ^{bc}	3.95 ^{defg}	3.256 ^{bcde}	62.90 ^{j}

Note: The values followed by the same letter show no statistically significant differences ($P < 0.05$)

The highest values for fruit flavonoid content (TFC) were 9.72 mg QE per 100 g of fresh weight ('Red Chief'), 7.79 mg QE per 100 g FW ('Po 55') and 7.30 mg QE per 100 g FW ('Red-Delicious'), whereas the least TFC concentration were observed in 'Po53' (0.63 mg QE per 100 g FW) and 'Burki Gala' (1.08 mg QE per 100 g FW). The same variability was often detected in various plants, including the apple cultivars (Lee et al., 2003; Geană et al., 2021). Some research reported that apple fruits were particularly rich in flavonoids like quercetin, catechin and epicatechin, which exhibited potent antioxidant properties and played a crucial role in neutralizing harmful free radicals in the body, reducing oxidative stress and potentially lowering the risk of chronic diseases such as cardiovascular ailments and certain cancers (Shehzadi et al., 2020; Ullah et al., 2020).

Moreover, fruit anthocyanin content of the twenty six apple cultivars ranged from 0.33 mg of C_3gE 100 g^{-1} of FW in the 'Golden Delicious' to 5.204 mg of C_3gE 100 g^{-1} on average in the 'Po46', 'Gala Royal-1' and 'Gold Rush' cultivars. Similar conclusions were detected in the other apple collection grown under different environmental conditions (Lister et al., 1996; Kunradi et al., 2009).

Similarly, the cultivars 'Red Chief' and 'Gala Royal-1' were characterized by the highest values of fruit antioxydant activity with the values of 90.53% and 85.08% respectively. However, the lowest AA values were detected in 'Elstar' (19.13%), 'Po41' (25.26%) and 'Po53' (31.72%). These results were in line with the work of Panzella et al. (2013) who reported values of apple fruit antioxidant activity between 16 and 82% in eight traditional apple cultivars native of the Campania Region (Southern Italy). On the other hand, Wolfe et al. (2003) reported that the antioxidant activity of the apple fruit varied greatly between the apple peel and the cortex region. In fact, the fruit peels all had significantly higher total antioxidant activities than the fruit flesh of four apple varieties growing under American environmental conditions. The differences in comparison with the results of the present study may be attributed to different apple cultivars and sample extraction methods used in the experiments. However, the results of our study are consistent with a number of other previous studies which state that the apples possess strong antioxidant activity and these values vary between apple cultivars (Petkovsek et al., 2007; Khanizadeh et al., 2008).

The results of this study on the biochemical attributes of apple genotypes indicate that the twenty six cultivars differ significantly in their maturity index, titratable acidity, total soluble solids value and content of total sugars, polyphenols, anthocyanins and flavonoids, consequently affecting their antioxidant activity. Moreover, a comparison with other research in the literature reveals considerable variations in these properties among the cited studies and the regions considered by the researchers. These disparities may be attributed to the specific geographical characteristics of different areas and diverse agronomical practices. Additionally, it is well-established that the chemical composition of apples is strongly influenced by the plant genotype (Petkovsek et al., 2007; Wu et al., 2007). Thus, the differences observed in the physical-chemical properties of apple cultivars can be attributed to the origin of the plant material, as all cultivars were grown under identical geographical conditions and subject to the same agronomic practices.

Vegetative Growth

The analysis of vegetative traits revealed notable variations among the 26 apple cultivars (Table 5): the cultivar 'Gala Royal-2' demonstrated the highest shoot growth with a value of 18.35 cm mLn^{-1}, indicating robust growth compared to other cultivars, whereas 'Starking Delicious' exhibited the lowest vegetative growth value (9.34 cm mLn^{-1}).

Vegetative growth in apple trees is an essential aspect of their development and overall productivity. However, many factors can affect vegetative growth in apple trees, such as nutrition (Mészáros et al., 2021), irrigation treatment (Faghih et al., 2019) and rootstock selection (Gioia et al., 2010). On the other hand, several researchers also report the high variation in shoots growth between apple cultivars, which is mainly due to the specific proprieties of each cultivar, such as the unbalance of indigenous hormonal level like nitrogen and cytokinins, the chlorophyll content, and the sucrose (Sapir et al., 2003; Malagoli et al., 2004; Khan et al., 2015).

Regarding foliage density of the tested cultivars, 'Po53' displayed the highest value (9.47 leaves per 10 cm of shoot), while 'Starkrimson' had the lowest value (3.51 leaves per 10 cm of shoot), indicating significant differences in leaf density among the cultivars. As for leaf area, a highly significant difference was reported between the apple cultivars: 'PO 55' showed the largest leaves (15.80 cm^2), whereas 'Red Chief' had the smallest leaves with an average of 8.36 cm^2.

The leaves number and area play a crucial role in the comprehensive characterization of apple cultivars. These leaf traits provide valuable insights into the physiological and morphological attributes of the trees, enabling the assessment of their growth, productivity and overall health. The number of leaves indicates the vigor and vitality of the cultivar, while the leaf area reflects its capacity for photosynthesis, nutrient assimilation and carbon fixation. Variations in leaf number and area can indicate genetic differences, responses to environmental conditions and potential adaptability to specific climates or management practices (Massonnet et al., 2008). Consequently, a thorough analysis of these leaf characteristics aids in the selection of optimal cultivars for different regions and agricultural practices, contributing to sustainable apple production and improved orchard management strategies (Zhang et al., 2023)

Correlation between Low Temperature Effects

To better understand the relationship between the apple fruit's physical-biochemical trait and the vegetative growth of the studied cultivars, all mean values of the analyzed properties were subjected to bivariate correlation using the Pearson coefficient. The significant correlations at the level of 0.05 or 0.01 are reported in Table 6. A significant negative correlation was observed between the apple fruit yield and vegetative growth with a correlation coefficient of r = –0.665. This correlation can be attributed to the phenomenon of resource allocation within the apple tree. In this sense, Albarracín et al. (2017) report that in plants, limited resources such as nutrients, carbohydrates and energy must be distributed between reproductive (fruit production) and vegetative (shoot growth) activities.

However, when a tree invests more resources into producing fruits, there is reduced availability of these resources for the growth and development of vegetative shoots, and vice versa. On the other hand, the variation of the juice soluble solid content under the Moroccan environmental conditions was negatively correlated with the variation of the leaf area with a correlation coefficient of $r = -0.507$. This result can be explained by the fact that the greater leaf area implies a higher capacity for photosynthesis and assimilation of nutrients. Consequently, resources that could have contributed to fruit soluble solids accumulation are allocated to support leaf expansion. Conversely, a smaller leaf area ensures higher resource partitioning towards fruit development, leading to enhanced total soluble solids content (Trad et al., 2013). However, a contradictory result was reported by TR Roper et al. (1987) on the sweet cherry trees and revealed that a high leaf area facilitated enhanced photosynthetic activity and subsequent translocation of sugars to the fruits, resulting in elevated total solids soluble content.

Table 5. Shoot growth, leaves density and leaf area of the twenty-six apple cultivars

Cultivar	Vegetative growth	Leaves density	Leaf area
Golden Delicious	13.45 ^{cd}	6.43 ^{cd}	9.39 ^{efg}
Po41	10.47 ^{ef}	5.39 ^{def}	10.54 ^{ef}
Starking Delicious	9.34 ^{f}	5.20 ^{def}	11.36 ^{de}
Red-Delicious	11.70 ^{de}	4.56 ^{fg}	10.47 ^{ef}
Red Chief	15.38 ^{bc}	7.38 ^{bc}	8.36 ^{g}
Po45	14.29 ^{bcd}	8.49 ^{abc}	12.45 ^{cd}
Po46	15.56 ^{bc}	5.09 ^{ef}	14.83 ^{ab}
Gala 47	15.92 ^{bc}	6.32 ^{de}	13.37 ^{bc}
Gala Royal-1	14.28 ^{bcd}	5.98 ^{def}	14.65 ^{ab}
Gala Galaxy	14.39 ^{bcd}	7.25 ^{bc}	9.46 ^{fg}
Starkrimson	10.36 ^{ef}	3.51 ^{g}	13.26 ^{bc}
Elstar	14.37 ^{bcd}	6.29 ^{de}	10.50 ^{ef}
Po52	14.30 ^{bcd}	4.03 ^{f}	12.86 ^{cd}
Po53	13.22 ^{cd}	9.47 ^{a}	10.53 ^{ef}
Gold Rush	15.35 ^{bc}	5.32 ^{def}	11.75 ^{cde}
Po 55	13.57 ^{cd}	4.93 ^{ef}	15.80 ^{a}
Er Rad	17.49 ^{ab}	5.03 ^{ef}	13.68 ^{bc}
Golden Reinders	14.55 ^{bcd}	7.48 ^{bc}	10.57 ^{ef}
Jeromine	12.43 ^{d}	8.84 ^{abc}	9.48 ^{fg}
P59	15.30 ^{bc}	5.48 ^{def}	10.56 ^{ef}
Burki Gala	13.56 ^{cd}	4.07 ^{f}	8.04 ^{g}
Gala Royal-2	18.35 ^{a}	6.40 ^{cd}	10.56 ^{ef}
Washington Spur	12.48 ^{d}	6.89 ^{c}	13.28 ^{bc}
Jeromine C2	13.50 ^{cd}	9.30 ^{ab}	11.67 ^{cde}
Gala-Val	12.47 ^{d}	5.39 ^{def}	10.53 ^{ef}
Fuji Zen	13.24 ^{cd}	6.47 ^{cd}	10.42 ^{ef}

Note: The values followed by the same letter show no statistically significant differences ($P < 0.05$)

Table 6. Matrix of coefficients correlations between mean values of all fruit physical-biochemical traits and vegetative properties involved in the study

	Y	FW	FS	FSL	TSS	TA	MI	SSC	TPC	FTC	TAC	AA	VG	LD	LA
Y	1														
FW	0.108	1													
FS	-0.075	**0.903****	1												
FSL	-0.034	-0.328	-0.310	1											
TSS	0.183	-0.396	-0.393	0.031	1										
TA	0.077	-0.372	-0.358	**0.686****	0.148	1									
MI	-0.283	-0.081	-0.020	-0.193	-0.040	-0.372	1								
SSC	-0.095	0.279	0.307	0.118	-0.287	-0.093	-0.054	1							
TPC	0.139	0.367	0.416	-0.352	-0.395	-0.402	0.073	0.147	1						
FTC	0.008	0.382	0.438	-0.345	-0.365	-0.378	-9.233	0.352	**0.690****	1					
TAC	-0.065	0.399	0.270	-0.253	-0.469	-0.230	0.063	-0.094	0.387	0.166	1				
AA	-0.082	0.454	**0.532****	-0.331	-0.273	-0.350	-0.112	0.334	**0.672****	**0.679****	0.387	1			
SL	**-0.665****	0.012	0.082	-0.054	-0.177	-0.102	0.301	0.210	0.041	0.017	0.188	-0.012	1		
LD	0.114	0.036	0.054	0.191	-0.054	0.174	0.248	-0.022	0.113	-0.089	-0.028	-0.116	0.122	1	
LA	-0.267	0.436	0.403	-0.143	**-0.507****	-0.252	-0.081	0.109	0.371	0.207	**0.546****	0.354	0.109	-0.266	1

Note: Significant and potential correlations are marked in bold. FY: fruit yield; FW: fruit weight; FSL: fruit stem length; TSS: total soluble solids content; TA: titratable acidity; MI: maturity index; TPC: total polyphenols content; FTC: total flavonoids content; SSC: soluble sugars content; TAC: total anthocyanins content; AA: antioxidant activity; SL: shoot length; LA: leaf area; NL: number of leaves per 10 cm of shoot;* Correlation is significant at the 0.05 level. ** Correlation is significant at the 0.01 level.

On the other hand, the specific relationship between leaf area and fruit TSS content can vary between different plant species, varieties, environmental conditions and cultural practices (Adiba et al., 2022; Adiba et al., 2023).

A significant positive correlation was observed between the total polyphenol content of the apple fruits and their content of the total flavonoids (r = 0.690) and antioxidant activity (r = 0.672). This indicates that the variation in the fruit's antioxidant activity between the apple cultivars is linked to the variation in the total phenolic content. In agreement with our finding, many studies on various apple cultivars suggest that total phenolic content plays a major role in antioxidant activity (Piagentini and Pirovani, 2017; Starowicz et al., 2020; Groth et al., 2021). These mentioned researchers supported the idea that the phenolic compounds were the main contributors to the antioxidant capacity in apple fruit. This result makes the apple cultivars with a high level of phenolic compounds display enhanced antioxidant activity beneficial for human health and well-being.

However, the significant correlation (r = 0.679) between the total flavonoids and the antioxidant activity of the apple fruit is explained by the fact that flavonoids are the most antioxidant phenolic compounds abundantly present in apple fruits. Similar data are reported by D'Abrosca et al. (2007) revealing that the apple flavonoids possess a high number of electron-donor hydroxyl groups and a large number of double bonds, properties that have been shown to increase the overall antioxidant activity of the apple fruits.

The variation of the apple fruit content on the anthocyanins was positively correlated with the variation of the leaf area with a significant coefficient of 0.546. In the same way, a high level of anthocyanins indicates a strong defense mechanism against stressors, such as intense sunlight or environmental stress, which often leads to an increase in leaf area. As the fruit develops, the higher anthocyanin content reflects better plant health and vigor, promoting greater leaf growth to support efficient photosynthesis and nutrient uptake. This correlation showcases the adaptability of apple trees to varying environmental conditions, reinforcing their resilience and reproductive success.

Cluster Analysis

The results of multivariate characterization of the twenty-six apple cultivars growing under Moroccan environmental conditions regarding all the quantified traits are depicted in Fig. 2. Evidently, these results reveal the existence of three principal clusters, each representing a set of highly homogeneous groups of the investigated apple genotypes. These clusters are primarily discerned based on their respective potential productivity, vegetative growth and biochemical attributes. Cluster CI

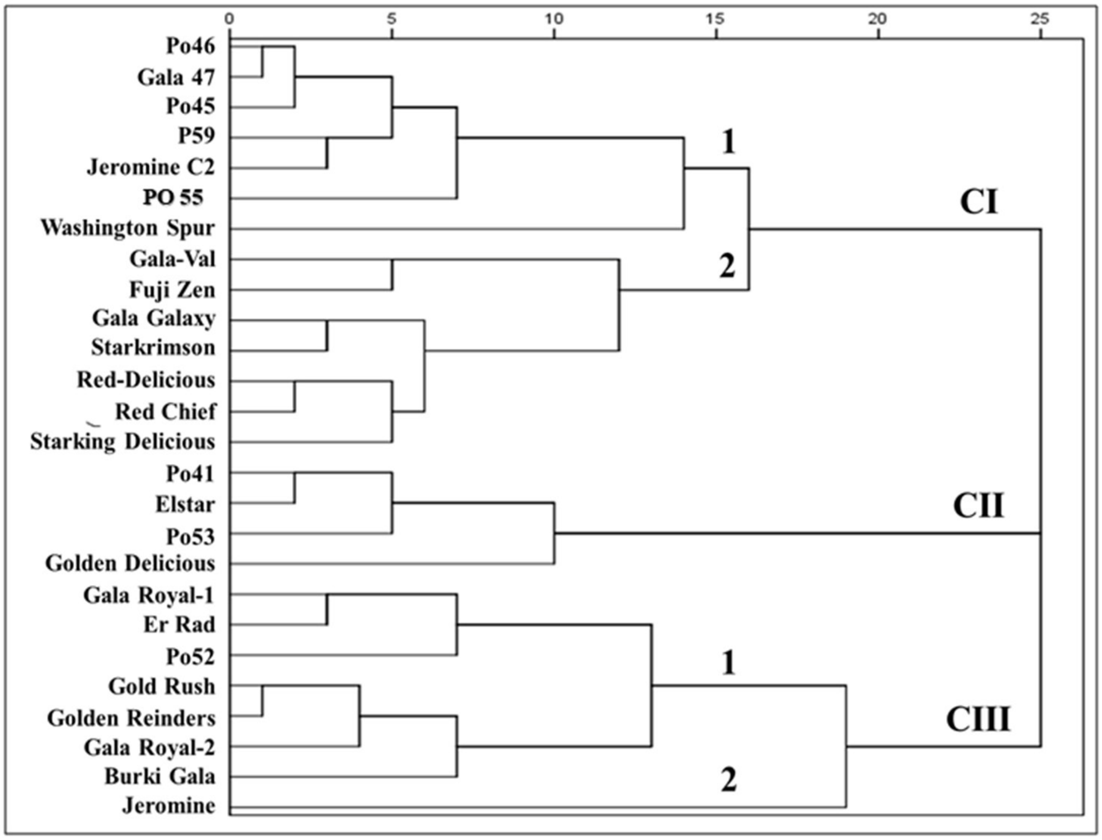

Figure 2. Cluster analysis of the studied apple cultivars based on the measured traits using Euclidean distance

contained fourteen genotypes, subdivided into two homogeneous and distinctive subgroups (CI-1 and CI-2). The first subgroup (CI-1) contained seven apple cultivars, which were characterized by an average fruit yield of 39.11 kg tree^{-1}, with big fruits and a high leaf area. Biochemically, the fruit of these cultivars was characterized by high anthocyanins content. The second subgroup (CI-2) was formed also by seven cultivars, where the lowest shoot growth and leaf density were detected. However, these cultivars were characterized by the highest total flavonoid content and the highest antioxidant activity in comparison with the other cultivars tested.

The second cluster (CII) included four cultivars 'Golden Delicious', 'Po41', 'Elstar' and 'Po53' where the highest titratable acidity and the lowest maturity index were detected. However, their fruits had the lowest flavonoid content and consequently the lowest antioxidant activity. The vegetative traits analysis reported that this group was characterized by the highest number of leaves per shoot. The third cluster (CIII) included eight cultivars, subdivided into two distinctive and homogeneous subgroups (CIII-1 and CIII-2). The subgroup CIII-1 was represented by seven cultivars in which a low fruit yield and size were detected. Thus, these cultivars were characterized by the highest anthocyanins content and antioxidant activity. The vegetative properties induce that these genotypes are characterized by a high shoot length with a low number of leaves and a low leaf area. The second subgroup (CIII-2) included only the 'Jeromine' cultivar in which the lowest fruit weight and the highest fruit maturity index were detected.

Conclusion

This research involved the evaluation of twenty-six different apple cultivars, focusing on their productive potential, vegetative growth, and the fruit's physical-biochemical properties. The findings demonstrate significant variations among the apple cultivars across all measured parameters. This result suggests a great variability existing even in the genetic pool of apple crops tested and indicates that cultivar is the main factor determining the yield, fruit quality and vegetative properties in apple trees. The 'Starking Delicious' cultivar presents the highest fruit yield and fruit weight, while, 'Burki Gala' and 'Jeromine' are characterized by the lowest fruit yield and fruit weight, respectively. Biochemically, 'Red-Delicious' is characterized by the highest total sugar content and the highest phenolic compounds, while flavonoid content and antioxidant activity are detected in the 'Red Chief' cultivar.

Overall, this study highlights the substantial impact of cultivar choice on fruit yield, as well as the physical, biochemical and vegetative attributes of apple trees. The findings contribute to advancing our understanding of the diverse apple cultivars and their potential applications in various fields, including breeding, cultivation and the food industry. This knowledge can aid researchers, farmers and industry professionals as well in making informed decisions regarding cultivar selection for optimal outcomes and utilization of apple cultivars in different contexts.

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CRediT Authorship Contribution Statement.

Hassane Boudad: performing most of the experiments and writing of the first version of the manuscript. **Atman Adiba:** performed some of the experiments and the statistical analysis. **Abdelmajid Haddioui:** contributed to supervision of work and review of the manuscript. **Mentag Rachid:** contributed to the editing of the manuscript. **El Fazazi Kaoutar:** contributed to the editing of the manuscript. **Najjari Sara:** performed some of the experiments. **Jamal Charafi:** conceptualization, investigation, performed most of the experiments, supervised the work and reviewed the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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