agronomy

MDPI

Article

A Comparison of Selected Biochemical and Physical Characteristics and Yielding of Fruits in Apple Cultivars (*Malus domestica* Borkh.)

Hong-Ki Yoon [1], Tomasz Kleiber [2], Zofia Zydlik [3], Krzysztof Rutkowski [3], Agnieszka Woźniak [4], Sławomir Świerczyński [3], Waldemar Bednarski [5], Jacek Kęsy [6], Łukasz Marczak [7], Jeong-Hak Seo [1], Taeg-Yong Choi [1], Kyeong-Jin Kang [1], Neisbe Ebru Kafkas [8], Jan Bocianowski [9], Philippe Jeandet [10] and Iwona Morkunas [4,*]

1 Chungcheongnam-do Agricultural Research and Extension Services, Research Division for Horticulture, 167, Chusa-ro, Shinam-myeon, Yesan-gun, Chungcheongnam-do 32418, Korea; grapeyoon@korea.kr (H.-K.Y.); seoapple@korea.kr (J.-H.S.); cty404@korea.kr (T.-Y.C.); kkj0531@korea.kr (K.-J.K)
2 Department of Plant Nutrition, Faculty of Horticulture and Landscape Architecture, Poznań University of Life Sciences, Wojska Polskiego 28, 60-624 Poznań, Poland; tomasz.kleiber@up.poznan.pl
3 Department of Dendrology, Pomology and Nursery Production, Faculty of Horticulture and Landscape Architecture, Poznań University of Life Sciences, Dąbrowskiego 159, 60-594 Poznań, Poland; zofia.zydlik@up.poznan.pl (Z.Z.); krzysztof.rutkowski@up.poznan.pl (K.R.); slawomir.swierczynski@up.poznan.pl (S.Ś)
4 Department of Plant Physiology, Faculty of Horticulture and Landscape Architecture, Poznań University of Life Sciences, Wołyńska 35, 60-637 Poznań, Poland; agnieszkam.wozniak@gmail.com
5 Institute of Molecular Physics, Polish Academy of Sciences, Smoluchowskiego 17, 60-179 Poznań, Poland; Waldemar.Bednarski@ifmpan.poznan.pl
6 Chair of Plant Physiology and Biotechnology, Nicolaus Copernicus University, Gagarina 9, 87-100 Toruń, Poland; Jacek.Kesy@umk.pl
7 Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12/14, 61-704 Poznań, Poland; lukasmar@ibch.poznan.pl
8 Department of Horticulture, Faculty of Agriculture, University of Cukurova, Balcalı, 01380 Adana, Turkey; ebru@cu.edu.tr
9 Department of Mathematical and Statistical Methods, Poznań University of Life Sciences, Wojska Polskiego 28, 60-637 Poznań, Poland; jboc@up.poznan.pl
10 Research Unit "Induced Resistance and Plant Bioprotection", UPRES EA 4707, Department of Biology and Biochemistry, Faculty of Sciences, University of Reims, P.O. Box 1039, 02 51687 Reims CEDEX, France; philippe.jeandet@univ-reims.fr
* Correspondence: iwona.morkunas@mail.up.poznan.pl or iwona.morkunas@gmail.com; Tel.: +48-61-846-6040; Fax: +48-61-848-7179

Received: 23 December 2019; Accepted: 19 March 2020; Published: 25 March 2020

Abstract: The aim of the research was to determine selected biochemical and physical characteristics and yielding of fruits in apple varieties (*Malus domestica* Borkh) such as Gala Schniga, Beni Shogun (Fuji) and Ligol, M.9 rootstock growing in the Wielkopolska region, Poland. high-performance liquid chromatography (HPLC) analysis revealed differences both in the profile and contents of soluble sugars and other metabolites detected in fruits of the tested apple varieties. The highest total saccharide content was found in fruits of cv. Gala Schniga, while leaves and shoots of this variety showed the lowest contents. Electron paramagnetic resonance (EPR) spectroscopy revealed the lowest contents of semiquinone radicals in apple fruits and the highest in leaves of apple trees. All organs of Schniga Gala apple trees were characterized by the highest levels of these radicals. Besides, gas chromatography-mass spectrometry (GC-MS) analysis of abscisic acid (ABA) revealed the highest levels of this molecule in shoots of apple trees, especially the Beni Shogun variety, while in fruits the levels were the lowest. Ligol fruits had the highest content of ABA. The percentage of injury observed after a low-temperature treatment and estimated on the basis of electrolyte leakage,

was shown to be the lowest in fruits of the Beni Shogun variety. In turn, the lowest average yield of apple fruits was recorded for the Ligol and Fuji varieties, with the highest in the case of the Gala Schniga variety. At the same time, a significant variability in the average weight of fruits was observed; the highest average mass of fruits was recorded for the Ligol variety, while it was lowest for Gala Schniga. Additionally, firmness evaluation of the fruits revealed that the firmest fruits were harvested from Gala apple trees, before the Beni Shogun and Ligol varieties. A significant variability was recorded in the red blush on fruit surfaces of the tested varieties, with Ligol fruits having the weakest blush. Altogether, these results indicate that fruits of the analyzed apple varieties differed both in terms of their biochemical composition and physical characteristics.

Keywords: *Malus domestica*; sugars; organic acids; sorbitol; semiquinone radicals; membrane injury index; abscisic acid; fruit yielding

1. Introduction

Biological properties of apple varieties are the basic factors determining the growth of trees, production and economic effects of their cultivation [1–7]. Therefore, the main goal of apple breeding programs is to obtain new apple genotypes characterized by high yields and good fruit qualities (shape, size, acidity, juiciness, sweetness, color) and low susceptibility/resistance of trees to fungal pathogens, as well as adaptation to climatic conditions of a given area [8–13]. In view of the above classical hybridizations performed, which involve not only major commercial cultivars but also some old local varieties and wild *Malus* species, the aim of apple breeding is to efficiently produce higher quality apples [9,14]. For example, advanced rootstock breeding programs combine molecular and conventional techniques to produce rootstocks that are dwarfing, productive, and tolerant to biotic and abiotic stresses. Foster et al. [15] showed how differing growing environments may modify the first expression of rootstock-induced scion dwarfing. It should be emphasized that among fruit trees, apples (*Malus* species) are important crops both in Poland and other countries worldwide, with an annual production in Poland in 2018 amounting to 3999.523 tons from 161.790 ha (data from FAOSTAT, Production of agricultural and horticultural crops in 2017, Warszawa 2018 https://stat.gov.pl/en/topics/agriculture-forestry/agricultural-and-horticultural-crops/). In turn, the total world production of apples in 2018 was 86142192 tons, an increase from approximately 4904305 ha (data from FAOSTAT). China, the United States, Turkey, Iran and Poland are the largest apple producers in the world, the latter country being the leading producer in Europe.

This paper highlights biochemical (profile and amounts of some metabolites, semiquinone radicals and abscisic acid levels and the release of electrolytes) and physical properties of apple fruits (mass and size), from Poland and those obtained in other countries, i.e., New Zealand and Japan.

Tang et al. [16] reported that the sugar content is one of the most important characteristics of fruits to ensure better customer experience, repeat purchases and it is one of the main attributes in quality grading of fruits [17]. Moreover, Aprea et al. [18] demonstrated that the search for increasing sweetness in apple breeding programs must take into account not only the sugar content, but also factors such as volatile compounds, texture parameters and polyphenols. Besides, due to the complexity of sweetness evaluation, malic acid and soluble solid content (SSC) are commonly used when assessing this trait. Different techniques have been proposed for sugar detection in apple fruits, e.g., chromatographic techniques such as gas chromatography (GC), high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), attenuated total reflectance spectroscopy in the mid-infrared region (ATR-MIR) coupled with chemometry [19,20], high-pressure capillary ion chromatography with pulsed amperometric detection [18] and, more recently, multispectral imaging [16]. In this study, metabolites (sugars, organic acids and total soluble solid contents) in fruits of various apple trees were identified and quantified using HPLC. In turn,

sorbitol was determined by GC-MS. An important indicator ensuring a better definition of sweetness is provided by the ratio between single sugars (mainly sucrose, glucose and fructose), sorbitol and organic acids rather than the ratio between total sugar and organic acid content [18]. Apart from the above-mentioned metabolites, we also determined the level of abscisic acid (ABA). As reported by Krost et al. [21], different levels of growth-regulating hormones seem to be present in apples for a specified phenotype. For example, in Columnar apple trees, levels of ABA and gibberellin are low, whereas the ratio of auxins/cytokinins is high. It is known that ABA plays an essential role in various aspects of the plant growth [22] and that it regulates plant responses to environmental stresses [23], while also regulating organic substance translocation and metabolism in plants [24,25]. Numerous reports showed that ABA enhances the assimilate unloading in the phloem of economic sink organs such as fleshy fruits and crop grains [26]. It has been demonstrated that ABA significantly promotes sugar unloading into the fruits [27]. Besides, ABA strongly activates ATPases especially those in the phloem cells of apple fruits [26].

Moreover, this work is the first report revealing the generation of semiquinone radicals (of organic origin) in the organs of apple trees. Semiquinone and phenoxyl radicals in plant cells are formed as a result of the oxidation of hydroxyl groups of phenols and polyphenols [28] and their generation is positively associated with other markers of oxidation, such as protein carbonyls and total peroxides [28]. Additionally, incorporation of these radicals in polymers such as lignin by combining with reactive oxygen species as well as their many other functions in plant defense response to biotic stresses has been well-documented [29–37]. Otherwise, determination and comparison of the macro- and microelement contents in fruits the apple varieties analyzed here, have been presented in our previous work [38].

The present study was thus undertaken to investigate and compare biochemical and physical indicators of organs in *M. domestica* varieties such as Gala Schniga, Beni Shogun (Fuji) and Ligol, grafted onto M.9 rootstock growing in the Wielkopolska region (Poland). 'M9' is still the main rootstock used in European apple fruit growing. In parallel, the yield and fruit quality of the above apple varieties were estimated.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

This study was conducted in 2017 in an orchard at the Agricultural and Pomiculture Experimental Farm in Przybroda (geographical location: 52°31′ northern latitude and 16°38′ eastern longitude). The farm is located in the Wielkopolska Plateau, where proper lessive soils formed of loamy sands are found lying over light boulder clay, with the floatable fraction content of 17–20%. The groundwater table for most of the vegetation season was 180–220 cm below the ground. The study was conducted on Ligol, Gala Schniga and Beni Shogun (Fuji) apple trees grafted on the M.9 virus-free rootstock, planted in 2009 at a 3.5 × 1 m spacing (number of trees: 2857 trees·ha^{-1}). The soil management system applied was herbicide fallow in rows of trees, which were 1.25 m wide, and sward in the interrows of 2.25 m in width. Apple trees were trained as spindles on an espalier with annual pruning as recommended. Fertilization and plant protection procedures were performed in accordance with the recommendations for commercial orchards. Due to poor fruit setting in the vegetation season of 2017, no fruitlet thinning was applied.

2.2. Description of Climatic Conditions

Climatic conditions were characterized based on data recorded at the weather station located in the Przybroda orchard. It results from these data that the winter of 2016/2017 was mild. The lowest real minimum temperature recorded in January was −15.5 °C. January was also the coldest winter month with a mean temperature of −2.7 °C, which was by 1.9 °C lower in comparison to the multiannual mean for that month (Table 1).

Table 1. Course of temperatures and rainfall in the growing season in the area of Przybroda.

Month	The Sum of Rainfall in mm			Average Temperature in °C		
	Average from 1982–2012	In 2017	Change in Relation to the Long-Term Average	Average from 1982–2012	In 2017	Change in Relation to the Long-Term Average
January	31.1	17.6	−13.5	−0.8	−2.7	−1.9
February	26.3	28.0	+1.7	−0.1	0.0	+0.1
March	34.3	35.0	+0.7	3.6	5.9	+2.3
April	28.0	36.4	+8.4	9.3	7.0	−2.3
May	48.0	31.2	−16.8	14.6	13.3	−1,3
June	63.5	85.6	+22.1	17.2	17.5	+0.3
July	78.8	182.4	+103.6	19.5	17.8	−1.7
August	61.9	80.0	+18.1	18.9	18.2	−0.7
September	41.0	47.2	+6.2	14.1	12.9	−1.2
October	32.0	56.8	+24.8	9.0	10.2	+1.2
November	37.2	35.0	−2.2	3.7	2.7	−1.0
December	39.0	40.6	+1.6	0.2	2.2	+2.0
Total/ Average	520.1	644.6	+124.5	9.1	8.8	-0.3

The spring of 2017 was cool, as indicated by lower mean temperatures in April and May (Figure 1). This contributed to poor bee flights and lesser fruit setting. The summer was also cold, as shown by lower mean temperatures in comparison to the multiannual mean. Lower temperatures were accompanied by greater rainfall. July was the month with the most abundant rainfall. In that month the total precipitation was by as much as 103.6 mm greater in comparison to the mean total precipitation for that month in the years 1982–2012 (Table 1). Despite considerable rainfall, as it results from Figure 1, in the vegetation period the so-called dry spells were experienced in April and May as well as August and September.

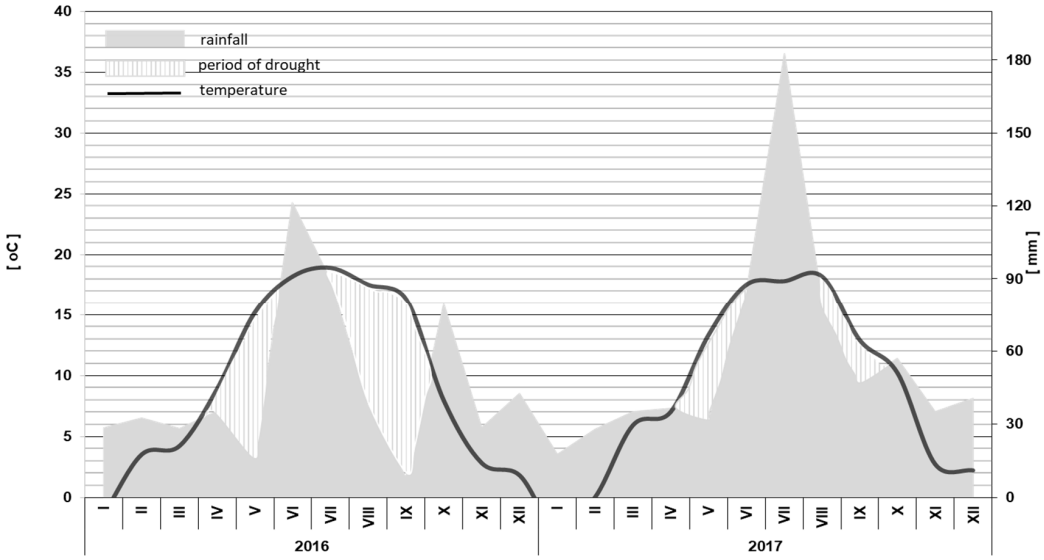

Figure 1. Climatogram for climatic conditions in Przybroda.

Moreover, as reported by Sofla et al. [14], other important objectives in apple breeding include adaptation to climatic conditions, tree growth habit and tree vigor, duration of juvenility and time of flowering. To ensure the greatest possible cropping, high and consistent yields of fruits uniform in size, shape and color for many years, fruit producers not only strive to improve tree training and orchard nutrition, but also use scion-rootstock combinations [39–41]. For example, Webster [42] reported that many rootstocks are used to propagate temperate fruit trees and improve tree vigor, beginning of fruit-bearing, tree productivity, fruit quality and adaptability to different environmental conditions. For over two thousand years, superior fruit tree genotypes have been grafted onto rootstocks thus

ensuring the genetic identity of desirable scions [43]. Recently, molecular techniques have been used to identify genes controlling interactions between scions and rootstocks.

2.3. Determination of Total Saccharide Content

Fruits (a total of 50) were collected from each experimental variant. 1-cm fragments were cut, from the skin deep into the mesocarp of pulp. Plant material (500 mg) was homogenized in 1.5 mL distilled water at 4 °C. The homogenate was centrifuged at 15,000 g and 4 °C for 15 minutes. 1 mL of the supernatant was added to 2 mL of cooled 0.02 anthrone reagent in sulfuric acid and heated at 90 °C for 14 minutes [44]. Under the influence of sulfuric acid, saccharides are converted into furfural derivatives, which together with anthrone, yield blue and green products. After incubation, the mixture was stirred and cooled. Absorbance was measured at 620 nm with a Jasco V-530 UV-spectrophotometer (JASCO Corp., Tokyo, Japan). The content of saccharides was calculated from a standard curve prepared for glucose. The final results, which were the means of four replicates for each experimental variant from one experiment, were expressed in mg of glucose per g of fresh weight.

2.4. Determination of Soluble Sugars and Organic Acids by HPLC

For the determination of sugars and organic acids, lyophilized and homogenized apple fruits (approximately 500 mg) were used and for each replicate. For the extraction of sugars, the plant material was boiled in 80% ethanol (v/v) according to the Miron and Schaffer method [45]. In turn, in the case of the extraction of organic acids, meta-phosphoric acid (3%) was used according to the method by Bozan et al. [46].

Sugars and organic acids were identified and quantified by high-performance liquid chromatography (HPLC) (Shimadzu LC 10A vp) consisting of an RID and UV detector. Individual sugars were detected using a Nucleosil NH2 analytical column (150 mm × 4.6mm i.d., 5 µm) (Shimadzu, Japan) at room temperature with a flow rate of 1 mL/min using 75% aqueous acetonitrile. For organic acids, a 250 mm × 4.6 mm i.d., 5 µm, reversed-phase Ultrasphere ODS analytical column (Beckman) operating at room temperature with a flow rate of 1 ml/min was used with 0.5% aqueous meta-phosphoric acid as the eluent.

Sugars and acids, together with small amounts of dissolved vitamins as well as fructans, proteins, pigments, phenolics, and minerals, are commonly referred to as soluble solids. The Total Soluble Solid (TSS) contents of samples were detected using a hand refractometer as "degrees Brix" (∘Brix), which is equivalent to the percentage (%). In these experiments, TSS contents (%) of apple samples were measured using the same samples as those dedicated to HPLC experiments after extraction. 1 g of homogenized samples were weighed and shaken with 10 mL double-distilled water using horizontal shaker for 5 minutes at room temperature. Supernatants were filtered using 0.45 µm nylon HPLC filter and measured using an ATAGO PAL 1digital refractometer.

2.5. Determination of Sorbitol by Gas Chromatography Coupled with Mass Spectrometry

2.5.1. Extraction

Plant material (150 mg) was ground in liquid nitrogen using a 30 Hz laboratory ball mill (1 min, 2 balls per 2 mL Eppendorf tube) and flooded with 1.4 mL 80% cooled methanol (MeOH, HPLC) [29,47]. Next, the samples were supplemented with 25 µL ribitol (1 mg/1 mL). Test tube contents were vortex-mixed in a thermomixer at 950 rpm for 10 min at room temperature, followed by centrifugation at 11.000× g for 10 min at 4 °C. The produced supernatant (250 µL) was transferred to Eppendorf tubes and evaporated in a speedvac at room temperature.

2.5.2. Derivatization

After sample desiccation in a desiccator, each sample was supplemented with 50 µL methoxyamine (20 mg/mL in dry pyridine) and vortex-mixed in a thermomixer for 1.5 h at 37 °C, afterwards it was

centrifuged for 10 s (short spin). Following centrifugation, the samples were supplemented with 80 µL MSTFA, again vortex-mixed in a thermomixer (30 min, 37 °C) and centrifuged at 11.000× g for 10 min. Prepared samples were transferred to inserts at 200 µL.

2.5.3. GC-MS Analyses

Sorbitol was identified and quantified using gas chromatography coupled with mass spectrometry (GC-MS) (TRACE 1310 GC oven with TSQ8000 triplequad MS from Thermo Scientific, USA) using a DB-5MS column (30 m × 0.25 mm × 0.25 µm, J&W Scientific, Agilent Technologies, Palo Alto, CA, USA). Gradient: 70 °C for 2 min, followed by 10 °C/min up to 300 °C (10 min). Injector 250 °C, interface 250 °C, source 250 °C, m/z range: 50–850, EI+, electron energy 70 eV. Sorbitol content was expressed as $\mu g \cdot g^{-1}$FW.

2.6. Determination of Semiquinone Radicals

Radicals were detected directly in apple tree organs (fruits, leaves and stems) using the electron paramagnetic resonance (EPR) technique [29–32,48]. Samples of 1 g fresh weight of apple fruits, stems and leaves were frozen in liquid nitrogen and lyophilized in a Jouan LP3 freeze dryer. The lyophilized material was transferred to EPR-type quartz tubes (diameter 4 mm). Electron paramagnetic resonance was measured at room temperature with a Bruker ELEXSYS X-band spectrometer (Rheinstetten, Germany). The EPR spectra were recorded as the first derivatives of microwave absorption. Microwave power of 2 mW and a 2 G magnetic field modulation were applied in all the experiments to avoid signal saturation and deformation. The EPR spectra were recorded for free radicals and Mn^{2+} ions in the magnetic field range of 3300–3360 G and with 4096 data points. To determine the number of paramagnetic centers in the samples the spectra were double-integrated and compared with the intensity of the standard Al_2O_3:Cr^{3+} single crystal with a known spin concentration [29,30,32,48–51]. Some background corrections of the spectra were introduced before and after the first integration to obtain a reliable absorption signal before the second integration. Concentrations of semiquinone radicals were calculated as the number of spins per 1 g of dry weight sample and the concentration of copper ions as the number of copper ions per 1 g of dry weight sample, respectively.

2.7. Detection of Abscisic Acid (ABA)

Frozen organs of apple trees (fruits, leaves and stems) were homogenized in liquid nitrogen and mixed with 20 mL of 80 % (v/v) methanol. Next, the mixture was transferred to Erlenmeyer flasks and a small amount of an anti-oxidant (BHT, butylhydroxytoluene) was added. Subsequently, 100 ng of deuterated ABA, [2H6]ABA, were added as the internal standard. The mixture was shaken overnight. The extract was then centrifuged and the supernatant was collected in evaporation flasks. For an additional centrifugation step, the remaining pellet was resolved two times in 10 mL of 80% (v/v) methanol, shaken for two hours and centrifuged again. This supernatant was then added to the evaporation flasks. Next, the extract was evaporated until all methanol was removed. The flasks were washed with additional H_2O and the extract was collected into centrifugation tubes. An appropriate amount of HCl was added for acidification until pH 2 was reached. Next, the extract was centrifuged and the supernatant was collected into Erlenmeyer flasks for solvent partition. The supernatant was partitioned three times against ethyl acetate. The organic phase was collected into evaporation flasks and dried under vacuum. The dry residue was then resolved in 5 mL 1 M formic acid (FA) and loaded on a Discovery®DSC-18 SPE cartridge (Supelco Inc., Bellefonte, PA, USA). These cartridges (columns) were preconditioned with 4 mL methanol and allowed to equilibrate with 4 mL of 1 M FA. The columns were subsequently washed with 4 mL 1 M FA, 4 mL of 20 % methanol in 1 M FA and finally phytohormones were eluted with 4 mL of 80% methanol in water. The eluate was evaporated to dryness, resolved in 200 µL of 20% acetonitrile in water and applied to HPLC for further sample purification. HPLC was run in a SUPELCOSIL ABZ+ PLUS column (250 x 4.5 mm, 5 µm particle size; Supelco). The samples were chromatographed with a linear gradient of 20-80% acetonitrile in 0.1 M

FA for 20 min, flow rate 1.0 mL/min at 22 °C. The fractions collected at 12 ± 0.5 min were evaporated to dryness and transferred to appropriate small glass tubes with 100% methanol. The methanol was then evaporated using N_2. Next, the residue was methylated with diazomethane dissolved in ethyl ether. Afterwards, ethyl ether was evaporated with N_2 and the residue was dissolved in 30 µL of 100% methanol. 1 µL of this sample was analyzed by GC/MS–SIM (Auto-System XL coupled to a TurboMass, Perkin-Elmer, Walthman, MA, USA) using a MDN-5 column (30 mm x 0.25 mm, 0.25 µm phase thickness; Supelco). The GC temperature program was set at 60 °C for 1 min, 60−250 °C at 10 °C/min, flow rate 1.5 mL/min, injection port was 280 °C, electron potential 70 eV. The retention times of ABA and [2H6]ABA were 14.07 and 14.3 min, respectively. The GC/MS was performed by monitoring mass/charge (m/z) 190 for endogenous ABA and 194 for [2H6]ABA according to the method described by Vine et al. [52].

2.8. Determination of Membrane Injury Index

Fruits, leaves and stems (10 pieces) were collected from each experimental variant and 1-cm fragments were cut. Then, 5 pieces from these organs were selected, each in 5 repetitions. After collecting the material, leaf, stem and apple pieces were washed three times in 10 mL of deionized water, then immersed in 20 mL of deionized water and kept for 24 h at 10 °C. The electrical conductivity of the effusate was measured. Then, the tissues were killed by autoclaving for 15 min, cooled down to 25 °C and electrical conductivity of the effusate was measured once again [53,54]. The same procedure was used for samples after 24 h treatment with negative temperature (−20 °C). Membrane injury was evaluated as an injury index in percentages, according to the formula proposed by Sullivan [55]:

$$I = [1-(1-TI/T2\ )/(1-C1/C2\ )] \times 100\ \%, \quad (1)$$

where C1 and C2 represent conductivity values of the samples (directly after plant material collection) before and after autoclaving, respectively; T1 and T2 represent the conductivity values of the samples after negative temperature treatment before and after autoclaving, respectively.

2.9. Yield and Determination of Fruit Quality

Apple trees matured in the orchard in the following order: Gala Schniga, Beni Shogun (Fuji) and Ligol. Fruit harvest dates were determined based on the starch decomposition rate and fruit firmness. When assessing yielding, the unit yield per tree ($kg \cdot tree^{-1}$) was used and converted to yield per hectare ($t \cdot ha^{-1}$).

The fruit yield quality was evaluated directly after harvesting, taking into account:

- fruit weight: 100 fruits were collected from each experimental variant and weighed accurate to 1 g;
- fruit firmness: measurements were taken on 100 fruits per four replication (a total of 400 fruits) for each experimental variant on the shaded side and intensive blush using a Fruit Hardness Tester (FHT-803) by Silverado Company, China;

This test is referred to as the Magness-Taylor test and consists in the penetration of apple fruit flesh to a depth of 8 mm with a plunger of 11 mm with a rounded tip. Values of measurements were given in $kg \cdot cm^{-2}$;

- Soluble solid content was measured on the same fruits, on which firmness was measured. Soluble solid contents were evaluated using a Digital Handheld Refractometer DR 101-60 (A. KRUSS Optronic GmbH, Hamburg Germany). Slices of fruit flesh were cut from apples on opposite sides of fruits, from which juice was pressed on a refractometer plate. Values of measurements were expressed in % °Brix;
- Potential acidity was measured using a pH-meter. The juice was pressed from apples, from which 5 mL samples were collected, 45 mL distilled water was added and 0.1 N NaOH were titrated

until pH 7.4 was reached. Based on the amount of used NaOH, the acid percentage content was given in malic acid;

- Fruit size was determined based on their calibration. A total of 100 fruits from each experimental variant were evaluated. Fruit diameter was measured using a template ruler graduated to 0.5 cm in a 5-point scale: 1–below 6.5 cm; 2–6.6–7.0 cm; 3–7.1–7.5 cm; 4–7.6–8.0 cm; 5–over 8.1 cm;
- Fruit color was assessed using a 5-point scale, taking into account the blush area. Evaluation was performed on 100 fruits from each experimental variant immediately after fruit harvest as a relative number (percentage of all assessed fruits). For color assessment, the percentage area of the skin blush was analyzed, i.e., 0–fruits with no blush; 1–<25% skin area covered by blush; 2–26–50% skin area covered by blush; 3–51–75% skin area covered by blush; 4–>75% skin area covered by blush; 5—100% skin area covered by blush.

2.10. Statistical Analysis

All determinations were conducted within three independent biological replicates per experimental variant, i.e., apple tree variety. Two-way analysis of variance (ANOVA) was used to verify the significance of means from independent replicates within a given experimental variant. Moreover, comparisons were related to the plant material variants. The significance of differences between the combinations was estimated based on Tukey's test at the significance level of $\alpha = 0.05$. The figures present data obtained as means of triplicates for each variant along with the standard deviation of the mean (SD). All biochemical analyses were conducted using the GenStat v. 18 statistical software package.

3. Results

3.1. Sugar Levels in Organs of Apple Trees

3.1.1. Total Saccharide Content in Organs of Apple Trees

The highest total sugar content was found in fruits of the Gala Schniga variety. In turn, the total sugar contents were similar in the fruits of Beni Shogun (Fuji) and Ligol. Moreover, in leaves and shoots, the highest sugar content was noted in the Ligol variety and the lowest in Gala Schniga (Figure 2).

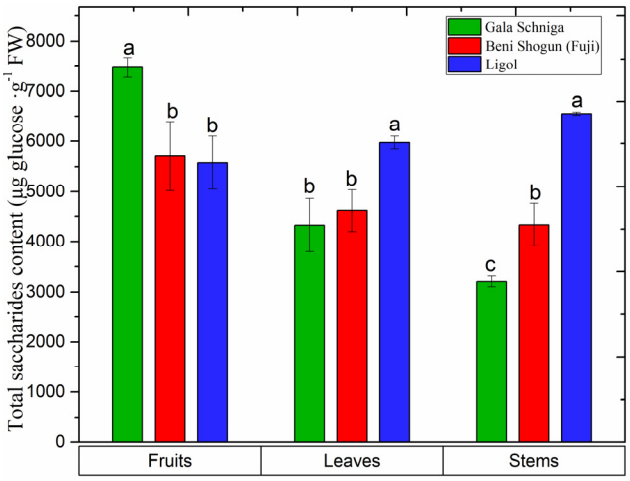

Figure 2. Total saccharide levels in organs of apple trees. Data were obtained within three independent biological replicates per experimental variant (apple trees variety) and two-way analysis of variance (ANOVA) was used. Statistically significant differences between the combinations were estimated based on Tukey's test at the significance level of $\alpha = 0.05$. Note.* Different letters in the fruits, leaves and stems represent significant differences at $\alpha = 0.05$ level of significance.

3.1.2. Soluble Sugars in Fruits of Apple Trees

HPLC analysis showed the highest concentration of sucrose in apple fruits of the Gala Schniga variety, although Tukey's test showed no statistically significant differences (at $\alpha = 0.05$) between the combinations. Moreover, apple fruits of the Beni Shogun (Fuji) variety were characterized by the highest amounts of glucose and fructose in comparison to the other varieties. The concentration of glucose in Beni Shogun (Fuji) apples was the above or about two times higher than in the Gala Schniga and Ligol varieties, respectively. Tukey's test revealed statistically significant differences between the combinations (Figure 3).

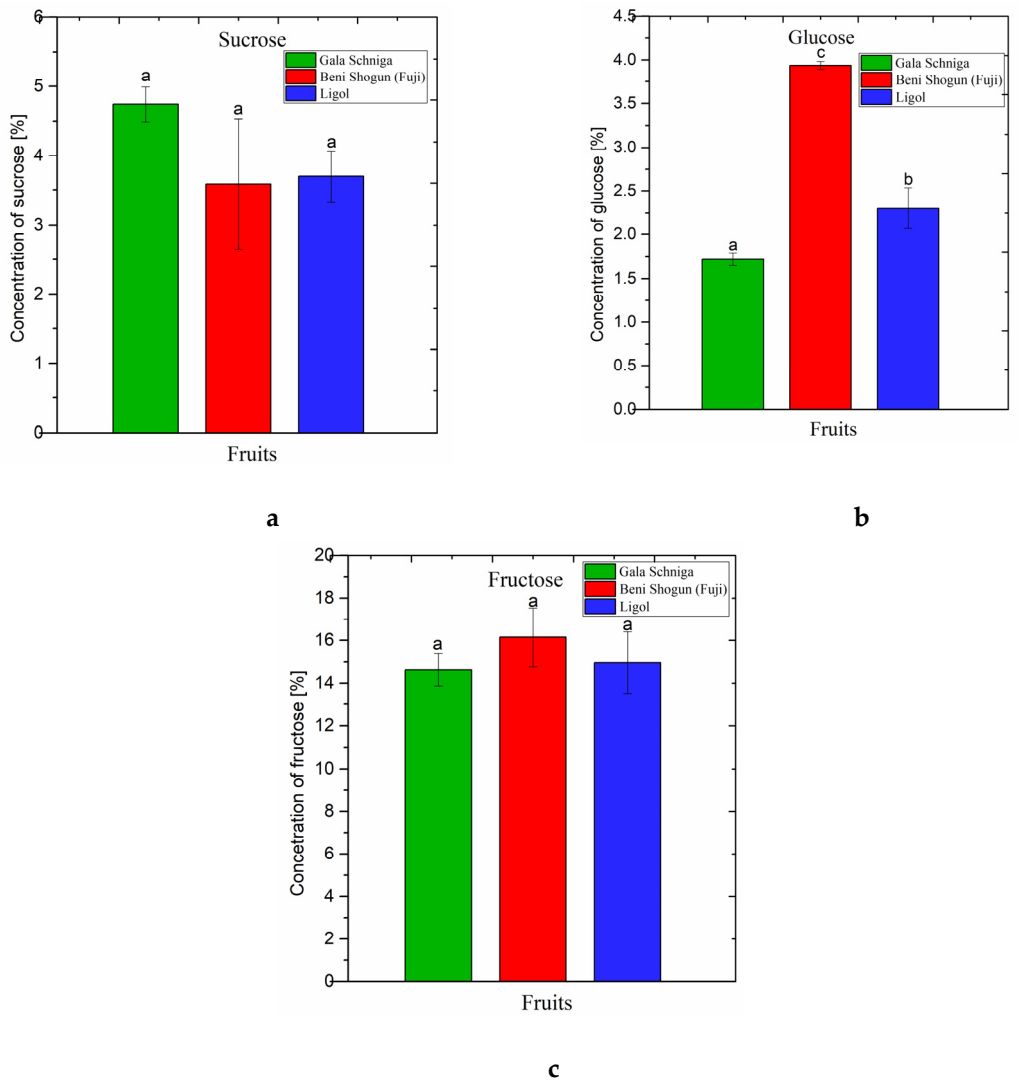

Figure 3. Sugar amounts in fruits of apple trees (**a**–**c**). The data were obtained within three independent biological replicates per experimental variant (apple trees variety) and two-way analysis of variance (ANOVA) was used. Statistically significant differences between the combinations was estimated based on Tukey's test at the significance level of $\alpha = 0.05$. Note:* Different letters in the fruits represent significant differences at $\alpha = 0.05$ level of significance.

3.2. Concentration of Organic Acids in Fruits of Apple Trees

HPLC analysis revealed the highest amounts of malic and L-ascorbic acids in apple fruits of the Ligol variety; statistically significant differences in comparison to other variants were noted. In turn, apple fruits of the Beni Shogun (Fuji) variety were characterized by a slightly higher level of malic acid than in the Gala Schniga variety (Figure 4).

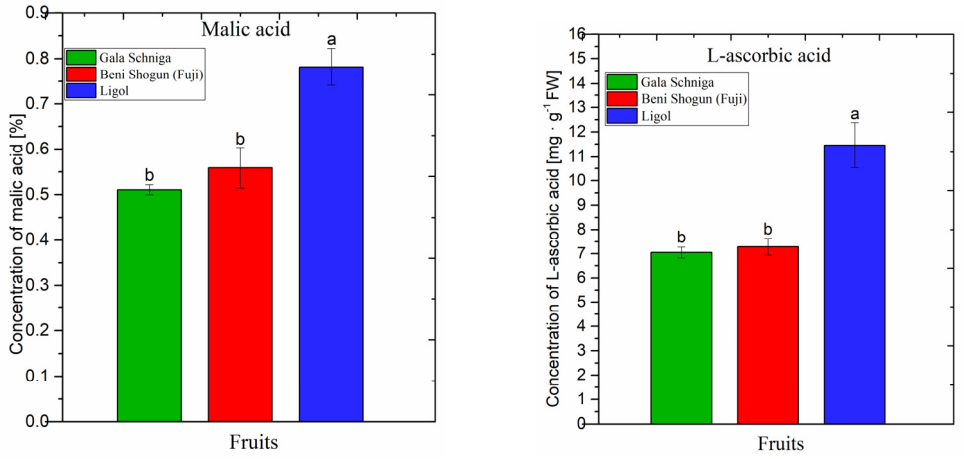

Figure 4. Concentration of organic acids in fruits of apple trees. The data were obtained within three independent biological replicates per experimental variant (apple trees variety) and two-way analysis of variance (ANOVA) was used. Statistically significant differences between the combinations were estimated based on Tukey's test at the significance level of $\alpha = 0.05$. Note:* Different letters in the fruits represent significant differences at $\alpha = 0.05$ level of significance.

3.3. Amounts of Sorbitol in Fruits of Apple Trees

The highest sorbitol level was noted in Beni Shogun (Fuji) fruits. In turn, similar contents of sorbitol were found in the Gala Schniga and Ligol variety fruits, which were significantly lower than in fruits of the Fuji variety (Figure 5).

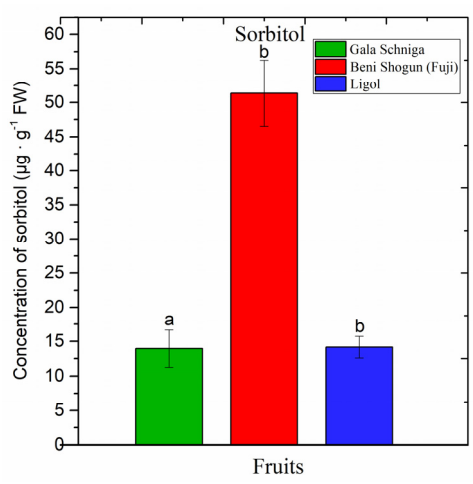

Figure 5. Relative amounts of sorbitol in fruits of apple trees. The data were obtained within three independent biological replicates per experimental variant (apple trees variety) and two-way analysis of variance (ANOVA) was used. Statistically significant differences between the combinations were estimated based on Tukey's test at the significance level of $\alpha = 0.05$. Note:* Different letters in the fruits represent significant differences at $\alpha = 0.05$ level of significance.

3.4. Total Solid Substance (TSS) Contents in Fruits of Apple Trees

Fruits of Beni Shogun (Fuji) contained the highest amounts of total solid substances (TSS), while the lowest amount of TSS was detected in Ligol fruits. However, these differences between variants were not statistically significant, as was estimated based on Tukey's test at the significance level of $\alpha = 0.05$ (Figure 6).

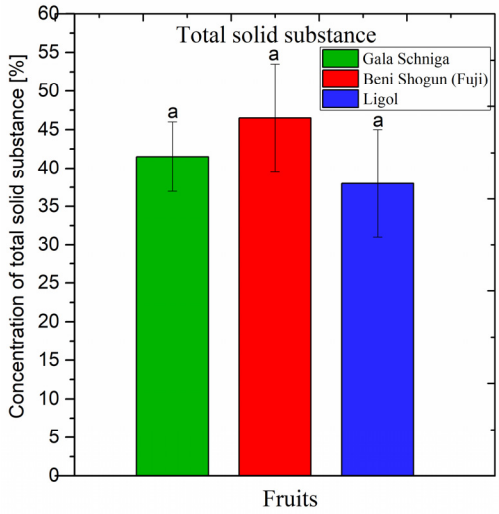

Figure 6. Total solid substance (TSS) contents in fruits of apple trees. The data were obtained within three independent biological replicates per experimental variant (apple trees variety) and two-way analysis of variance (ANOVA) was used. Statistically significant differences between the combinations were estimated based on Tukey's test at the significance level of $\alpha = 0.05$. Note:* Different letters in the fruits represent significant differences at $\alpha = 0.05$ level of significance.

3.5. Concentration of Semiquinone Radicals in Organs of Apple Trees

EPR analysis showed generally the highest level of semiquinone radicals (of organic origin) in all organs of Gala Schniga and the lowest in Ligol. Moreover, an overall comparison shows that the lowest contents of these radicals were found in fruits and the highest ones in leaves of apple trees (Figure 7).

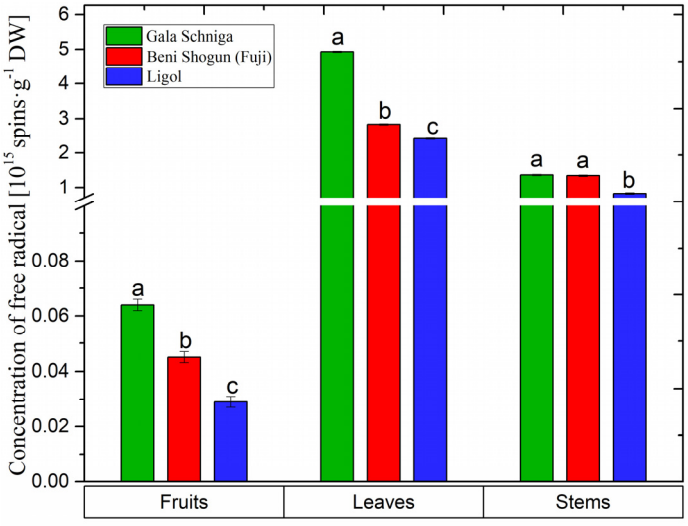

Figure 7. Concentration of semiquinone radicals in organs of apple trees. The data were obtained within three independent biological replicates per experimental variant (apple trees variety) and two-way analysis of variance (ANOVA) was used. Statistically significant differences between the combinations were estimated based on Tukey's test at the significance level of $\alpha = 0.05$. Note:* Different letters in the fruits, leaves and stems represent significant differences at $\alpha = 0.05$ level of significance.

3.6. Abscisic Acid Levels in Organs of Apple Trees

Ligol fruits were characterized by the highest ABA level, while marked differences were observed in fruits of Gala Schniga and Fuji Benishogun, but significantly lower than for the Ligol variety. In turn, in leaves, the highest ABA levels were recorded in the Schniga Gala variety, while in stems, ABA levels

were highest for the Fuji Benishogun variety. Besides, when comparing the ABA concentration in apple tree organs, the highest levels were recorded in shoots (Figure 8).

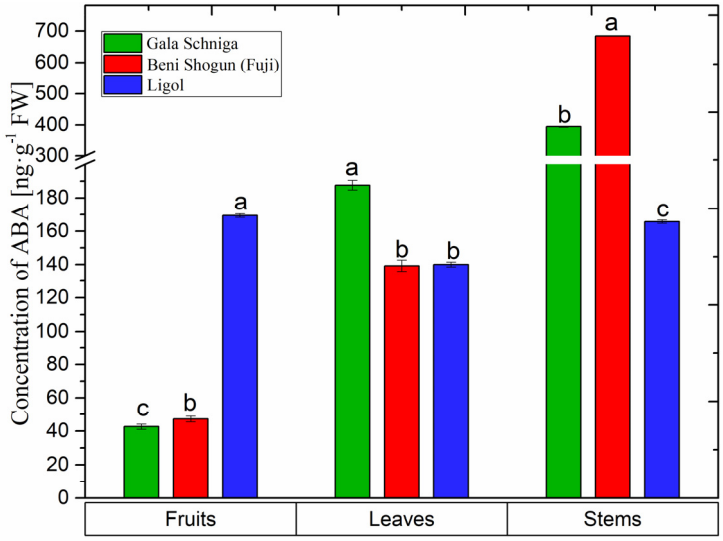

Figure 8. Concentration of abscisic acid (ABA) in organs of apple trees. The data were obtained within three independent biological replicates per experimental variant (apple trees variety) and two-way analysis of variance (ANOVA) was used. Statistically significant differences between the combinations were estimated based on Tukey's test at the significance level of $\alpha = 0.05$. Note:* Different letters in the fruits, leaves and stems represent significant differences at $\alpha = 0.05$ level of significance.

3.7. Release of Electrolytes from Organs of Apple Trees

The percentage of injury following a low-temperature treatment (−20°C) estimated on the basis of electrolyte leakage was lowest in the fruits of Fuji Benishogun. In turn, the highest electrolyte leakage was observed in fruits of the Polish variety, i.e., Ligol. The release of electrolytes from apple shoots was also highest in the Ligol variety (Figure 9).

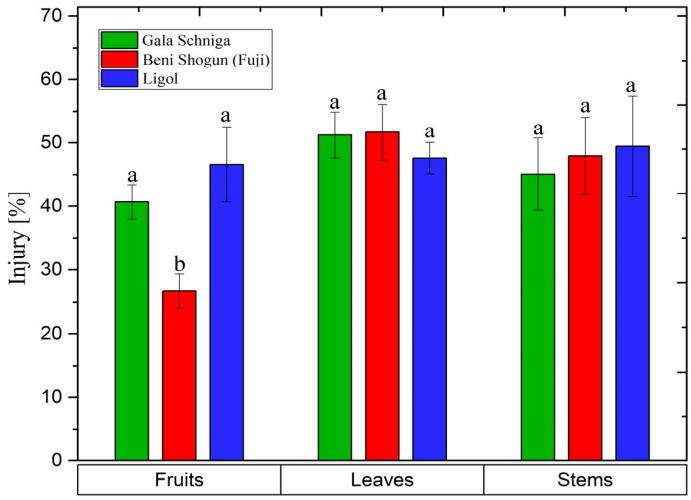

Figure 9. The percentage of injury (based on electrolyte leakage) in organs of apple trees after low-temperature treatment (−20°C). The data were obtained within three independent biological replicates per experimental variant (apple trees variety) and two-way analysis of variance (ANOVA) was used. Statistically significant differences between the combinations were estimated based on Tukey's test at the significance level of $\alpha = 0.05$. Note:* Different letters in the fruits, leaves and stems represent significant differences at $\alpha = 0.05$ level of significance.

3.8. Yields and Quality of Fruits

Analysis of the yields accomplished in 2017 showed a significant variability (Table 2). The highest yield was recorded for the Gala Schniga variety. In turn, both Fuji Benishogun and Ligol varieties showed comparable yields though being two-fold lower than for Gala Schniga. The lowest yield was recorded for Ligol trees, as it was 2.5 and 8.5-fold lower than those obtained from Fuji Benishogun and Gala Schniga trees, respectively. Moreover, fruit quality evaluation also showed significant variation among the varieties studied. The highest average weight of fruits was recorded for Ligol, while it was lowest for Gala Schniga. This latter variety was also characterized by the lowest weight among 100 fruits from the evaluated varieties. In contrast, the Ligol fruits were the heaviest among 100 fruits from all the evaluated varieties. Moreover, the maximum weight of 1 fruit in the Gala Schniga and Fuji Benishogun varieties did not differ significantly. Additionally, firmness evaluation showed that the firmest fruits were harvested from Gala apple trees, before that recorded for the Fuji Benishogun and Ligol varieties. The lowest minimum fruit firmness among all the varieties examined, was found in Ligol. Additionally, analysis of the soluble solid contents displayed no significant variations between all the analyzed varieties though a slight trend towards an increase in the soluble solid contents was observed in the fruits of Fuji Benishogun. The maximum soluble solid content was highest in Fuji Benishogun, while it was lowest in Gala Schniga. In turn, blush in fruit skin analysis revealed the most extensive blush in fruits of Gala Schniga, in which blush exceeded 75% area in all fruits. Ligol fruits showed the weakest color development, as the area covered by blush was max. 50% in 63% fruits (Table 3). Irrespective of the above, an apple fruit size analysis was also performed taking into account their diameter (Table 4). Size class over 7.0 cm in diameter was found in 59% Gala Schniga fruits, while it was assigned to 100% for Fuji Benishogun fruits. In the case of Ligol, 100 % fruits had a diameter of min. 7.5 cm, while 58 % had a diameter exceeding 9.0 cm.

Table 2. Yields and quality of fruit.

Variety	Mean	Minimum	Maximum	Standard Deviation
		Yield (kg per tree)		
Gala Schniga	12.86 b	3.50	28.40	4.71
Fuji Benishogun	6.49 a	1.00	19.50	4.21
Ligol	6.21 a	0.40	18.30	4.30
		Average fruit weight (g)		
Gala Schniga	156.4 a	121.2	242.6	24.9
Fuji Benishogun	194.9 b	151.4	263.1	30.6
Ligol	295.9 c	208.6	420.9	45.2
		Firmness kg/cm^2		
Gala Schniga	7.18 c	5.50	8.90	0.60
Fuji Benishogun	6.02 a	4.90	7.60	0.54
Ligol	6.22 b	4.50	7.90	0.65
		Total soluble solid content °Brix		
Gala Schniga	12.13 a	10.30	13.80	0.66
Fuji Benishogun	12.86 b	10.90	15.80	1.01
Ligol	12.63 b	10.60	15.50	0.89
		pH of juice		
Gala Schniga	4.10 c	4.13	4.07	5.50
Fuji Benishogun	4.01 b	4.03	3.99	5.56
Ligol	3.70 a	3.72	3.69	5.40
		Acidity as malic acid content %		
Gala Schniga	0.33 a	0.31	0.37	0.02
Fuji Benishogun	0.37 b	0.33	0.43	0.03
Ligol	0.52 c	0.49	0.54	0.02

Table 3. Blush in fruit skin (% area).

Variety	No Blush	<25%	26–50%	51–75%	>75%	100%
	%					
Gala Schniga	0	0	0	0	25	75
Fuji Benishogun	0	2	17	61	20	0
Ligol	0	24	39	31	6	0

Table 4. Size classes according to fruit diameter.

Variety	6.5 cm	7.0 cm	7.5 cm	8.0 cm	8.5 cm	9.0 cm	>9.0 cm
	%						
Gala Schniga	41	46	8	5	0	0	0
Fuji Benishogun	0	12	53	24	11	0	0
Ligol	0	0	5	15	11	11	58

4. Discussion

The present study is the first to investigate and compare biochemical and physical indicators and yields of fruits in *M. domestica* Borkh such as Gala Schniga, Beni Shogun (Fuji), i.e., varieties originating from various countries (New Zealand and Japan) and the Polish variety Ligol, M.9 rootstock. In this study, we focused mainly on apple fruits, but additionally, analyses for some indicators were also performed on leaves and shoots of apple trees in order to obtain comprehensive information on the relationships between the organs of a given apple variety. The results showed that these apple varieties differ in terms of biochemical composition, physical properties and accomplished yields.

Namely, spectrophotometric measurements of the total saccharide contents showed the highest levels of these metabolites in fruits of Gala Schniga, while leaves and shoots of this apple variety had the lowest total sugar contents (Figure 2). In parallel, Gala Shniga contained the highest sucrose content as it was recorded by HPLC (Figure 3). In turn, the Ligol variety was characterized by the lowest saccharide levels in fruits and their highest contents in leaves and shoots (Figure 2). HPLC analysis made it also possible to assess the profile of these sugars (Figure 3) as well as those of other metabolites (organic acids) (Figure 4) found in apple tree fruits. It has been demonstrated that fruits of the tested apple varieties contained various concentrations of sugars (Figure 2) Fuji fruits being characterized by their highest contents of glucose and fructose among the apple varieties.

In this study, we found a positive interdependence between the total saccharide content of fruits and yields (expressed as kg fruits per tree) in the case of the Gala Schniga variety (Figure 1 and Table 3). At the same time, these fruits were also the firmest. In turn, the low total saccharide content in Ligol fruits was accompanied by a two-fold lower yield in comparison to the Schniga Gala. Moreover, our study showed that the yield harvested from Ligol trees was many times lower than those of the Gala Schniga and Beni Shogun (Fuji) varieties (Table 3). At the same time, the highest total saccharide content in Gala Schniga fruits was accompanied by the lowest acidity of fruits (expressed as the percentage of malic acid content) among the apple varieties. The Polish Ligol variety which showed the highest acidity of fruits was indeed characterized by its greatest content in organic acids such as malic acid and L-ascorbic acid (Figure 4). In addition, organic acid levels in Gala Schniga and Beni Shogun did not differ significantly. Moreover, these varieties were characterized by a similar mean value of juice pH. In conclusion, analysis of the sugar contents and profiles in fruits revealed above all significant quantitative differences between the apple varieties studied. As reported by Wosiacki et al. [56], a significant change in the sugar contents of fruit results from differences in varieties, maturity, climate, growing regions, seasons and storage conditions. Analysis of the concentration of the total reducing sugars in authentic Brazilian apple juices has revealed that clarified juices contained high contents of fructose, followed by sucrose and glucose [56]. In our study, the highest fructose content was recorded in Fuji fruits, notwithstanding the fact that it was estimated based on Tukey's test

at the significance level of $\alpha = 0.05$. Sweetness and sourness have been recognized as important drivers of apple consumer preferences [57,58]. For this reason, the sweetness is one of the most important fruit sensory quality traits which is taken into account in breeding programs. Aprea et al. [18] found that the sorbitol content correlates with perceived sweetness better than any other single sugar or even the total sugar content. In the present study, GC-MS analysis revealed the highest sorbitol content in Fuji fruits compared to the other varieties (Figure 5). In Fuji fruits, we need to stress the highest concentrations of total solid substances (TSS) (Figure 6), while the lowest TSS content was found in Ligol fruits. Fuzfai et al [59] have developed a GC-MS gas method for the simultaneous quantitation of mono-, di- and trisaccharides, sugar alcohols, carboxylic and amino acids found in apple fruits, measured as their trimethylsilyl-(oxime) ether/ester derivatives. Moreover, as reported by Hu et al. [19], among the spectroscopic techniques, nuclear magnetic resonance (NMR) and near-infrared (NIR) spectroscopy are also useful tools for characterizing sugars. For example, NMR analysis provides exhaustive information concerning the number of sugar residues, monosaccharide composition, anomer configuration, glycofraction bonds and substituent position, among others.

A positive interdependence between the concentration of semiquinone radicals (Figure 7) and the level of Mn was reported in Gala Shinga fruits [38]. In turn, fruits of this Polish variety also contained a high level of this microelement [38], whereas the concentration of semiquinone radicals was the lowest. A positive correlation was found between the total sugar contents in Gala Schniga fruits and the levels of semiquinone radicals detected using EPR. A similar trend was noted in our earlier studies [29]. In turn, no relationship between the ABA level and the total sugar content was found in fruits of the apple varieties studied in this work. A negative interdependence between glucose and ABA was found in fruits of the Beni Shogun (Fuji) variety. We detected low ABA levels in Fuji Benishogun and Gala Schniga fruits, while the highest content was recorded in Ligol fruits. Additionally, the highest electrolyte leakage after a low-temperature treatment (−20 °C) was observed in fruits of the Polish variety Ligol and the highest ABA content. High ABA levels are found in aging tissues and in plants growing under stress conditions [60,61]. The phytohormone ABA also plays a crucial role in fruit development and ripening [62]. Some authors reported that a mechanism occurs in plants to coordinate multiple ABA sources and signaling pathways as well as to regulate transport in order to provide appropriate cellular ABA levels under variable developmental stages and environmental conditions. ABA is degraded by the oxidation of one of the methyl groups in the 6-position and cyclization to phaseic acid [63]. The concentration of ABA in tissues is also regulated by the intensity of synthesis and the formation of glucosyl conjugates [64]. Data reported by Onik et al. [65] suggested that in addition to ethylene, ABA and other hormones also play key roles in regulating apple fruit ripening and that they may interact with the ethylene signaling pathway. Plant hormones are reportedly considered to be closely linked with fruit development as well as ripening [66]. Through the response of different hormones, significant ripening regulations seem to be controlled primarily by ethylene and ABA [67,68]. In the present study, our attention also draws to lowest the percentage of injury after −20 °C treatment and the highest level of monosaccharides (glucose and fructose) in fruits of Fuji Benishogun, which may indicate the protective role of these sugars to low temperature.

Moreover, the cool spring in 2017 contributed to the weak flight of bees. Apple blossoms are adapted to insect-dustiness [69]. The limited presence of bees contributed to poor pollination of flowers, which was reflected in a significant reduction in yields (Table 2). According to Lech [69], in the case of non-fertilization of ovules at the stage of effective pollination by insects, degeneration of embryo sacs and their descent are observed. The lowest yields were collected from Ligol and Fuji Benishogun trees (the average tree yield was 6.21 kg and 6.49 kg, respectively), which gives 17.7 and 18.5 $t \cdot ha^{-1}$, respectively). The highest yield was collected from Gala Schniga trees, amounting to 12.86 kg/tree, which is equivalent to 36.7 $t\ ha^{-1}$. The level of tree yielding in the growing season of 2017 may not be considered as satisfactory. According to economic analyses, the average apple yield from 1 ha should be around 60 tons. Poor yielding in the growing season of 2017 was also connected with a significant variation in the weight of fruit (Table 2). The largest fruits with an average weight of 295.9 g were

collected from trees of the Ligol variety. Beni Shogun (Fuji) fruits were lighter by an average of 100 g. The smallest fruits with an average weight of 156.4 g were collected from trees of the Gala variety, which is classified as small-fruited [70].

An important element of fruit quality is its firmness. Among the tested varieties, the firmest fruits were harvested from Gala Schniga trees (Table 2). Fruit firmness of the Beni Shogun variety was the lowest. Fruit firmness is a feature very often associated with fruit size and calcium content, which in the growing season was low due to unfavorable climatic conditions, despite the use of foliar fertilization. The influence of weather conditions on the quality and storage stability of fruit was underlined by Failla et al. [71], while Ahmadi-Afzadi [72] draws attention to the great importance of genetic characters of the variety.

Fruits of the tested varieties did not significantly differ in terms of their soluble solid contents (Figure 6, Table 2). However, a significant relationship was found between apple varieties and juice pH (Table 2). The lowest juice pH was recorded in the Ligol variety. Hydrolytic acidity of fruits based on malic acid levels showed the highest content of that acid in fruits of the Ligol variety and the lowest in Gala Schniga and Fuji (Table 2, Figure 4). Acid and soluble solid contents in fruits largely determine their suitability for consumption or industrial purposes. During the ripening of fruits, their acidity is reduced. Additionally, the course of weather conditions also has an impact on fruit color (Table 3). Analysis of fruit blush demonstrated the largest blush surface in fruits of the Gala Schniga variety. In turn, Ligol fruits were characterized by the smallest blush surface, which did not exceed 50% of the surface in 63% of fruits. As can be seen from our results, the Schniga Gala variety was characterized by a total dark red color. These data may confirm the results of Mizani and Hajnajari [73], who showed that the genetic stability of mutants is threatened by different degrees of climate adaptation, which may affect changes in firmness, weight or color of their fruits. In addition, the size of fruits, especially their diameter, is one of the criteria taken into account when assessing the marketability of fruit. Retail operators demand fruit of apple varieties with large fruits (Ligol) with a minimum diameter of 7.5 cm, while for varieties classified as small-fruited (Gala Schniga and Fuji) this minimum is of 7.0 cm [74]. In Schniga Gala 59% of the fruits evaluated qualified as the size class above 7.0 cm, while in the Fuji variety it was 100% (Table 4). For Ligol 100% of fruits had a diameter of minimum. 7.5 cm, while 58% had a diameter of more than 9.0 cm.

5. Conclusions

The obtained results showed that the investigated apple varieties differed in their biochemical composition, physical properties and yields. The analyzed apple varieties differed in the profiles of sugars and their respective contents. Moreover, the highest levels of glucose and the lowest percentages of injury as a result of low-temperature impact in fruits of Beni Shogun (Fuji) were observed. Monosaccharide accumulation in Fuji fruits might have a protective action. Additionally, the highest concentration of sorbitol as the sugar alcohol and the lowest organic acid contents in fruits of Beni Shogun variety were found. Moreover, a positive interdependence was noted between the total saccharide content in Gala Schniga fruits and the level of semiquinone radicals. There was no relationship between ABA levels and the total saccharide contents in fruits of the analyzed apple varieties. Besides, a positive interdependence between D-glucose and ABA was observed in Ligol fruits, where the percentage of injury after a low-temperature treatment was the highest. Besides, fruit weight and firmness were dependent on the variety. The largest fruits were harvested from Ligol trees, while Gala Schniga trees produced the firmest fruits. Soluble solid contents did not vary significantly between the analyzed varieties.

Author Contributions: H.-K.Y. as the head of the project from the Korean side took part in the discussion on the research results, analyzed and interpreted the data and corrected the manuscript; I.M. wrote and prepared the manuscript, directed the implementation of physiological and biochemical analyses, and analyzed and interpreted the data; A.W., a Ph.D. of I.M. prepared all samples for physiological and biochemical analyses and prepared all the figures. Ł.M. contributed to measurements of sorbitol concentrations by GC-MS; J.K. contributed to measurements of abscisic acid concentrations, W.B. contributed to measurements of semiquinone radicals concentrations by

EPR; T.K., as the head of the project from the Polish side; Z.Z. designed experiments conducted in the orchard, took part in the collection of fruits, wrote and prepared a part of the manuscript regarding the evaluation of the yield and quality of fruits, analyzed and interpreted the data and prepared figures; K.R. conducted work in the orchard, collected plant research material, evaluated the yield and quality of fruits and participated in the statistical analysis of results; S.Ś. performed work in the orchard and collected plant research material, J.-H.S., T.-Y.C. and K.-J.K. took part in the discussion on the research results and they are co-investigators in the project; N.E.K. contributed to measurements of metabolite concentrations by HPLC and volatiles by HS/SPME/GC/MS; J.B. contributed to performing statistical analysis; P.J. made formal analysis, edited the whole manuscript and proofreading this manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: The study was supported by the Cooperative Research Project between the Poznań University of Life Sciences (PULS), the Faculty of Horticulture and Landscape Architecture in Poznań and Chungcheongnam-do Agricultural Research and Extension Services (CNARES) of the Rural Development Administration (RDA) of the Republic of Korea.

Conflicts of Interest: The authors declare no conflicts of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the research results.

References

1. Goddrie, P.W.; Kemp, H. Cultivar testing with apple. *Wilherminadorp Res. St Ann. Rep.* **1991**, 48–54.
2. Ugolik, M.; Kantorowicz-Bąk, M.; Ugolik, D. Evaluation of the Growth and Yield of Several New Apple Varieties on the M.9 Rootstock. In Proceedings of the 33 Polish National Scientific Conference of Institute of Pomology and Floriculture, Skierniewice, Poland, 30 August–1 September 1994; pp. 86–87.
3. Wrona, D.; Sadowski, A.; Dziuban, R. Comparison of Seven Varieties of VF Apple on M.9 Rootstock. In Proceedings of the 33 Polish National Scientific Conference of Institute of Pomology and Floriculture, Skierniewice, Poland, 30 August–1 September 1994; pp. 81–89.
4. Włodarczyk, P. *Selected Factors Affecting the Early Start Fruiting Apple Trees*; Akademia Rolnicza Lublin: Lublin, Poland, 1996.
5. Błaszczyk, J.; Poniedziałek, W. Growth and Yielding of 20 Apple Varieties in the Kraków Region. In Proceedings of the 37 Polish National Scientific Conference of Judical Institute of Pomology and Floriculture, Skierniewice, Poland, 25–27 August 1998; pp. 363–365.
6. Makosz, E. The Vision of the Fruit Market in Poland. In Proceedings of the 44 Congress of the Fruit-Growers' Market Fruit, Skierniewice, Poland, 27 October 2005; pp. 5–13.
7. Kapłan, M.; Wociór, S. The study of the yield of four apple varieties on the Sandomierska Upland. *Zesz Nauk. Inst. Sadow. Kwiac. Ski.* **2002**, 69–73.
8. Lewandowski, M.; Żurawicz, E. Effect of genotype on germination of *Malus domestica* seeds. In *Natural and Induced Variability in the Genetic Improvement of Horticultural Plants*; UTP in Bydgoszcz: Bydgoszcz, Poland, 2007; pp. 73–80.
9. Laurens, F. Review of the current apple breeding programmes in the world: Objectives for scion cultivar improvement. *Acta Hortic.* **1998**, *484*, 163–170. [CrossRef]
10. Sansavini, S.; Donati, F.; Costa, F.; Tartarini, S. Advances in apple breeding for enhanced fruit quality and resistance to biotic stresses:new varieties for the european market. *J. Fruit Ornam. Plant Res.* **2004**, *12*, 40.
11. Kellerhals, M. Introduction to Apple (*Malus* × *domestica*). In *Genetics and Genomics of Rosaceae*; Folta, K.M., Gardiner, S.E., Eds.; Springer: New York, NY, USA, 2009; pp. 73–84. ISBN 978-0-387-77490-9.
12. Kumar, S.; Volz, R.K.; Chagné, D.; Gardiner, S. Breeding for apple (*Malus* × *domestica* Borkh.) fruit quality traits in the genomics era. In *Genomics of Plant Genetic Resources*; Tuberosa, R., Graner, A., Frison, E., Eds.; Springer: Dordrecht, The Netherlands, 2014; pp. 387–416. ISBN 978-94-007-7574-9.
13. Bekbergen, A. Marker Assisted Breeding and Screening of Apple Scab Resistance (*Vf* Gene) from Columnar Apple Seedlings by PCR. Master's Thesis, University of Eastern Finland, Joensuu/Kuopio, Finland, 2016.
14. Sofla, H.S.; Zamani, Z.; Talaei, A.R.; Fatahi, M.R.; Nazari, S.A.; Farokhzad, A.R.; Gharghani, A.; Asgarzadeh, M. Introduction of New Promising Apple Genotypes: A Study of Quality Attributes of Apple in Crosses between Iranian Early Ripening and Exotic Late Ripening Apple Cultivars. *Int. J. Fruit Sci.* **2016**, *16*, 210–224. [CrossRef]
15. Foster, T.M.; Celton, J.-M.; Chagné, D.; Tustin, D.S.; Gardiner, S.E. Two quantitative trait loci, Dw1 and Dw2, are primarily responsible for rootstock-induced dwarfing in apple. *Hortic. Res.* **2015**, *2*, 15001. [CrossRef]

16. Tang, C.; He, H.; Li, E.; Li, H. Multispectral imaging for predicting sugar content of 'Fuji' apples. *Opt. Laser Technol.* **2018**, *106*, 280–285. [CrossRef]
17. Choi, D.; Cho, H.-T.; Lee, Y. Expansins: Expanding importance in plant growth and development. *Physiol. Plant.* **2006**, *126*, 511–518. [CrossRef]
18. Aprea, E.; Charles, M.; Endrizzi, I.; Laura Corollaro, M.; Betta, E.; Biasioli, F.; Gasperi, F. Sweet taste in apple: The role of sorbitol, individual sugars, organic acids and volatile compounds. *Sci. Rep.* **2017**, *7*, 44950. [CrossRef]
19. Hu, W.; Sun, D.-W.; Pu, H.; Pan, T. Recent developments in methods and techniques for rapid monitoring of sugar metabolism in fruits: Rapid monitoring of sugar metabolism. *Compr. Rev. Food Sci. Food Saf.* **2016**, *15*, 1067–1079. [CrossRef]
20. Leopold, L.; Diehl, H.; Socaciu, C. Quantification of glucose, fructose and sucrose in apple juices using ATR-MIR spectroscopy coupled with chemometry. *Bull. Univ. Agric. Sci. Vet. Med. Cluj-Napoca Agric.* **2009**, *66*, 9.
21. Krost, C.; Petersen, R.; Schmidt, E.R. The transcriptomes of columnar and standard type apple trees (*Malus* x *domestica*)—A comparative study. *Gene* **2012**, *498*, 223–230. [CrossRef] [PubMed]
22. Rock, C.; Quatrano, R. The role of hormones during seed development. In *Plant Horomnes: Physiology, Biochemistry and Molecular Biology*; Davies, P.J., Ed.; Kluwer Academic Publishers: Norwell, MA, USA, 1995; pp. 671–697.
23. Davies, W.J.; Zhang, J. Root Signals and the Regulation of Growth and Development of Plants in Drying Soil. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1991**, *42*, 55–76. [CrossRef]
24. Brenner, M.L. The role of hormones in photosynthate partitioning and seed filling. In *Plant Hormones and Their Role in Plant Growth and Development*; Davies, P.J., Ed.; Martinus-Nijhoff: Dordrecht, The Netherlands, 1987; pp. 474–493.
25. Wayne, H.L.; John, D.E. Sugar alcohol metabolism in sinks and sources. In *Photoassimilate Distribution in Plants and Crops: Source-Sink Relationships*; Zamski, E., Schaffer, A.A., Eds.; Marcel Dekker Inc.: New York, NY, USA, 1996; pp. 185–207.
26. Peng, Y.-B.; Lu, Y.-F.; Zhang, D.-P. Abscisic acid activates ATPase in developing apple fruit especially in fruit phloem cells. *Plant Cell Environ.* **2003**, *26*, 1329–1342. [CrossRef]
27. Lu, Y.M.; Zhang, D.P.; Yan, H.Y. Sugar unloading mechanism in the developing apple fruit. *Acta Hortic. Sin.* **1999**, *26*, 141–146.
28. Barbehenn, R.V.; Poopat, U.; Spencer, B. Semiquinone and ascorbyl radicals in the gut fluids of caterpillars measured with EPR spectrometry. *Insect Biochem. Mol. Biol.* **2003**, *33*, 125–130. [CrossRef]
29. Formela, M.; Samardakiewicz, S.; Marczak, Ł.; Nowak, W.; Narożna, D.; Bednarski, W.; Kasprowicz-Maluśki, A.; Morkunas, I. Effects of endogenous signals and *Fusarium oxysporum* on the mechanism regulating genistein synthesis and accumulation in yellow lupine and their impact on plant cell cytoskeleton. *Molecules* **2014**, *19*, 13392–13421. [CrossRef]
30. Mai, V.C.; Bednarski, W.; Borowiak-Sobkowiak, B.; Wilkaniec, B.; Samardakiewicz, S.; Morkunas, I. Oxidative stress in pea seedling leaves in response to *Acyrthosiphon pisum* infestation. *Phytochemistry* **2013**, *93*, 49–62. [CrossRef]
31. Morkunas, I.; Bednarski, W.; Kopyra, M. Defense strategies of pea embryo axes with different levels of sucrose to *Fusarium oxysporum* and *Ascochyta pisi*. *Physiol. Mol. Plant Pathol.* **2008**, *72*, 167–178. [CrossRef]
32. Morkunas, I.; Bednarski, W. *Fusarium oxysporum*-induced oxidative stress and antioxidative defenses of yellow lupine embryo axes with different sugar levels. *J. Plant Physiol.* **2008**, *165*, 262–277. [CrossRef]
33. Esteban-Carrasco, A.; Lopez-Serrano, M.; Zapata, J.M.; Sabater, B.; Martin, M. Oxidation of pehnolic compounds from *Aloe barbadensis* by peroxidase activity: Possible involvement in defence rection. *Plant Physiol. Biochem.* **2001**, *39*, 521–527. [CrossRef]
34. Hammerschmidt, R. Phenols and plant-pathogen interactions: The saga continues. *Physiol. Mol. Plant Pathol.* **2005**, *66*, 77–78. [CrossRef]
35. Barbehenn, R.; Cheek, S.; Gasperut, A.; Lister, E.; Maben, R. Phenolic Compounds in Red Oak and Sugar Maple Leaves Have Prooxidant Activities in the Midgut Fluids of *Malacosoma disstria* and *Orgyia leucostigma* Caterpillars. *J. Chem. Ecol.* **2005**, *31*, 969–988. [CrossRef] [PubMed]

36. Pearce, N.J.G.; Perkins, W.T.; Westgate, J.A.; Gorton, M.P.; Jackson, S.E.; Neal, C.R.; Chenery, S.P. A compilation of new and published major and trace element data for NIST SRM 610 and NIST SRM 612 glass reference materials. *Geostand. Newsl. J. Geostand. Geoanalysis* **1997**, *21*, 115–144. [CrossRef]
37. Borowiak-Sobkowiak, B.; Woźniak, A.; Bednarski, W.; Formela, M.; Samardakiewicz, S.; Morkunas, I. *Brachycorynella asparagi* (Mordv.) induced—Oxidative stress and antioxidative defenses of *Asparagus officinalis* L. *Int. J. Mol. Sci.* **2016**, *17*, 1740. [CrossRef]
38. Zydlik, Z.; Rutkowski, K.; Świerczyński, S.; Morkunas, I.; Yoon, H.-K.; Seo, J.-H.; Kang, K.; Kleiber, T. The effect of climatic conditions in successive plant growing seasons on the response of selected varieties of apple trees (Malus domestica Borkh.). *J. Elem.* **2020**, *25*, 205–224.
39. Vercammen, J. Search for a more dwarfing rootstock for apple. *Acta Hortic.* **2004**, *658*, 313–318. [CrossRef]
40. Robinson, T. Advances in apple culture worldwide. *Rev. Bras. Frutic.* **2011**, *33*, 37–47. [CrossRef]
41. Yordanov, A.; Tabakov, S.; Kaymakanov, P. Comparative study of Wavit®rootstock with two plum and two apricot cultivars in nursery. *J. Agric. Sci. Belgrade* **2015**, *60*, 159–168. [CrossRef]
42. Webster, A.D.; Wertheim, S.J. Comparisons of species and hybrid rootstocks for European plum cultivars. *J. Hortic. Sci.* **1993**, *68*, 861–869. [CrossRef]
43. Marini, R.P.; Fazio, G. Apple rootstocks: History, physiology, management, and breeding. *Hortic. Rev.* **2018**, *45*, 197–312.
44. Björnesjö, K.B. Analysis of protein-bound serum polysaccharides with anthrone reagent. *Scand. J. Clin. Lab. Invest.* **1955**, *6*, 147–152. [CrossRef] [PubMed]
45. Miron, D.; Schaffer, A.A. Sucrose Phosphate Synthase, Sucrose Synthase, and Invertase Activities in Developing Fruit of *Lycopersicon esculentum* Mill. and the Sucrose Accumulating *Lycopersicon hirsutum* Humb. and Bonpl. *Plant Physiol.* **1991**, *95*, 623–627. [CrossRef] [PubMed]
46. Bozan, B.; Tunalier, Z.; Kosar, M.; Altintas, A.; Baser, K.H.C. Quantitative Analysis of Vitamin C in Rose Hip Products Collected from Local Markets in Turkey. In Proceedings of the XI Symposium Plant Originated Crude Drugs, Ankara, Turkey, 22–24 May 1996; pp. 258–266.
47. Morkunas, I.; Woźniak, A.; Formela, M.; Mai, V.C.; Marczak, Ł.; Narożna, D.; Borowiak-Sobkowiak, B.; Kühn, C.; Grimm, B. Pea aphid infestation induces changes in flavonoids, antioxidative defence, soluble sugars and sugar transporter expression in leaves of pea seedlings. *Protoplasma* **2016**, *253*, 1063–1079. [CrossRef] [PubMed]
48. Bednarski, W.; Ostrowski, A.; Waplak, S. Low temperature short-range ordering caused by Mn^{2+} doping of $Rb_3H(SO_4)_2$. *J. Phys. Condens. Matter* **2010**, *22*, 225901. [CrossRef] [PubMed]
49. Morkunas, I.; Garnczarska, M.; Bednarski, W.; Ratajczak, W.; Waplak, S. Metabolic and ultrastructural responses of lupine embryo axes to sugar starvation. *J. Plant Physiol.* **2003**, *160*, 311–319. [CrossRef] [PubMed]
50. Morkunas, I.; Bednarski, W.; Kozłowska, M. Response of embryo axes of germinating seeds of yellow lupine to *Fusarium oxysporum*. *Plant Physiol. Biochem.* **2004**, *42*, 493–499. [CrossRef]
51. Morkunas, I.; Formela, M.; Floryszak-Wieczorek, J.; Marczak, Ł.; Narożna, D.; Nowak, W.; Bednarski, W. Cross-talk interactions of exogenous nitric oxide and sucrose modulates phenylpropanoid metabolism in yellow lupine embryo axes infected with *Fusarium oxysporum*. *Plant Sci.* **2013**, *211*, 102–121. [CrossRef]
52. Vine, J.H.; Noiton, D.; Plummer, J.A.; Baleriola-Lucas, C.; Mullins, M.G. Simultaneous quantitation of indole 3-acetic acid and abscisic acid in small samples of plant tissue by gas chromatography/mass spectrometry/selected ion monitoring. *Plant Physiol.* **1985**, *85*, 419–422. [CrossRef]
53. Dexter, S.T.; Tottingham, W.E.; Graber, L.F. Investigation of the hardiness of the plants by measurement of electricial conductivity. *Plant Physiol.* **1932**, *7*, 63–78. [CrossRef]
54. Bandurska, H. Does proline accumulated in leaves of water deficit stressed barley plants confine cell membrane injuries? II. Proline accumulation during hardening and its involvement in reducing membrane injuries in leaves subjected to severe osmotic stress. *Acta Physiol. Plant.* **2001**, *23*, 483–490. [CrossRef]
55. Sullivan, C.Y. Techniqes for measuring plant drought stress. In *Drought Injury an Resistance in Crops*; Larson, K.L., Eastin, J.D., Eds.; Crop Science Society of America: Madison, WI, USA, 1971; pp. 1–18.
56. Wosiacki, G.; Nogueira, A.; Denardi, F.; Vieira, R.G. Sugar composition of depectinized apple juices. *Semin. Ciênc. Agrár.* **2007**, *28*, 645–652. [CrossRef]
57. Endrizzi, I.; Torri, L.; Corollaro, M.L.; Demattè, M.L.; Aprea, E.; Charles, M.; Biasioli, F.; Gasperi, F. A conjoint study on apple acceptability: Sensory characteristics and nutritional information. *Food Qual. Prefer.* **2015**, *40*, 39–48. [CrossRef]

58. Bonany, J.; Buehler, A.; Carbó, J.; Codarin, S.; Donati, F.; Echeverria, G.; Egger, S.; Guerra, W.; Hilaire, C.; Höller, I.; et al. Consumer eating quality acceptance of new apple varieties in different European countries. *Food Qual. Prefer.* **2013**, *30*, 250–259. [CrossRef]
59. Füzfai, Z.; Katona, Z.F.; Kovács, E.; Molnár-Perl, I. Simultaneous identification and quantification of the wugar, Sugar Alcohol, and Carboxylic Acid Contents of Sour Cherry, Apple, and Ber Fruits, as Their Trimethylsilyl Derivatives, by Gas Chromatography−Mass Spectrometry. *J. Agric. Food Chem.* **2004**, *52*, 7444–7452. [CrossRef] [PubMed]
60. Kopcewicz, J.; Lewak, S. *Plant Physiology*; Wydawnictwo Naukowe PWN: Warsaw, Poland, 2002.
61. Woźniak, A.; Drzewiecka, K.; Kęsy, J.; Marczak, Ł.; Narożna, D.; Grobela, M.; Motała, R.; Bocianowski, J.; Morkunas, I. The influence of lead on generation of signalling molecules and accumulation of flavonoids in pea seedlings in response to pea aphid infestation. *Molecules* **2017**, *22*, 1404. [CrossRef]
62. Leng, P.; Yuan, B.; Guo, Y. The role of abscisic acid in fruit ripening and responses to abiotic stress. *J. Exp. Bot.* **2013**, *65*, 4577–4588. [CrossRef]
63. Frankowski, K.; Wilmowicz, E.; Kućko, A.; Sidłowska, M.; Kesy, J.; Kopcewicz, J. Abscisic acid metabolism. *Postepy Biochem.* **2013**, *59*, 83–88.
64. Kitahata, N.; Asami, T. Chemical biology of abscisic acid. *J. Plant Res.* **2011**, *124*, 549–557. [CrossRef]
65. Onik, J.C.; Hu, X.; Lin, Q.; Wang, Z. Comparative transcriptomic profiling to understand pre- and post-ripening hormonal regulations and anthocyanin biosynthesis in early ripening apple fruit. *Molecules* **2018**, *23*, 1908. [CrossRef]
66. Seymour, G.B.; Østergaard, L.; Chapman, N.H.; Knapp, S.; Martin, C. Fruit ripening and development. *Annu. Rev. Plant Biol.* **2013**, *64*, 219–241. [CrossRef]
67. McAtee, P.; Karim, S.; Schaffer, R.; David, K. A dynamic interplay between phytohormones is required for fruit development, maturation, and ripening. *Front. Plant Sci.* **2013**, *4*, 79. [CrossRef]
68. Giovannoni, J.J. Fruit ripening mutants yield insights into ripening control. *Curr. Opin. Plant Biol.* **2007**, *10*, 283–289. [CrossRef] [PubMed]
69. Lech, W. Flowering and fruiting of fruit plants. *Zeszyty Naukowe Akademii Rolniczej im. Hugona Kołłątaja w Krakowie* **2000**, *70*, 5–17.
70. Żurawicz, E. *Pomology, Varieties of Fruit Plants*; PWRiL: Warsaw, Poland, 2003.
71. Failla, O.; Treccani, C.P.; Mignani, I. Water status, growth and calcium nutrition of apple trees in relation to bitter pit. *Sci. Hortic.* **1990**, *42*, 55–64. [CrossRef]
72. Ahmadi-Afzadi, M. Genetic and Biochemical Properties of Apples that Affect Storability and Nutritional Value. Ph.D. Thesis, Balsgård, Swedish University of Agricultural Sciences, Uppsala, Sweden, 2012.
73. Mizani, A.; Hajnajari, H. Genetic stability assessment of apple mutants "Fuji Kiku 8" and "Gala Schniga" during adaptation trials in Iran. *Acta Hortic.* **2015**, *1074*, 111–118. [CrossRef]
74. Mika, A.; Buler, Z. Modifying apple spindle trees to improve fruit quality. *Acta Sci. Pol. Hortorum Cultus* **2015**, *14*, 12.