horticulturae

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Intraspecific Hybridization and Heritability of Biometric and Biochemical Traits in F1 Blueberry (*Vaccinium corymbosum* L.) Hybrids

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Abstract: Blueberry breeding requires a significant commitment of time, skilled labour, and financial resources, but it is essential to develop new cultivars that can meet challenges such as climate change, disease resistance, and changing market preferences. Intraspecific hybridisationis a widely used breeding strategy to increase genetic diversity, broaden the selection base, and develop new cultivars. By crossing different varieties and making advanced selections, breeders can introduce desirable traits such as improved fruit quality, increased yield, improved disease resistance and greater adaptability to environmental conditions. The present study aimed to evaluate the heritability of some key biometric and biochemical parameters inblueberry hybrids derived from intraspecific crosses to assess their inheritance patterns. The results can guide breeders in selecting parent combinations that maximise genetic gain, ultimately supporting the advancement of commercial blueberry production. The 'Delicia × 4/6' hybrid combination showed excellent performance for total polyphenol content, flavonoids, tannins, and ascorbic acid, with high genetic gain and near complete heritability, making it a promising candidate for improving antioxidant activity. The 'Azur × Northblue' hybrid had favourable total anthocyanin and tannin content, but an unfavourable sugar and ascorbic acid profile.The 'Simultan × Duke' hybrid combination showed the highest genetic gain for total soluble solids and firmness, together with high positive heterosis and heterotic progress, highlighting its potential for high-quality cultivars suitable for mechanical harvesting and storage. This research provides valuable insights into the efficiency of intraspecific hybridisationin the development of new blueberry cultivars with improved agronomic and nutritional qualities.

Keywords: breeding; crosses; genetic gain; heterosis; heterotic progress; highbush blueberry

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Academic Editor: Yuepeng Han

Received: 1 May 2025
Revised: 31 May 2025
Accepted: 2 June 2025
Published: 4 June 2025

Citation: Hera, O.; Sturzeanu, M.; Vijan, L.E. Intraspecific Hybridization and Heritability of Biometric and Biochemical Traits in F1 Blueberry (*Vaccinium corymbosum* L.) Hybrids. *Horticulturae* **2025**, *11*, 630. https://doi.org/10.3390/horticulturae11060630

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1. Introduction

Cultivated blueberries (*Vaccinium corymbosum*), a tetraploid species native to North America, are prized for their large, sweet fruits that are 2–4 times larger than their wild counterparts. It is increasingly sought after for several key factors, including the fruit's delicious flavour profile, nutritional value, and ability to be used in a variety of forms, both fresh and processed [1]. Compared to other blueberry species, such as the black blueberry (*V. fuscatum*) and the wild blueberry (*V. myrtillus*), the cultivated highbush blueberry has a superior nutritional content; the fruit is rich in vitamins, minerals, and antioxidants, and offers multiple health benefits [2].

The genus *Vaccinium* L. is characterised by a high level of intraspecific variation, thus displaying a notable degree of diversity. This genus, which belongs to the *Ericaceae* [3], is considered to be ancient, with estimates suggesting between 150 and 450 species [4], which have adapted to a wide range of environmental conditions. The majority of *Vaccinium* species are endemic to tropical regions, particularly open mountain slopes, as previously documented by Luby (1991) [5]. This observation suggests that the genus has evolved a variety of adaptations to survive in such specific ecological niches. However, it is noteworthy that the majority of species are found in subtropical, temperate, and boreal regions of the northern hemisphere, where they are commonly associated with cooler climates [6,7].

Recent advances in genetics, biotechnology, bioinformatics, and artificial intelligence have had a significant impact on the development of crops with improved yield, disease resistance, and adaptability to environmental change [8,9]. However, despite remarkable advances in crop yield, disease resistance, and adaptability, a concomitant decline in genetic diversification has been observed in many crop species [10–12]. As a result, the future of plant breeding will be focused on meeting the challenges of climate change, ensuring global food security, and promoting environmentally sustainable agriculture [13].

The traditional way of breeding plants relies on three interdependent main activities: introduction (obtaining new genetic material from different places to increase the gene pool), selection (finding and growing plants that have the desired traits), and hybridisation(crossing selected plants to combine good traits in the offspring). Modern breeding practices build on these principles, using tools such as marker-assisted selection and genomic editing to further refine and speed up the process. Intraspecific pollination in blueberry has been shown to generate significant levels of genetic diversity, which is beneficial for broadening the selection base and breeding new cultivars with desirable traits. Introducing genetic variation from different species has also been shown to increase blueberry disease resistance and tolerance to environmental stresses, improve flavour profile, and extend fruit shelf life [14].

Historical records indicate that the breeding of blueberries commenced with the incorporation of wild *Vaccinium* species. Early pioneers in this field, such as Frederick Coville and Elizabeth White, recognised the valuable traits of wild species, including hardiness, superior flavour, and adaptability, which could be used to develop new varieties for commercial production. In addition, selective breeding through cross-pollination of different species has led to the improvement of many beneficial traits, including increased yield, disease resistance, and overall performance [15,16].

The progress in blueberry breeding globally reflects continuous adaptation to diverse climatic conditions and market demands. Each region tailors breeding to local conditions, aiming for disease resistance, higher yields, and better adaptability [17–19]. This diversification of varieties contributes to the sustainability of the blueberry industry and meets the needs of consumers worldwide. In the U.S., varieties like Southern Highbush thrive in warmer climates, while Northern Highbush and Lowbush varieties are bred for cold regions. Canadaemphasises cold-hardy and high-yielding varieties for shorter growing seasons. Australia focuses on Southern Highbush varieties for export, aiming for fruit quality and low-input growing. In Europe, breeders focus on drought- and heat-resistant varieties and early-ripening cultivars. In Asia, Japan develops disease-resistant varieties, while tropical regions like China breed varieties for year-round production.

In Europe, significant advancements in blueberry cultivation commenced in 1929 with Dr. Hermann Heermann, who established one of the first highbush blueberry plantations in Germany [20]. In Romania, the blueberry breeding programme was initiated in 1980 under the leadership of Dr. Paulina Mladin at the Research Institute for Fruit Growing

in Pitesti-Maracineni [21]. The programme has focused on breeding varieties that meet market requirements, including larger fruit size and a longer ripening period. The initiative has successfully produced several Romanian blueberry varieties [21,22].

The firmness of blueberries is crucial for maintaining their quality after harvest. Firmer berries are more resilient and less susceptible to damage during harvesting, transportation, and storage [23]. This trait also extends the fruit's shelf life, reducing spoilage rates and enabling longer distribution periods. Breeding for firmness helps to overcome the logistical challenges of transporting blueberries globally while ensuring they maintain their quality until reaching consumers. Consequently, this characteristic is a key focus in breeding programmes centred on commercial-scale production [24].

Higher soluble solids, which correlate with increased sugar content, directly impact fruit flavour, a key factor in consumer satisfaction [25]. Sweetness is one of the most sought-after qualities in fresh blueberries, and higher sugar levels align with consumer preferences for sweeter fruits [23]. Breeding for higher soluble solids is particularly valuable for fresh-market sales, as sweeter berries tend to fetch a higher price. Furthermore, higher sugar content can enhance the flavour of processed blueberry products, such as jams and juices, thereby expanding the market potential for the fruit.

Balanced acidity is another important factor in determining blueberry flavour. While slight acidity can enhance flavour and make the fruit more refreshing, excessive acidity can reduce consumer acceptance. Blueberries with balanced sweetness and acidity are more likely to be favoured by consumers, making this trait highly relevant for market success. Moreover, managing acidity levels through breeding improves the fruit's overall postharvest quality, as overly acidic fruits may not have the same shelf life or shipping tolerance as those with a more balanced flavour.

The presence of bioactive compounds such as anthocyanins, flavonoids, and carotenoids enhances the health benefits of blueberries, setting them apart in the market [26]. As consumers become more health-conscious, demand is increasing for functional foods, which offer health benefits beyond basic nutrition. Blueberries are already considered a superfood thanks to their high levels of bioactive compounds, which give them their antioxidant properties. These properties have been linked to various health benefits, including anti-inflammatory effects and improved heart health [1,21]. Therefore, breeders prioritise these compounds to cater to the growing trend of health-conscious consumer preferences. Higher levels of these compounds can also improve the visual appeal of blueberries, as anthocyanins give the fruit its vibrant blue colour [27].

For the past 40 years, the genetic breeding of blueberries to produce high-yielding, frost-resistant cultivars with large, aromatic fruits has been a key objective of research programmes in Romania. The quality of the fruit depends on the genetic factors inherent in each cultivar, as well as environmental influences, cultural practices, harvesting methods, and storage in temperature-controlled facilities. Apart from commercial considerations for fruit intended for fresh consumption, characteristics such as taste, average berry weight (over 2.5 g), firmness, ripening in bunches on the bush, colour, aroma, and consistency are also important. Resistance/tolerance to specific diseases and suitability for mechanical harvesting are also important factors [28–30].

In terms of market acceptance and postharvest quality, all these traits are interconnected. To meet consumer preferences, blueberries must taste good, look appealing, and be able to withstand transportation and storage [31]. Therefore, breeding efforts focus on improving traits that enhance shelf life and resilience to handling and long-distance transport. These traits are essential to ensure that the fruit reaches markets in optimal condition, whether for fresh consumption or processing into value-added products.

Heritability is undoubtedly a pivotal parameter in the assessment of trial precision and the estimation of response to selection. This is a method of quantifying the impact of genes on a specific trait. It can also be thought of as an estimate of the probability that a particular trait will change from one generation to the next if there is pressure to select for that trait. By estimating heritability, plant breeders can determine the reliability of the results of an experiment in terms of genetic differences. It is generally accepted that a higher heritability value indicates that selection will be more effective, given that genetic variation exerts a greater influence than environmental factors [4,32,33].

In plant breeding, it is common practice to collect information ona genotype [34,35]. This approach ensures that the estimates accurately reflect the true genetic potential of the genotypes being studied. The primary objectives of highbush blueberry breeding have been the selection of aromatic cultivars with good storage resistance, an optimal fruiting period, resistance to diseases and pests, and adaptability to mechanised harvesting [36]. The objective of the present article is to analyse the inheritance of biometric and biochemical traits in the F1 progeny of blueberry hybrids. The specific traits of interest include fruit dimensions, firmness, total soluble solids, total acidity, DPPH radical scavenging activity, sugar content, ascorbic acid content, polyphenol content, and other bioactive compounds. The present article aims to provide valuable information on the variability of these traits in blueberry hybrids and to contribute to the improvement of selection techniques for breeding varieties with superior biometric and biochemical traits. The study will investigate the mechanisms of inheritance and the variation in these traits.

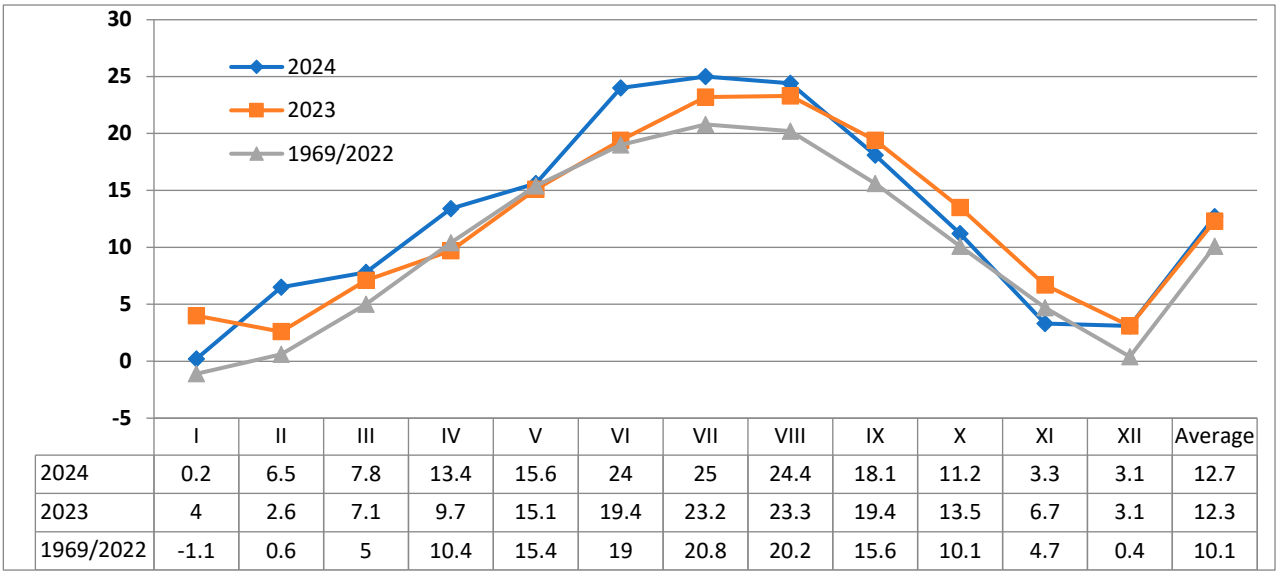
2. Materials and Methods

2.1. Location and Conditions of the Study

The experiment was conducted at the Research Institute for Fruit Growing Pitesti-Maracineni, Arges, Romania (44.898533° N, 24.859348° E), within the Department of Small Fruits and Strawberries, and tested in a hybrid field (Figure S1). The institute was founded in 1967 and has since developed nine new blueberry cultivars. The genotypes were planted at a spacing of 3 m × 1 m in a mixture of soil and peat. The soil composition included 17.6% clay, 1.84% organic matter, and a pH of 5.8. The experimental plot was irrigated using sprinklers, and Cropmax (0.5 L/ha) was applied as a foliar fertiliser. The blueberries were harvested at full maturity stage, determined visually by their uniform colouration, between 12 June and 5 July (2022), 11 June and 1 July (2023), and 17 June and 7 July (2024). A WatchDog 900 ET weather station, situated in proximity to the experimental plot, was used to monitor climatic conditions. The Pitesti-Arges region has a humid continental climate, classified as Cfbx.

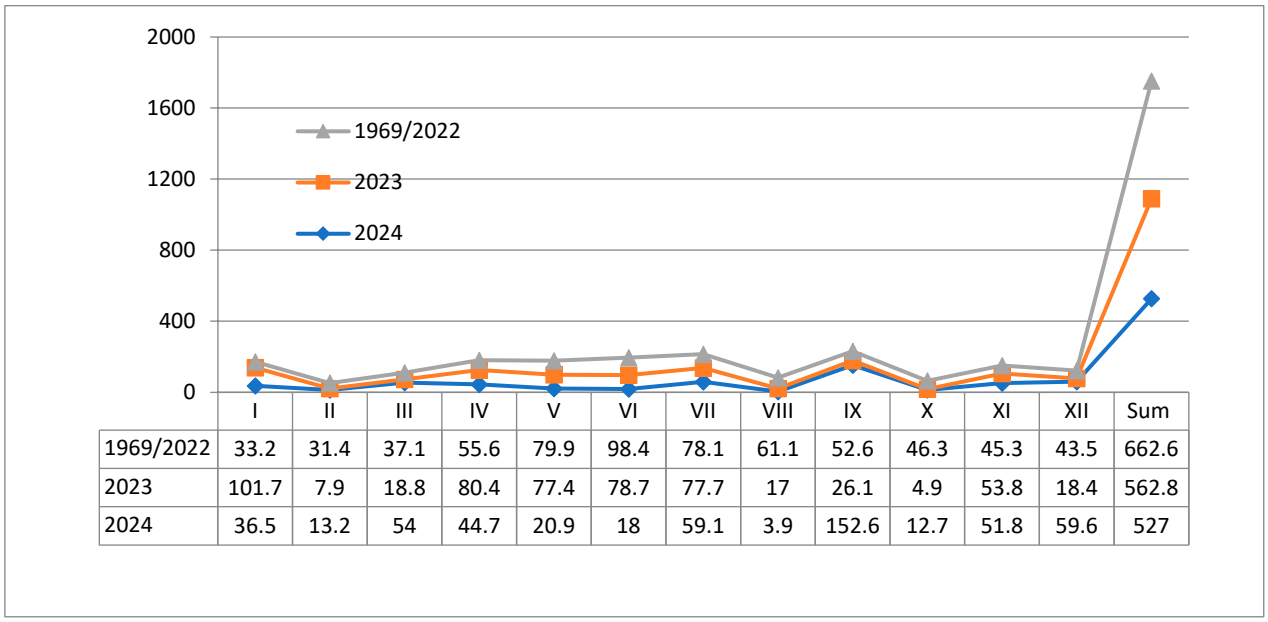
By analysing the data on temperatures and precipitation for 2024, 2023, and the historical average (1969–2022) for the Pitesti-Maracineni weather station (Figures 1 and 2), we can observe several significant trends that reflect local and regional climate changes, with potential implications for blueberry cultivation. The year 2024 shows a pattern of warmer temperatures coupled with more variable precipitation, with extremely dry periods (e.g., August) followed by intense rainfall in some months (e.g., September). The higher summer temperatures in 2024 are much above the historical averages, suggesting a possible shift toward warmer and potentially more erratic seasonal patterns in the region. These conditions could negatively affect blueberry growth, especially during prolonged drought periods, as blueberries are sensitive to temperature extremes and require consistent water availability. Blueberries thrive in a moderate water regime and stable temperatures, so the increasing heat and erratic precipitation could put additional stress on the plants. In contrast, 2023 showed more stable temperature patterns, with temperatures close to historical norms, but it was much wetter, particularly in the first half of the year. This more consistent

rainfall could have supported better water availability for blueberry cultivation, which is crucial for plant health. A wetter year would be beneficial for blueberries, providing them with the moisture needed for healthy growth, particularly during the fruiting period. The milder winter and wetter spring could have favoured the development of blueberry plants, but this precipitation pattern may not be sustainable in the long term, especially considering global warming trends. The fluctuations in precipitation observed in 2024 may present additional challenges for water management in blueberry farming.



	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Average
2024	0.2	6.5	7.8	13.4	15.6	24	25	24.4	18.1	11.2	3.3	3.1	12.7
2023	4	2.6	7.1	9.7	15.1	19.4	23.2	23.3	19.4	13.5	6.7	3.1	12.3
1969/2022	-1.1	0.6	5	10.4	15.4	19	20.8	20.2	15.6	10.1	4.7	0.4	10.1

Figure 1. Monthly average temperature (°C) in the experimental field of the Research Institute for the Fruit Growing Pitesti-Maracineni.



	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Sum
1969/2022	33.2	31.4	37.1	55.6	79.9	98.4	78.1	61.1	52.6	46.3	45.3	43.5	662.6
2023	101.7	7.9	18.8	80.4	77.4	78.7	77.7	17	26.1	4.9	53.8	18.4	562.8
2024	36.5	13.2	54	44.7	20.9	18	59.1	3.9	152.6	12.7	51.8	59.6	527

Figure 2. Monthly atmospheric precipitation (mm) in the experimental field of the Research Institute for the Fruit Growing Pitesti-Maracineni.

Overall, the data from 2024 and 2023 suggest a trend toward warmer years, with 2024 standing out for higher temperatures and drier conditions in the summer months, which may point to shifts linked to climate change or changing regional weather patterns.

2.2. PlantMaterial

All F1 blueberry hybrids (Figures 3 and 4) were the result of intraspecificcrosses between different cultivars, including three Romanian cultivars ('Simultan', 'Delicia', and 'Azur'), two American cultivars ('Duke' and 'Northblue'), and advanced selections used as parents. The genotypes used as parents were obtained through breeding programmes involving intraspecific hybridisation.

♀	♂	F1 HYBRIDS	
Azur × 6/38	⟶	Azur × 6/38	260
Azur × 4/6	⟶	Azur × 4/6	257
Azur × Duke	⟶	Azur × Duke	289
Azur × Northblue	⟶	Azur × Northblue	243
Delicia × 4/6	⟶	Delicia × 4/6	227
Delicia × Duke	⟶	Delicia × Duke	238
Delicia × Northblue	⟶	Delicia × Northblue	321
Simultan × Duke	⟶	Simultan × Duke	41
Simultan × Northblue	⟶	Simultan × Northblue	248
total number of hybrids ≈ 2124			

Figure 3. The hybridisationscheme was reinterpreted in accordance with the approach outlined by Wannemuehler et al. (2019) [37].



Figure 4. Fruit of F1 hybrids from controlled crosses ((**a**) = 'Simulan × Duke'; (**b**) = 'Simulan × Northblue'; (**c**) = 'Azur × Duke'; (**d**,**e**) ='Azur × 4/6'; (**f**) = 'Delicia × 4/6'; (**g**) = 'Azur × Northblue'; (**h**) = 'Delicia × Duke'; (**i**) = 'Delicia × Northblue').

The 'Simultan' cultivar stands out due to its early ripening season, which occurs between 25 June and 25 July. This is comparable towell-known cultivars such as 'Weymouth' and 'Duke'. This early harvest window makes it an attractive option for growers looking to start their berry season before anyone else.

The 'Delicia' cultivar is distinguished by its early fruiting. It begins to bear fruit in the second year after planting, producing 0.8–1.0 kg of fruit per bush. As the plants mature, their productivity increases significantly, reaching 2.5–4.0 kg per bush.

The 'Azur' cultivar is renowned for its impressive and consistent productivity, yielding 2.5–4.0 kg of fruit per bush. This makes it a reliable choice for growers seeking a substantial harvest year after year. Its large, visually appealing berries boast a light blue colour accentuated by a thick bloom.

The 'Northblue' cultivar is renowned for its consistently high productivity, with an average yield of 4.5 kg per bush. This reliable output makes it a popular choice among breeders.

The 'Duke' cultivar produces high-quality fruit: medium-sized, light blue berries with a thick bloom. These berries boast excellent organoleptic properties, offering an appealing balance of flavour and texture.

The '4/6' is a well-established variety renowned for its consistently high productivity. It produces 3.0–4.5 kg of fruit per bush. This makes it a valuable and reliable choice for growers who want to produce high-quality fruit in large quantities.

The '6/38' is a renowned variety known for its high quality. It produces large, blue-purple berries that have excellent organoleptic properties. These visually appealing berries are also rich in total polyphenols, making them a nutritious choice.

The main blueberry varieties used in this study of cross combinations were selected based on the following criteria:

- Fruit quality and attractiveness: 'Simultan' and 'Delicia';
- Late ripening and good storage: 'Azur';
- Earliness: 'Duke', 'Simultan', and '4/6';
- Resistance/tolerance to specific diseases: 'Delicia' and 'Azur'.

These crosses were performed as part of the breeding cycle, following the standard scheme described by Wannemeuhler et al. (2019) [37].

2.3. Chemicals and Reagents

Acetone, n-hexane, ethanol, methanol, sodium hydroxide, sodium carbonate, sodium nitrite, disodium phosphate, aluminium chloride, hydrochloric acid, ascorbic acid, gallic acid, citric acid, concentrated sulphuric acid, phenol, Folin–Ciocalteu reagent, catechinhydrate, 2,6-dichloroindophenol (DCPIP), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were used in this experimental study. All these reagents were purchased from Merck, Darmstadt, Germany.

2.4. Obtaining the Alcoholic Extracts

A total of 1 g of plant material was treated with 10 mL of ethanol or methanol and mixed for 2 min at 3000 rpm in a Corning Incorporated Vortex Mixer VX-200 system (Corning Life Sciences, Tewksbury, MA, USA). The mixture was then placed in an ultrasonic bath (model ULTR-2L0-001, manufactured by Labbox Labware, Migjorn, Spain) and left for 30 min at a frequency of 40 kHz. Finally, after spinning at 6000 rpm for 30 min in a LabnetSpectrafuge 6c centrifuge (Labnet International Inc., Edison, NJ, USA), the upper layer of the mixture was separated by filtration through a Whatman No. 1 filter.

2.5. Obtaining the Aqueous Extracts

A total of 1 g of plant material was mixed with 10 mL of distilled water in a Corning Incorporated Vortex Mixer VX-200 system for 2 min. The mixtures were then subjected to ultrasound at 99 °C for 30 min. Finally, the top layer of the mixture was separated by filtration through a Whatman No. 1 filter.

2.6. Methods of Analysis

Fruit length and diameter were measured with a digital calliper and are expressed in millimetres.

Mean fruit weight was determined by weighing a sample of 50 fruits from each genotype, and it is expressed in grams per fruit (g/fruit).

Firmness was measured on both parts of the fruit using a Bareiss HPE II non-destructive penetrometer (Bareiss North America Inc., Stouffville, ON, Canada) with a 25 cm^2 anvil and is expressed in hardness parameter (HPE) units.

Soluble solids were determined using a Kruss DR201-95 digital handheld refractometer (A. Krüss Optronic GmbH, Hamburg, Germany) and the results are expressed as °Brix at 20 °C.

The pH was measured in freshly extracted blueberry juice at 20 °C using a Consort C-561 multimeter (Consort nv, Turnhout, Belgium).

Total acidity (TTA) was determined by the titrimetric method, using 0.1 mol/L sodium hydroxide solution and 1% alcoholic phenolphthalein solution. The results are expressed as a percentage of citric acid (%).

The total sugar content (TSC) was determined by the method proposed by Stamin et al. (2024) [38] using 0.5 mL of an equimolecular mixture of aqueous extract and distilled water. A quantity of 2.5 mL of concentrated sulphuric acid was added to the mixture, followed by 0.5 mL of a 5% phenol solution. The samples were then placed in a water bath at 100 °C for 15 min. The absorbance of the samples was measured at 490 nm, and the results are expressed as grams of glucose equivalents (GluE)/100 g fresh weight (FW).

The total polyphenol content (TPC) was determined according to the method proposed by Stamin et al. (2024) [38], using 0.5 mL of ethanolic extract. Polyphenols react with phosphotungstic acid in an alkaline medium to form a blue coloured compound with maximum absorbance at 765 nm. The results are expressed as mg gallic acid equivalent (GAE)/100 g fresh weight (FW).

The total flavonoid content (TFC) was determined in accordance with the methodology proposed by Giura et al. (2019) [39] using 1 mL ofethanolic extract. Flavonoids react with aluminium chloride to form a yellow-orange-coloured compound with maximum absorbance at 510 nm. The results are expressed as mg catechin equivalent (CE)/100 g of fresh weight (FW).

The ascorbic acid content was determined by the colourimetricmethod proposed by Hera et al. (2023) [1], using 0.2 mL of ethanolic extract and 2,6-dichloroindophenol (DCPIP) at a pH of 3–4.5. Reduction in DCPIP by ascorbic acid results in a decrease in the absorbance maximum at 520 nm. The results are expressed as mg ascorbic acid/100 g fresh weight (FW).

The total anthocyanin content (TAC) was determined by the pH differential method proposed by Lee et al. (2005) [40], with minor modifications, using 0.5 mL of ethanolic extract. The method quantifies the total monomeric anthocyanin content as the anthocyanin chromophore undergoes a reversible structural change as a function of pH. Absorbance at 520 nm was measured 30 min after sample preparation at pH 0.6 (2% hydrochloric acid) and pH 3.5 (phosphate buffer containing 0.1 M citric acid and 0.2 M disodium phosphate). The results are expressed as cyanidin-3-O-glucoside equivalents (C3-GE)/100 g fresh weight (FW).

Lycopene and β-carotene contents were determined using the methodology established by Tudor-Radu et al. (2016) [41]. Carotenoids were extracted from a mixture of 3 g of plant material in 25 mL of a solution of hexane, ethanol, and acetone in a volumetric ratio of 2:1:1 using a magnetic stirrer (ISOLAB Laborgeräte GmbH, Eschau, Germany) for 30 min. The mixture was then mixed with 10 mL of distilled water and stirred for a further

15 min. After the separation of the phases, the supernatant was analysed. The results are expressed as mg lycopene or β-carotene per 100 g fresh weight (FW), using the molar extinction coefficients of both compounds at 470 and 503 nm.

The antioxidant activity was evaluated by measuring the free radical scavenging capacity of DPPH, following the methodology proposed by Mazilu et al. (2022) [42]. A solution of DPPH in methanol (0.116 mM) was prepared, and 2.97 mL of the DPPH solution was mixed with 0.03 mL of methanolic blueberry extract. The mixture was allowed to stand for 30 min at room temperature. The absorbance at 517 nm was then measured, and the percentage of DPPH radical scavenging activity (RSA%) was calculated.

2.7. Statistical Analysis

All analyses were performed in triplicate, and data are presented as mean ± standard deviation (SD). The statistical analysis was conducted using Excel 2021 (Microsoft Office 2021 Pro Plus) with the XLSTAT extension and SPSS Trial Version 28.0 (SPSS Inc., Chicago, IL, USA). Depending on the experimental design, appropriate statistical methods were used, including one-way and two-way analysis of variance (ANOVA) to compare differences between groups and when multiple variables or interactions between factors were involved. Multiple comparison tests were conducted using Duncan's multiple range test to identify significant differences between the tested groups.

Broad-sense heritability (H^2) is defined as the proportion of phenotypic variation that is accounted for by total genetic variation [43]. It takes into account all forms of genetic variance, including additive, dominant, and epistatic effects:

$$H^2 = V_G / V_P$$

where V_G represents total genetic variance (which includes V_{GA}, V_{GD}, and V_{GI}) and V_P represents total phenotypic variance (which includes V_G, V_E, and V_{G*E}) [44]. Additive genetic variance (V_{GA}) is the contribution of individual alleles to the variation in a trait; this is the portion that can be passed on from the genitor to the descendants. Dominant genetic variance (V_{GD}) is the variance due to dominant genetic interactions between alleles at the same locus. Epistatic genetic variance (V_{GI}) is the variance due to interactions between alleles at different loci (epistasis). The article by Sestras and Sestras [45] reviews comparative studies on the heritability of quantitative traits relevant to selecting apple varieties for breeding programmes. These traits are crucial for developing varieties that are resistant and productive, and that possess characteristics that consumers prefer.

Environmental variance (V_E) is defined as the proportion of variance in traits attributable to environmental factors. In contrast, genetic × environmental interaction variance (V_{G*E}) is the portion of variance in traits that is accounted for by the interaction between genetic and environmental factors.

By decomposing genetic and phenotypic variance into its components, researchers can better estimate how much of a trait is inherited and how different types of genetic interactions and environmental factors influence that trait. The half-sibling method allows accurate heritability estimates in the absence of complete parental information for the population under study [45,46]. The relationship between half-siblings is useful for separating genetic and environmental effects because half-siblings have less genetic overlap (sharing on average 25% of their genes) than full siblings (sharing 50% of their genes). This relationship allows estimation of the proportion of phenotypic variance due to genetic variation (both broad-sense and narrow-sense heritability), assuming that all siblings have been exposed to the same technological and environmental conditions, which allows isolation of genetic effects.

The genotypic coefficient of variation (GCV) is used to measure the relative variability of genotypic data and is calculated by dividing the genotypic standard deviation (S_G) by the trait mean ($\overline{x}$) in half-sib families.

$$G_{CV} = (S_G/\overline{x}) \times 100$$

The result is expressed as a percentage to give an idea of how much variation exists relative to the trait mean.

3. Results and Discussion

Table 1 shows the variation in biometric parameters among different F1 blueberry hybrid combinations, demonstrating how the parental genotypes influence these key fruit size characteristics. The coefficient of variation (CV%) is an important indicator of the stability and consistency of the traits analysed. A low CV indicates low variability and stable performance, which is ideal for large-scale production and increases the predictability of fruit quality. Conversely, a high CV indicates greater variability, which can lead to unpredictable fruit characteristics and may be less favourable for selecting hybrids for commercial production. The coefficient of variation for fruit taste among the analysed families was relatively high, consistent with the results of Sestras and Sestras (2023) [45].

Table 1. Biometric parametersin F1 blueberry hybrids in different combinations of hybrids according to parents.

Hybrid Combination (Parents ♀× ♂)	Berry Length, mm		Berry Diameter, mm		Berry Weight, g		Firmness, HPE Units	
	Mean ± SD	CV%	Mean ± SD	CV%	Mean ± SD	CV%	Mean ± SD	CV%
Azur × 6/38	11.90 ± 0.60 ^{a*}	0.25	14.43 ± 0.85 ^{c}	0.23	1.68 ± 0.31 ^{bc}	0.76	20.44 ± 1.75 ^{d}	0.22
Azur × 4/6	12.05 ± 0.82 ^{a}	0.29	15.08 ± 1.14 ^{abc}	0.26	1.82 ± 0.23 ^{b}	0.73	17.31 ± 0.86 ^{e}	0.24
Azur × Duke	12.67 ± 1.00 ^{a}	0.25	16.75 ± 1.61 ^{a}	0.22	1.75 ± 0.18 ^{bc}	0.74	20.46 ± 1.85 ^{d}	0.22
Azur × Northblue	12.19 ± 0.40 ^{a}	0.26	16.30 ± 0.65 ^{ab}	0.24	2.19 ± 0.21 ^{a}	0.68	15.89 ± 1.13 ^{e}	0.25
Delicia × 4/6	11.51 ± 0.07 ^{a}	0.29	14.78 ± 0.33 ^{bc}	0.26	1.58 ± 0.04 ^{bc}	0.80	15.94 ± 0.30 ^{e}	0.25
Delicia × Duke	12.67 ± 0.99 ^{a}	0.25	15.45 ± 1.09 ^{abc}	0.22	1.91 ± 0.22 ^{ab}	0.72	23.56 ± 1.76 ^{b}	0.22
Delicia × Northblue	11.53 ± 0.5 ^{a}	0.26	16.51 ± 0.34 ^{ab}	0.24	1.67 ± 0.12 ^{bc}	1.09	21.06 ± 0.77 ^{cd}	0.96
Simultan × Duke	12.57 ± 0.30 ^{a}	0.28	16.62 ± 0.49 ^{a}	0.25	2.19 ± 0.14 ^{a}	0.69	26.37 ± 1.52 ^{a}	0.53
Simultan × Northblue	12.36 ± 1.38 ^{a}	0.25	15.45 ± 1.09 ^{abc}	0.21	1.42 ± 0.06 ^{c}	0.82	23.01 ± 1.33 ^{bc}	0.21

* Means with the same letter are not significantly different at the 5% level.

The results obtained for the analysed hybrids are generally consistent with the published literature on the parent cultivars and other known blueberry varieties [47–53]. This confirms the quality, agronomic potential, and commercial viability of the developed hybrids. All hybrid combinations had relatively similar berry length values, ranging from 11.51 mm to 12.67 mm, and were placed in group 'a', indicating that berry length did not differ significantly between hybrid combinations. 'Delicia × 4/6' had the shortest berries with 11.51 mm, while 'Simultan × Duke' and 'Delicia × Duke' had the longest berries with 12.67 mm. The largest berry diameters were observed in 'Azur × Duke' and 'Simultan × Duke', with 16.75 mm and 16.62 mm, respectively, classified in group 'a', suggesting that these combinations produced berries with the largest diameter. The smallest diameter was found in 'Azur × 6/38' with 14.43 mm, classified in group 'c', indicating that this combination produces smaller berries compared to the others. The other hybrid combinations, such as 'Azur × Northblue' and 'Delicia × Northblue', showed moderate diameters between 16.30 mm and 16.51 mm, classified in group 'ab'. Significant differences were observed in the 'Azur × Duke' and 'Simultan × Duke' combinations.

The heaviest berries were found in 'Simultan × Duke' and 'Azur × Northblue', weighing 2.19 g and placed in group 'a' of the large fruit size. On the other hand, the smallest

berries were recorded in 'Simultan×Northblue' (1.42 g), placed in group 'c', suggesting that this combination tends to produce smaller fruits. Significant differences were observed in the 'Azur × Northblue' and 'Simultan × Duke' combinations. These were the only combinations of hybrids that differed from the others, except for the 'Delicia × Duke' hybrid combination. With regard to firmness, 'Simultan × Duke' showed the highest firmness, with berries measuring 26.37 HPE units, making it the best performing cultivar; the variety was classified in group 'a', indicating the presence of a superior textural profile in its fruit. On the other hand, the berries of 'Azur × 4/6', 'Delicia × 4/6', and 'Azur × Northblue' (17.31, 15.94, and 15.89 HPE units, respectively) were found to have the softest texture, as indicated by their grouping in 'e'. This finding indicates that these fruits are susceptible to damage and require careful handling and storage to ensure optimum freshness and quality. On average, analysis of the fruit firmness indicator showed that there were significant differences between the 'Simultan × Duke' combination and the other nine hybrid combinations that were studied. Most of the other hybrid combinations, such as 'Delicia × Duke' and 'Azur × Duke', show moderate values for both berry weight and firmness, indicating a balance between size and texture.The CV% values give an indication of the uniformity of berry size and texture (firmness) within each hybrid. Lower CV% values, such as those for 'Simultan × Duke' for both berry weight and firmness, indicate more uniformity in the fruit, which is generally desirable in commercial blueberry production [54]. On the other hand, higher CV% values, such as those for 'Delicia × Northblue' (1.09% for berry weight and 0.96% for firmness), indicate greater variability, which is undesirable for consistent fruit quality.

The physicochemical properties of the analysed fruits, such as total soluble solids, pH, total acidity, and sugar content, are consistent with the published data on blueberry cultivars found in the specialised literature [47,48,50]. 'Duke' and 'Northblue' have TSS values ranging from 11 to 16 °Brix, which is also the range observed in hybrids with these cultivars as parents [47,48]. The measured pH range (3.1–3.9) is typical of blueberry fruits, while variations in total acidity (0.07–0.76%) reflect the characteristics of the cultivars and the effects of hybridisation. Furthermore, the total sugar content is comparable to that reported for the cultivars in the literature [47,48], which highlights the quality of the developed hybrids. The total soluble solids values of the analysed hybrids (11.81–15.82 °Brix) are comparable to the values reported for the 'Duke' and 'Northblue' cultivars (11–16 °Brix) [47,48]. These values are crucial for the fruits' sweetness and marketability. According to Kalt et al. (1999) [50], the fruit's pH level ranges from 3.16 to 3.95, falling within the normal range for blueberries. This suggests that the fruit strikes a balance between acidity and sweetness. Total acidity ranges from 0.03% to 0.76%, comparable to values reported in specialised literature [48]. The 'Duke' and 'Northblue' cultivars have an acidity level ranging from 0.3% to 0.8%. This parameter is important for flavour and fruit preservation. The total sugar content of the hybrid fruits ranged from 3.81 to 11.14 g/100 g, similar to values reported for 'Duke' and 'Northblue' [47]. This confirms the high nutritional and commercial value of the fruits.

The data presented in Table 2 and Figure S2 show notable differences in total soluble solids (TSS) and pH among different F1 blueberry hybrids, reflecting the influence of parental genotypes on these key fruit quality traits. Statistical analysis of the recorded soluble solids values of the fruits from the hybrid combinations revealed significant differences between the 'Simultan × Northblue' combination and all the other hybrid combinations, except 'Delicia × Northblue'. 'Simultan × Northblue' had the highest TSS value (15.82 °Brix), suggesting that it may offer the sweetest fruit among the hybrid combinations. Similarly, 'Delicia × 4/6' (14.67 °Brix) also showed relatively high sweetness, indicating its potential for sweeter fruit production. On the other hand, 'Simultan × Duke' and 'Azur × 4/6' had lower TSS values, resulting in less sweet fruit.The hybrid combination 'Azur × 4/6' had the

highest pH (3.95). In contrast, 'Delicia × Northblue' had the lowest pH (3.16), indicating a more acidic fruit composition, which may contribute to a more acidic flavour profile. The remaining hybrid combinations showed a moderate pH range of 3.3 to 3.68. As can be seen in Table 2, there were no significant differences in pH value between the different hybrid combinations. Overall, the hybrid combinations with 'Simultan' and 'Delicia' as female parents, especially with 'Northblue' or '4/6' as male parents, showed a favourable sweet-sour balance, making them promising candidates for blueberry breeding programmes aimed at optimising fruit flavour.

Table 2. Total soluble solids (TSS),pH, total acidity (TA), and total sugar content (TSC) in F1 blueberry hybrids in different combinations of hybrids according to parents.

Hybrid Combination (Parents ♀× ♂)	TSS, °Brix		pH		TA, %		TSC, g GluE/100 g	
	Mean ± SD	CV%	Mean ± SD	CV%	Mean ± SD	CV%	Mean ± SD	CV%
Azur × 6/38	13.71 ± 0.71 ^{bcd*}	0.27	3.30 ± 0.04 ^{ab}	0.55	0.07 ± 0.06 ^{c}	4.57	8.42 ± 0.57 ^{b*}	0.35
Azur × 4/6	12.41 ± 1.61 ^{cd}	0.28	3.95 ± 1.05 ^{a}	0.52	0.55 ± 0.21 ^{ab}	1.35	8.07 ± 0.68 ^{b}	0.37
Azur × Duke	13.08 ± 1.02 ^{bcd}	0.28	3.51 ± 0.05 ^{ab}	0.53	0.47 ± 0.04 ^{b}	1.42	4.74 ± 0.49 ^{de}	0.45
Azur × Northblue	12.98 ± 1.11 ^{bcd}	0.25	3.68 ± 0.16 ^{ab}	0.53	0.46 ± 0.09 ^{b}	1.35	4.35 ± 0.60 ^{e}	0.46
Delicia × 4/6	14.67 ± 0.59 ^{ab}	0.26	3.46 ± 0.09 ^{ab}	0.54	0.03 ± 0.01 ^{c}	5.29	10.61 ± 0.83 ^{a}	0.33
Delicia × Duke	12.93 ± 1.25 ^{bcd}	0.28	3.29 ± 0.07 ^{ab}	0.55	0.56 ± 0.09 ^{ab}	1.37	3.81 ± 0.64 ^{e}	0.51
Delicia × Northblue	14.20 ± 1.24 ^{abc}	0.79	3.16 ± 0.12 ^{b}	0.96	0.76 ± 0.18 ^{a}	1.30	6.60 ± 0.91 ^{c}	0.85
Simultan × Duke	11.81 ± 0.81 ^{d}	0.29	3.61 ± 0.05 ^{ab}	0.53	0.63 ± 0.15 ^{ab}	1.32	11.14 ± 0.26 ^{a}	0.31
Simultan × Northblue	15.82 ± 1.31 ^{a}	0.26	3.42 ± 0.20 ^{ab}	0.54	0.51 ± 0.14 ^{b}	1.39	5.89 ± 0.83 ^{cd}	0.42

* Means with the same letter are not significantly different at the 5% level.

The one-way analysis of variance (ANOVA) revealed statistically significant differences ($p < 0.05$) among the F1 hybrid combinations for all four analysed biochemical parameters: total soluble solids (TSS), pH, total acidity (TA), and total sugar content (TSC). These results suggest that the genetic combination has a significant influence on the biochemical composition of blueberry fruits. Total sugar content (TSC) and total acidity (TA) showed significant differences between the hybrid combinations (Table 2 and Figure S2), indicating a strong genetic influence on these fruit quality traits. The hybrid combinations with the highest sugar content were 'Simultan × Duke' (11.14 g GluE/100 g) and 'Delicia × 4/6' (10.61 g GluE/100 g). These two combinations belonged to the 'a' significance group and showed significant differences compared to the others. These values underline their ability to produce fruit with a sweet taste, a much sought-after quality for direct consumption. On the other hand, the hybridcombinations 'Delicia × Duke' (3.81 g GluE/100 g) and 'Azur × Northblue' (4.35 g GluE/100 g) had the lowest sugar values, belonging to the 'e' and 'de'groups, respectively, indicating a reduced sweetness profile. For acidity, the highest value was recorded for 'Delicia × Northblue' (0.76%), in group 'a', indicating a tangier flavour profile. Conversely, the lowest acidity values were recorded for 'Delicia × 4/6' (0.03%) and 'Azur × 6/38' (0.07%), both in group 'c', indicating a milder, sweeter flavour profile. In terms of coefficient of variation, TSC values showed minimal variability, indicating consistent performance. Conversely, TA values showed increased variability, particularly in combinations such as 'Delicia × 4/6' and 'Azur × 6/38', which may affect flavour stability in large-scale production.

The values of total polyphenol content (TPC) and total flavonoid content (TFC) of the F1 blueberry hybrids (Table 3 and Figure S3) show that the hybrid combination 'Delicia × 4/6' had the highest values for TPC (807.48 mg GAE/100 g) and TFC (191.53 mg CE/100 g), significantly higher than other hybrid combinations, indicating a greater capacity to accumulate these bioactive compounds. Conversely, the combinations with 'Azur', especially 'Azur × 4/6' and 'Azur × Duke', showed lower values for both TPC

and TFC compared to the others, making them less favourable in terms of these bioactive compounds. Furthermore, the coefficient of variation (CV%) showed minimal variability in most of the combinations, indicating a relatively consistent result. The combination 'Delicia × 4/6' deserves particular attention, as it had a lower CV%, indicating greater stability in total polyphenol and flavonoid content. These results could be valuable for future research aimed at selecting blueberry hybrid combinations with higher content of antioxidant compounds.

Table 3. Total polyphenol content (TPC), total flavonoid content (TFC), total tannin content (TTC), and total anthocyanin content (TAC) in F1 blueberry hybrids in different combinations of hybrids according to parents.

Hybrid Combination (Parents ♀× ♂)	TPC, mg GAE/100 g		TFC, mg CE/100 g		TTC, mg GAE/100 g		TAC, mg C3-GE/100 g	
	Mean ± SD	CV%	Mean ± SD	CV%	Mean ± SD	CV%	Mean ± SD	CV%
Azur × 6/38	374.01 ± 43.65 ^{b*}	0.06	120.86 ± 16.97 ^{bc}	0.09	246.10 ± 37.86 ^{ab}	0.06	122.53 ± 61.06 ^{bc}	0.09
Azur × 4/6	472.89 ± 102.89 ^{b}	0.05	90.56 ± 7.66 ^{c}	0.10	228.27 ± 65.45 ^{b}	0.07	128.09 ± 17.79 ^{bc}	0.09
Azur × Duke	542.62 ± 120.87 ^{ab}	0.05	108.14 ± 20.93 ^{bc}	0.10	326.29 ± 64.19 ^{ab}	0.06	144.20 ± 11.79 ^{abc}	0.08
Azur × Northblue	497.15 ± 126.56 ^{b}	0.05	117.19 ± 15.32 ^{bc}	0.09	394.99 ± 147.75 ^{a}	0.06	234.03 ± 99.26 ^{a}	0.07
Delicia × 4/6	807.48 ± 272.49 ^{a}	0.04	191.53 ± 54.09 ^{a}	0.08	260.39 ± 47.69 ^{ab}	0.07	195.50 ± 35.69 ^{abc}	0.08
Delicia × Duke	536.58 ± 86.17 ^{ab}	0.05	140.08 ± 7.78 ^{b}	0.09	263.09 ± 70.01 ^{ab}	0.06	172.22 ± 29.02 ^{abc}	0.08
Delicia × Northblue	568.01 ± 105.65 ^{ab}	0.66	153.50 ± 25.51 ^{ab}	0.68	258.94 ± 129.52 ^{ab}	0.68	218.44 ± 79.98 ^{ab}	0.68
Simultan × Duke	640.02 ± 278.50 ^{ab}	0.04	152.84 ± 13.30 ^{ab}	0.08	279.68 ± 34.19 ^{ab}	0.06	178.12 ± 24.68 ^{abc}	0.08
Simultan × Northblue	512.14 ± 67.96 ^{ab}	0.05	130.52 ± 10.95 ^{bc}	0.09	374.23 ± 64.57 ^{ab}	0.05	98.39 ± 19.01 ^{c}	0.12

* Means with the same letter are not significantly different at the 5% level.

According to Duncan's multiple range test ($p < 0.05$), the 'Delicia × 4/6' hybrid combination had the highest TPC (807.48 mg GAE/100 g), which was significantly higher than that of most other combinations except for 'Azur × 6/38', 'Azur × 4/6' and 'Azur × Northblue'. The lowest TPC value was found in 'Azur × 6/38' (374.01 mg GAE/100 g). Similar trends were observed in TFC, with 'Delicia × 4/6' again showing the highest value (191.53 mg CE/100 g), which was significantly higher than that of most other combinations except for 'Delicia × Northblue' and 'Simultan × Duke'. The high TPC value observed in the 'Duke' cultivar is widely recognised in the specialised literature as one of its defining characteristics in terms of antioxidant potential. This makes it a valuable benchmark for improving polyphenol content in selection programmes. Similarly, hybrids with 'Duke' as one of the parents have a high concentration of phenolic compounds, making them valuable candidates for breeding programmes that aim to enhance the nutritional value of fruit.

From the perspective of relative values, crossing the 'Duke' cultivar with Romanian genotypes such as 'Simultan' or 'Delicia' produced hybrids with higher levels of anthocyanin. This suggests that the 'Duke' cultivar positively influences the accumulation of anthocyanin pigments in its offspring, thereby improving the nutritional and aesthetic qualities of the fruit. However, the 'Azur × Northblue' combination produced an even higher anthocyanin content (234.03 mg/100 g), despite not involving the 'Duke' cultivar. This highlights the potential of other genetic combinations for selecting this trait. The total polyphenol content (TPC) of the hybrids ranged from 374 to 807 mg GAE/100 g, which is consistent with the reported values for the 'Duke' and 'Northblue' cultivars [47,49]. Furthermore, the high total anthocyanin content (TAC) contributes to the fruits' antioxidant capacity [52].

The biochemical values determined for the 'Duke', 'Northblue', 'Azur', 'Delicia', and 'Simultan' cultivars are consistent with those reported in the literature [47,48,50,55]. For example, the estimated anthocyanin content of 'Duke' is approximately 350 mg C_3-GE/100 g [55], which is comparable to the levels observed in hybrids with 'Duke' as a parent ('Azur ×

Duke' − 144 mg C_3-GE/100 g). 'Northblue', with reported values ranging from 200 to 230 mg C_3-GE/100 g [48], is reflected in 'Azur × Northblue' hybrids, which have a content of 234 mg C_3-GE/100 g, and the phenolic and vitamin C content is also comparable. This confirms the superior quality of hybrids containing these cultivars [47,50]. According to reported RSA values [52], the 'Duke' cultivar's high antioxidant activity is also present in hybrids containing it.

One-way ANOVA revealed statistically significant differences ($p < 0.05$) in total phenolic content (TPC), total flavonoid content (TFC), total tannin content (TTC), and total anthocyanin content (TAC) among the F1 blueberry hybrids. This suggests that genetic combinations have a strong influence on the antioxidant profile of the fruits. The analysis of total tannin content (TTC) and total anthocyanin content (TAC) in F1blueberry hybrids (Table 3 and Figure S3) shows significant variability among the genotypes, highlighting the influence of the parental lines on these biochemical traits. 'Azur × Northblue' was characterised by the highest values for both TTC (394.99 mg GAE/100 g) and TAC (234.03 mg C3-GE/100 g), placing it in the 'a' significance group for both traits. This result indicates that the cross has significant potential for nutritional and functional applications due to its increased levels of tannins and anthocyanins. Significant differences in TTC were only observed between the 'Azur × Northblue' and 'Azur × 4/6' hybrid combinations.However, significant differences in TAC were observed between the 'Azur × Northblue' hybrid and the 'Azur × 6/38', 'Azur × 4/6' and 'Simultan × Northblue' hybrids. The concentration of total anthocyanin was similar to the study by Rossi in 2022 [27], with content ranging from 50.60 ± 11.77 to 322.54 ± 13.71 mg/100 g FW. Conversely, 'Simultan × Northblue' showed an intriguing trade-off: while its TTC was elevated (374.23 mg GAE/100 g), its TAC was the lowest of all hybrid combinations (98.39 mg C3-GE/100 g) and was the only hybrid combination classified in the 'c' group for TAC. This result suggests the possibility of a genetic decoupling between tannin and anthocyanin biosynthesis in this particular combination. The 'Delicia × Northblue' hybrid combination showed intermediate values for both traits, but a high variability (CV = 0.68%), which may affect the consistency and predictability of its performance under different growing conditions.

A one-way analysis of variance (ANOVA), followed by a Duncan multiple range test, revealed significant differences in lycopene, β-carotene, ascorbic acid, and radical scavenging activity (RSA) among the F1 hybrid combinations ($p < 0.05$). These variations emphasise the impact of genetic background on the accumulation of antioxidant compounds in the studied blueberry hybrids.

The evaluation of carotenoid content in F1 blueberry hybrids (Table 4 and Figure S4) revealed statistically significant variations in both lycopene and β-carotene levels among different parental combinations. The hybrid combination 'Delicia × 4/6' showed the highest lycopene content (0.12 mg/100 g), closely followed by 'Delicia × Duke' (0.11 mg/100 g), both grouped in the 'a' significance category, indicating their superior potential for carotenoid accumulation. On the other hand, 'Azur × 4/6' had the lowest lycopene value (0.06 mg/100 g), classified in group "b". In this case, the 'Delicia × 4/6' and 'Delicia × Duke' hybrid combinations showed significant differences compared to the 'Azur × 4/6' combination. For β-carotene, 'Azur × 4/6' had the highest mean value (0.90 mg/100 g), but the large standard deviation and high CV% indicate high variability and reduced stability. On the other hand, 'Delicia × 4/6' combined high β-carotene content (0.22 mg/100 g) with low variability, highlighting it as the most stable and promising genotype for carotenoid enrichment. In the case of β-carotene, values ranged from 0.05 to 0.90 mg/100 g. The 'Azur × 4/6' hybrid combination had the highest β-carotene content. However, this value also had a high standard deviation, indicating greater variability. There were significant differences between the 'Azur × 4/6' combination and the

'Azur × Northblue' combination. Overall, the hybrid combinations with 'Delicia' as the female parent, especially those with 4/6 as the male parent, tended to show improved and more consistent carotenoid profiles. These results are valuable for breeding programmes aimed at improving the nutritional quality of blueberries through increased carotenoid content. 'Delicia × 4/6' has relatively consistent levels of both lycopene and β-carotene, as indicated by its low CV% values, which may be beneficial in achieving a more uniform nutritional profile in the fruit. 'Azur × 4/6', on the other hand, has a greater variability in both lycopene and β-carotene content, which may indicate less predictability. The hybrid combinations 'Azur × Duke' and 'Simultan × Duke' show moderate consistency in carotenoid content, making them potentially stable choices for breeding programmes aiming for balanced carotenoid profiles.

Table 4. Total carotenoid (lycopene and β-carotene) content, ascorbic acid content and DPPH radical scavenging activity (RSA%) in F1 blueberry hybrids in different combinations of hybrids according to parents.

Hybrid Combination (Parents ♀× ♂)	Lycopene, mg/100 g		β-Carotene, mg/100 g		Ascorbic Acid Content, mg/100 g		RSA, %	
	Mean ± SD	CV%	Mean ± SD	CV%	Mean ± SD	CV%	Mean ± SD	CV%
Azur × 6/38	0.09 ± 0.02 ^{ab*}	3.38	0.14 ± 0.06 ^{ab}	2.67	12.54 ± 0.58 ^{a}	0.29	0.19 ± 0.01 ^{b}	2.26
Azur × 4/6	0.06 ± 0.04 ^{b}	5.06	0.90 ± 1.24 ^{a}	1.95	6.44 ± 0.84 ^{cd}	0.35	0.19 ± 0.00 ^{bc}	2.28
Azur × Duke	0.09 ± 0.02 ^{ab}	3.41	0.11 ± 0.04 ^{ab}	2.92	2.04 ± 0.51 ^{e}	0.40	0.20 ± 0.01 ^{ab}	2.27
Azur × Northblue	0.08 ± 0.02 ^{ab}	3.14	0.05 ± 0.02 ^{b}	3.68	7.77 ± 1.24 ^{bc}	0.39	0.19 ± 0.00 ^{bc}	2.27
Delicia × 4/6	0.12 ± 0.01 ^{a}	2.96	0.22 ± 0.02 ^{ab}	2.39	8.71 ± 0.89 ^{b}	0.37	0.18 ± 0.01 ^{d}	2.41
Delicia × Duke	0.11 ± 0.04 ^{a}	3.00	0.13 ± 0.06 ^{ab}	2.88	11.70 ± 0.94 ^{a}	0.33	0.08 ± 0.01 ^{e}	3.72
Delicia × Northblue	0.07 ± 0.03 ^{ab}	2.66	0.14 ± 0.07 ^{ab}	2.04	5.20 ± 0.63 ^{d}	0.88	0.20 ± 0.01 ^{a}	1.89
Simultan × Duke	0.07 ± 0.01 ^{ab}	4.04	0.09 ± 0.02 ^{ab}	3.70	11.87 ± 2.64 ^{a}	0.32	0.18 ± 0.01 ^{cd}	2.35
Simultan × Northblue	0.08 ± 0.03 ^{ab}	3.66	0.09 ± 0.02 ^{ab}	3.39	5.94 ± 0.39 ^{cd}	0.45	0.18 ± 0.00 ^{d}	2.35

* Means with the same letter are not significantly different at the 5% level.

The analysis of ascorbic acid content and DPPH radical scavenging activity (Table 4 and Figure S4) in F1 blueberry hybrids revealed statistically significant differences. The highest ascorbic acid levels were observed in 'Azur × 6/38' (12.54 mg/100 g), 'Simultan × Duke' (11.87 mg/100 g), and 'Delicia × Duke' (11.70 mg/100 g). The three varieties were grouped in significance class "a", suggesting a high potential for use in breeding programmes aimed at increasing ascorbic acid content. The lowest ascorbic acid content was found in 'Azur × Duke' (2.04 mg/100 g), classified in group 'e', indicating a lower nutritional value in terms of ascorbic acid. In general, the hybrid combinations resulting from the combination of 'Delicia' and 'Simultan' as female parents have been observed to produce offspring with higher levels of ascorbic acid. In contrast, 'Azur × Duke' has been shown to produce offspring with significantly lower levels of ascorbic acid. The vitamin C content of the hybrid combinations is consistent with the literature data [50], confirming the superior quality of hybrids including these cultivars. The high antioxidant activity of the 'Duke' cultivar is also reflected in the hybrids containing it, according to reported RSA values [52]. In terms of radical scavenging activity (RSA), the values were relatively consistent among the hybrid combinations, ranging from 0.08% to 0.20%. However, the 'Delicia × Northblue' and 'Azur × Duke' hybrid combinations had significantly higher RSA percentages (both 0.20%), while the 'Delicia × Duke' hybrid combination had the lowest value (0.08%). This suggests that RSA is not necessarily directly correlated with levels of individual antioxidants. According to Duncan's multiple range test, the 'Delicia × Northblue' hybrid combination had the highest RSA value, which was significantly higher than that of most other combinations, with the exception of 'Azur × Duke'. The highest DPPH radical

scavenging activity was recorded in 'Delicia × Northblue' (0.20%), which belongs to group "a", indicating a higher antioxidant potential. In contrast, 'Delicia × Duke' (0.08%) showed the lowest DPPH radical scavenging activity, belonging to group 'e', despite its high ascorbic acid content. This finding indicates that the antioxidant activity may not be directly proportional to the ascorbic acid content, suggesting the potential involvement of other bioactive compounds. The CV% values demonstrate the consistency or variability of ascorbic acid content and the DDPH radical scavenging activity in the diverse F1 blueberry hybrids. Lower values of CV% are indicative of more consistent levels of these nutrients, which could be beneficial for consistent fruit quality. Conversely, higher CV% values are indicative of greater variation, which may be suggestive of diminished predictability in the nutritional composition of the fruit.

The size of the fruit is among the most desirable traits in blueberry breeding, as it directly influences market acceptance and consumer preference. The results presented in Table 5 demonstrate a diverse array of genetic responses, contingent on the specific parental combinations, thus underscoring the potential for cultivar development. The values of heterosis and the broad-sense heritability of some traits demonstrate that, through judiciously choosing parents, blueberry breeding in the desired direction can be successful [54]. When crossed with either 'Duke' or 'Northblue', 'Azur' has been shown to have a significant effect on fruit size elongation. The 'Azur × Northblue' cross exhibited an exceptional heritability of 0.97, suggesting that berry length in this hybrid combination is predominantly governed by additive genetic factors. Heterosis, also known as hybrid vigour, is defined as an increase in performance over the parental varieties. The heterosis values for berry length were predominantly moderate or negative. The 'Azur × 4/6' hybrid combination exhibited a positive heterosis of 9.70%, indicative of hybrid vigour. 'Delicia × Northblue', despite having a high ΔG (22.23), showed negative heterosis (−21.54%), meaning that the hybrid combination performed below the mean of the parental values. Heritability was found to be significant for both the 'Simultan × Duke' (0.86) and 'Simultan × Northblue' (0.84) hybrid combinations. This finding suggests that deliberate selection for berry length in these hybrid combinations may prove to be a productive avenue of research. Strong genetic gains were observed for 'Azur × 4/6' (25.07), 'Simultan × Duke' (22.24), and 'Delicia × Northblue' (18.28). The high values of ΔG in these combinations indicate a considerable potential for increasing fruit diameter. The heritability for diameter was found to be exceptionally high in 'Delicia × Northblue' (0.97) and 'Simultan × Duke' (0.89), reinforcing the importance of additive genetics in these crosses. Positive values for heterosis were observed in 'Delicia × 4/6' (10.25), 'Simultan × Duke' (9.68), 'Azur × 6/38' (5.41), 'Simultan × Northblue' (5.06), and 'Azur × 4/6' (5.06), suggesting true hybrid improvements beyond the parental means. In contrast, 'Azur × Duke' and 'Azur × Northblue' showed negative heterobeltiosis with −10.21 and −11.54, respectively, suggesting a decrease in diameter performance compared to the superior parent. Despite these negative hp values, the relatively high heritability in the same combinations suggests potential for selection if breeding is continued through further generations. 'Azur × Duke' and 'Azur × Northblue' are particularly good for berry length, with high genetic gains and heritability. The 'Simultan' line is known for its ability to produce hybrid combinations with high heritability and positive heterosis, especially for diameter. Overall, these results show that we can make big improvements in plant genetics by choosing the right parents.

Research has demonstrated that two further key characteristics, namely berry weight and firmness, have a direct impact on marketability, consumer appeal and postharvest quality. The fruit weight data presented in Table 6 show significant variation between the hybrid combinations. Similar data were recorded using narrow-sense heritability estimates for yield and quality traits in the CSIRO Table Grape Breeding Pro-

gramme led by Wei et al. (2003) [56]. The highest recorded genetic gain was observed in the 'Azur × Northblue' cross (1.71), indicating a significant potential for fruit size improvement from this particular cross. This was followed by 'Delicia × Northblue' (1.21) and 'Azur × 4/6' (0.91); these values indicate the usefulness of both 'Azur' and 'Delicia' as maternal lines in combination with either '4/6' or 'Northblue' for increasing fruit weight. The high heritability (H^2) values for berry weight, particularly in 'Delicia × 4/6' (0.97), 'Simultan × Duke' (0.84), and 'Azur × 6/38' (0.83), indicate that a substantial proportion of the phenotypic variation in this trait is genetically determined. Consequently, selection for berry weight in early generations could be a very effective strategy for these crosses. However, heterosis values were predominantly negative in most combinations, e.g., 'Azur × 4/6' (−49.67) and 'Delicia × 4/6' (−35.63), indicating that the performance of hybrid combinations was often lower than the average of the two parents. The exceptions are few, but 'Simultan × Duke' is a notable example of positive heterosis, with a recorded increase in berry weight of 8.06% compared to the parental mean.

Table 5. Statistical analysis of the length and diameter of berries in F1 blueberry hybrids in different combinations of hybrids according to parents.

Hybrid Combination (Parents ♀× ♂)	Berry Length, mm				Berry Diameter, mm			
	ΔG	H^2	Heterosis, %	hp	ΔG	H^2	Heterosis, %	hp
Azur × 6/38	15.74	0.53	−20.24	−1.38	10.55	0.23	5.41	0.91
Azur × 4/6	18.29	0.88	9.70	0.53	25.07	0.87	5.06	0.25
Azur × Duke	31.88	0.79	−9.80	−1.75	12.13	0.62	0.54	−10.21
Azur × Northblue	23.14	0.97	0.45	8.25	11.24	0.22	0.44	−11.54
Delicia × 4/6	16.79	0.62	0.58	−5.32	16.91	0.45	10.25	0.45
Delicia × Duke	13.48	0.22	−4.12	2.54	13.12	0.68	−5.21	2.54
Delicia × Northblue	22.23	0.64	−21.54	3.54	18.28	0.97	5.61	2.25
Simultan × Duke	5.80	0.86	−9.80	−1.78	22.24	0.89	9.68	3.26
Simultan × Northblue	0.40	0.84	7.06	2.47	0.30	0.54	5.06	2.47

Table 6. Statistical analysis of weight and firmness of berries in F1 blueberry hybrids in different combinations of hybrids according to parents.

Hybrid Combination (Parents ♀× ♂)	Berry Weight, g				Firmness, HPE Units			
	ΔG	H^2	Heterosis, %	hp	ΔG	H^2	Heterosis, %	hp
Azur × 6/38	0.73	0.83	−30.24	−8.38	12.55	0.32	3.41	0.29
Azur × 4/6	0.91	0.45	−49.67	−15.25	16.64	0.46	7.34	0.87
Azur × Duke	0.81	0.64	−21.54	−5.39	33.82	0.62	9.14	1.35
Azur × Northblue	1.71	0.79	−3.14	−1.16	8.85	0.22	1.27	0.11
Delicia × 4/6	0.88	0.97	−35.63	−65.65	2.13	0.87	−5.09	−1.32
Delicia × Duke	0.41	0.62	−16.62	−2.57	1.23	0.64	−11.55	0.13
Delicia × Northblue	1.21	0.72	−27.97	−5.41	1.79	0.50	−4.43	−0.75
Simultan × Duke	0.40	0.84	8.06	1.47	3.08	0.97	77.97	14.27
Simultan × Northblue	0.71	0.74	−30.94	−4.80	38.04	0.66	10.38	0.31

Heritability estimates of 0.22–0.97 for firmness and 0.45–0.97 for fruit weight suggest a strong genetic basis for selection. These estimates are supported by the findings of Wang et al. (2008) [47], Wang and Stretch (2001) [57], and Montanari et al. (2022) [51]. The presence of a high ΔG value for firmness (up to 38.04) indicates significant genetic variability, which is essential for successful breeding programmes.

Firmness, a key trait for storage and mechanical harvesting, showed some strong results. The most significant genetic gain was identified in the cross 'Simultan × Northblue' (38.04), closely followed by the cross 'Azur × Duke' (33.82). These combinations show

great promise for breeding programmes aimed at improving fruit texture and storability. A noteworthy observation is the remarkably high heterosis for firmness exhibited by 'Simultan × Duke' (77.97%), which also demonstrated the highest heterobeltiosis (14.27). This finding indicates that this hybrid combination outperforms the average performance of its parents and exhibits a substantial margin of superiority over the superior parent. Consequently, 'Simultan × Duke' is considered a prime candidate for breeding firm, high-quality fruit. Other positive values of heterosis for firmness were found in 'Azur × 4/6' (7.34%) and 'Simultan × Northblue' (10.38%), thereby serving to reinforce the role of 'Simultan' as a strong parent for the improvement of texture. In conclusion, the 'Azur × Northblue' and 'Delicia × Northblue' cultivars showed high genetic gains and heritability for berry weight, although heterosis was generally negative, underscoring the necessity of meticulous parental selection to enhance this trait. The 'Simultan × Duke' cross showed a remarkable genetic gain, hybrid vigour and high heritability for firmness, making it a prime target for breeders aiming to enhance firmness.

Statistical analysis of total soluble solids (TSS) and pH in F1 blueberry hybrids (Table 7) provides important insights into the genetic potential of different parental combinations to improve fruit quality. 'Simultan × Northblue' recorded the highest genetic gain (23.66), indicating significant potential for increasing sugar content in the fruit. This was closely followed by 'Azur × Duke' (18.18) and 'Azur × Northblue' (15.50), both of which also showed favourable TSS levels. High TSS is directly related to fruit sweetness and consumer preference, making these combinations particularly valuable in commercial breeding programmes. In addition, the heritability of TSS was found to be moderate to high in a number of crosses, including 'Simultan × Duke' (0.87) and 'Simultan × Northblue' (0.74). This finding suggests that a significant proportion of the variation in the trait is due to additive genetic factors that can be reliably transmitted to future generations. Positive heterosis values, such as those observed in 'Simultan × Northblue' (11.59%) and 'Delicia × Northblue' (3.23%), reflect hybrid vigour and indicate that these progeniesexceed the average of their parents in terms of TSS content.

Table 7. Statistical analysis of (TSS) and pH in F1 blueberry hybrids in different combinations of hybrids according to parents.

Hybrid Combination (Parents ♀× ♂)	TSS, °Brix				pH			
	ΔG	H^2	Heterosis, %	hp	ΔG	H^2	Heterosis, %	hp
Azur × 6/38	5.92	0.36	−5.98	−2.51	0.96	0.59	−7.92	−2.38
Azur × 4/6	10.14	0.49	−14.88	−6.25	1.66	0.45	10.25	3.15
Azur × Duke	18.18	0.61	−2.97	−0.67	2.93	0.68	−5.21	−0.84
Azur × Northblue	15.50	0.38	1.27	0.11	3.58	0.78	11.82	2.26
Delicia × 4/6	15.02	0.69	−4.31	−1.48	2.13	0.87	−5.09	−1.32
Delicia × Duke	11.10	0.65	−10.05	0.10	1.23	0.64	−11.55	0.13
Delicia × Northblue	13.42	0.44	3.23	0.22	1.79	0.50	−4.43	−0.75
Simultan × Duke	5.54	0.87	−20.36	−1.69	3.08	0.97	77.97	14.27
Simultan × Northblue	23.66	0.74	11.59	0.68	1.86	0.59	2.32	0.34

The highest genetic gain for pH was found in the cross 'Azur × Northblue' (3.58), closely followed by the cross 'Simultan × Duke' (3.08) and the cross 'Azur × Duke' (2.93). In particular, 'Simultan × Duke' showed an exceptional performance with a heterosis value of 77.97% and a remarkably high heritability of 0.97, confirming the strong genetic control of this trait in the hybrid combination. The strong positive heterosis for pH in 'Azur × Northblue' (11.82%) further supports the effectiveness of using 'Northblue' as a parent for improving acidity balance, which is desirable for both taste and storage. In con-

trast, some crosses showed negative heterosis, such as 'Azur × 4/6' and 'Delicia × Duke', indicating that not all combinations are equally beneficial for these traits. In conclusion, the 'Simultan × Northblue' hybrid combination was shown to have superior performance for TSS, while the 'Simultan × Duke' cross had a significantly higher pH. The observation of high heritability in many crosses underlines the capacity for effective selection and genetic improvement.

As shown in Table 8, the genetic variability in total sugar content (TSC) and total acidity (TA) among F1 blueberry hybrids derived from different parental crosses is evident. The highest genetic gain (ΔG) values for TSC were observed in the 'Simultan × Duke' (20.66) and 'Delicia × 4/6' (15.13) hybrid combinations, suggesting a high potential for sugar improvement in these hybrid combinations. Heritability (H^2) was increased in most combinations, particularly in 'Azur × 4/6' (0.98), 'Delicia × 4/6' (0.97), and 'Azur × 6/38' (0.96), indicating a significant genetic contribution to sugar accumulation. Positive heterosis was found in 'Delicia × 4/6' (107.79%) and 'Simultan × Duke' (74.65%), indicating a significant level of hybrid vigour in these combinations. Heterotic progress (hp) was negative in the majority of hybrid combinations, including 'Delicia × 4/6' (−1.14) and 'Azur × Northblue' (−1.16), suggesting that despite high heterosis, improvement over the better parent may be limited or even regressive in some cases.The cross 'Simultan × Duke' had the highest heterotic progress (7.97) for TSC, establishing its value as a selection for targeted sugar improvement. The genetic gain (ΔG) values for TA were generally minimal, indicating a limited response to selection. The highest value (0.14) was observed in Delicia × Northblue, although this value was modest. Heritability (H^2) showed a range from moderate to low, with a few exceptions such as 'Azur × 6/38' (0.84) and 'Azur × Duke' (0.53). This finding suggests that environmental factors may have a more significant influence on acidity expression. The hybrid combination 'Simultan × Duke' has a very high TSC (20.66 mg GluE/100 g), suggesting positive transgressivity, exceeding the values of the parents.

Table 8. Statistical analysis oftotal sugar content (TSC) and total acidity (TA) in F1 blueberry hybrids in different combinations of hybrids according to parents.

Hybrid Combination (Parents ♀× ♂)	TSC, mg GluE/100 g				TA, %			
	ΔG	H^2	Heterosis, %	hp	ΔG	H^2	Heterosis, %	hp
Azur × 6/38	11.16	0.96	−3.92	−0.78	0.05	0.84	−91.84	−7.42
Azur × 4/6	11.66	0.98	27.55	0.88	0.02	0.30	−29.61	−2.25
Azur × Duke	2.45	0.30	−32.87	−1.97	0.04	0.53	−24.88	−0.68
Azur × Northblue	5.61	0.60	−33.41	−1.16	0.07	0.47	−51.33	−8.59
Delicia × 4/6	15.13	0.97	107.79	−1.14	0.04	0.30	−95.41	−8.83
Delicia × Duke	0.82	0.22	−34.66	−68.63	0.01	0.22	−12.68	−0.37
Delicia × Northblue	1.45	0.10	26.09	−0.29	0.14	0.28	−18.55	−2.27
Simultan × Duke	20.66	0.64	74.65	7.97	0.01	0.30	0.99	0.03
Simultan × Northblue	2.01	0.22	1.67	0.08	0.03	0.35	−44.94	−5.01

The majority of the hybrid combinations had a negative heterosis value for TA, especially 'Delicia × 4/6' (−95.41%), 'Azur × 6/38' (−91.84%), and 'Azur × Northblue' (−51.33%), which is advantageous if lower acidity is desired. Heterotic progress (hp) was predominantly negative, confirming the idea that acidity tends to decrease in hybrid combinations. However, 'Simultan × Duke' showed a slightly positive hp (0.03), although ΔG was minimal. The most promising hybrid combination overall is 'Simultan × Duke', which shows the highest values for genetic gain and heterosis and heterotic progress in TSC, while maintaining a balanced acid profile. 'Delicia × 4/6' showed a high potential for sugar

accumulation, but its heterotic progress was negative compared to the superior parent. This may be due to the very strong initial characteristics of the superior parent. In general, sugar content seems to be a highly heritable trait, whereas acidity is more environmentally influenced and shows less genetic responsiveness. Negative heterosis and hp for TA in most hybrid combinations indicate that breeding can effectively reduce acidity, a desirable trait for improving fruit palatability.

Table 9 shows that the highest values of genetic gain (ΔG) for TPC were recorded in the combinations 'Delicia × 4/6' (12,635.23) and 'Azur × Duke' (5885.38), showing that there is a high potential for phenolic accumulation through genetic improvement. Heritability (H^2) reached its highest values (0.99) in the combinations with 'Delicia' and 'Simultan', indicating a strong genetic influence and a high efficiency of selection. The highest positive hybrid vigour was observed in 'Delicia × 4/6' (144.58%), showing a large increase in phenolic content. Heterotic progress (hp) was notably positive in 'Delicia × Duke' (13.96), making this cross particularly promising for breeding programmes aimed at increasing polyphenol content. The highest ΔG values for TFC were observed in 'Delicia × 4/6' (1242.42) and 'Delicia × Northblue' (882.59), showing a strong genetic capacity to improve flavonoid content. The values for how well these traits can be passed on from parent to offspring were also high (0.99 and 0.95, respectively), confirming that these traits are reliably inherited. However, in contrast to TPC, heterosis for TFC was often negative, especially in combinations such as 'Azur × 4/6' (−51.81%) and 'Azur × Duke' (−15.64%), suggesting a more complex genetic control of flavonoid content. However, the positive hp values in 'Delicia × Duke' (12.85) and 'Delicia × Northblue' (1.72) show that these hybrid combinations could be further improved by selection. The parental genotype 'Delicia' was found to be a superior contributor for both TPC and TFC, showing consistently high values for ΔG, H^2 and positive heterosis. The high heritability observed across several combinations is indicative of strong genetic determinism and supports the hypothesis that selection is effective in early generations. The substantial heterogeneity observed in heterosis and heterotic progress across diverse hybrid combinations underscores the significance of precise parental pairing, underscoring the necessity for targeted breeding strategies contingent upon the targeted trait (e.g., phenolic content, antioxidant potential, or stress resistance).

Table 9. Statistical analysis of total phenolic content (TPC) and total flavonoid content (TFC) in F1 blueberry hybrids in different combinations of hybrids according to parents.

Hybrid Combination (Parents ♀× ♂)	TPC, mg GAE/100 g				TFC, mg CE/100 g			
	ΔG	H^2	Heterosis, %	hp	ΔG	H^2	Heterosis, %	hp
Azur × 6/38	1663.66	0.64	−0.72	−0.10	326.74	0.46	−17.42	−0.12
Azur × 4/6	4284.15	0.65	52.41	3.32	151.76	0.34	−51.81	−2.62
Azur × Duke	5885.38	0.64	43.59	8.85	449.82	0.51	−15.64	−8.73
Azur × Northblue	3326.18	0.64	12.54	0.67	575.86	0.64	−15.44	−2.64
Delicia × 4/6	12,635.23	0.99	144.58	6.35	1242.42	0.99	10.93	0.40
Delicia × Duke	3842.13	0.78	32.20	13.96	370.84	0.59	8.69	12.85
Delicia × Northblue	3450.63	0.54	20.92	−1.80	882.59	0.95	11.92	1.72
Simultan × Duke	4960.18	0.99	18.60	0.69	355.90	0.99	0.82	0.05
Simultan × Northblue	4990.09	0.77	−15.59	0.22	542.61	0.63	−19.77	−2.01

As shown in Table 10, the variability of TTC and TAC in F1 blueberry hybrids is evident. The highest genetic gains (ΔG) for TTC were recorded in 'Azur × 6/38' (2800.99), 'Azur × Duke' (2706.44), and 'Simultan × Duke' (2743.21), suggesting that these crosses have strong genetic potential for improving tannin accumulation. Heritability (H^2) values

were consistently high in most combinations, particularly in 'Simultan × Duke' (0.99) and 'Delicia × 4/6' (0.93), indicating reliable trait inheritance and high selection efficiency. Positive heterosis was observed in the following crosses: 'Azur × Northblue' (64.84%), 'Delicia × 4/6' (42.36%), and 'Azur × 4/6' (40.17%), indicating beneficial hybrid effects in these crosses. The highest heterotic progress (hp) for TTC was observed in 'Azur × Northblue' (4.02) and 'Delicia × 4/6' (3.42), confirming their breeding value for increasing tannin content.

Table 10. Statistical analysis of total tannin content (TTC) and total anthocyanin content (TAC) in F1 blueberry hybrids in different combinations of hybrids according to parents.

Hybrid Combination (Parents ♀× ♂)	TTC, mg GAE/100 g				TAC, mg C3-GE/100 g			
	ΔG	H^2	Heterosis, %	hp	ΔG	H^2	Heterosis, %	hp
Azur × 6/38	2800.99	0.82	−5.75	−0.32	737.34	0.82	−43.49	−1.19
Azur × 4/6	956.13	0.41	40.17	1.72	836.67	0.98	−39.97	−1.03
Azur × Duke	2706.44	0.66	8.75	0.27	1580.01	0.99	−31.95	−0.80
Azur × Northblue	2149.43	0.60	64.84	4.02	954.90	0.51	12.53	0.30
Delicia × 4/6	1799.84	0.93	42.36	3.42	1350.21	0.93	13.24	0.54
Delicia × Duke	1050.68	0.75	−13.32	−0.42	531.95	0.59	−0.51	−0.02
Delicia × Northblue	601.13	0.95	6.70	0.46	1139.90	0.95	30.57	1.08
Simultan × Duke	2743.21	0.99	−12.09	−0.35	488.83	0.99	−13.26	−0.35
Simultan × Northblue	542.61	0.63	−19.77	−2.01	523.35	0.69	−32.69	−0.35

The highest ΔG values for TAC were found in 'Azur × Duke' (1580.01), 'Delicia × 4/6' (1350.21) and 'Delicia × Northblue' (1139.90), indicating a strong selection potential in these combinations. Heritability peaked in 'Azur × 4/6' (0.98), 'Azur × Duke' (0.99) and 'Simultan × Duke' (0.99), indicating a dominant genetic component in the control of anthocyanin levels. Negative heterosis was frequently observed, especially in 'Azur × 6/38' (−43.49%), 'Azur × 4/6' (−39.97%), 'Azur × Duke' (−31.95%), and 'Simultan × Northblue' (−32.69%), which could indicate non-additive genetic effects or unfavourable parental interactions. On the other hand, 'Delicia × Northblue' (30.57% and 1.08) and 'Azur × Northblue' (12.53% and 0.30) showed positive heterosis and hp, making them promising crosses for improving anthocyanin content. Furthermore, the 'Azur'-based hybrid combinations showed both increased genetic gains and heritability, especially in crosses with 'Duke' and 'Northblue', suggesting considerable potential for improving tannin and anthocyanin content. 'Delicia × 4/6' and 'Delicia × Northblue' showed a balanced performance in all parameters, in particular their consistently high heritability and positive heterotic effects. Despite the high genetic gain and heritability for tannin content shown by 'Simultan × Duke', heterosis and hp were found to be negative, which may limit its usefulness without further selection strategies. Heritability was found to be high in the majority of crosses, indicating the feasibility of improving traits by selection. However, heterotic performance showed significant variation, highlighting the need for precise parental combinations.

Table 11 shows that the highest ΔG values were observed in the cross 'Delicia × 4/6' (0.02) for both lycopene and β-carotene, suggesting a potential for genetic improvement in this hybrid combination. However, the ΔG values for most combinations were quite low, indicating relatively limited genetic gain for carotenoid content in the hybrid combinations. The heritability (H^2) for lycopene was found to be moderate to high, with notable values observed in 'Azur × 4/6' (0.85), 'Simultan × Duke' (0.83) and 'Delicia × 4/6' (0.75). These results suggest that lycopene is under strong genetic control, which means that it is more likely to be passed on to subsequent generations through selection. Heterosis showed negative values for the majority of the hybrid combinations. This is illustrated by the

hybrid combinations 'Azur × 6/38' (−56.50) and 'Simultan × Northblue' (−57.80) for lycopene, 'Azur × Northblue' (−91.21) and 'Simultan × Duke' (−85.51) for β-carotene and indicates that in many cases the performance of the hybrid combination for carotenoid content is below the parental average. This may provide a valuable opportunity to select for hybrid combinations with improved expression of these traits.

Table 11. Statistical analysis oftotal carotenoids in F1 blueberry hybrids in different combinations of hybrids according to parents.

Hybrid Combination (Parents ♀× ♂)	Lycopene, mg/100 g				β-Carotene, mg/100 g			
	ΔG	H^2	Heterosis, %	hp	ΔG	H^2	Heterosis, %	hp
Azur × 6/38	0.01	0.49	−56.50	−3.07	0.04	0.83	−73.11	−4.40
Azur × 4/6	0.01	0.85	−52.76	−167.84	0.15	0.47	76.17	4.22
Azur × Duke	0.00	0.51	−55.38	−3.25	0.03	0.72	−74.25	−3.49
Azur × Northblue	0.02	0.52	−65.71	−19.22	0.11	0.77	−91.21	−20.15
Delicia × 4/6	0.02	0.75	−43.78	−1.73	0.02	0.75	−43.78	−1.73
Delicia × Duke	0.01	0.50	−46.95	−2.22	0.02	0.64	−68.70	−5.90
Delicia × Northblue	0.18	0.57	−69.42	−0.58	0.32	0.66	−73.58	−30.49
Simultan × Duke	0.01	0.83	−60.44	−51.13	0.01	0.83	−85.51	−2.47
Simultan × Northblue	0.01	0.57	−57.80	−4.35	0.04	0.76	−80.24	−4.74

The study conducted by Hera et al. (2023) [1] highlighted that hybridisationbetween the 'Simultan' and 'Duke' cultivars can lead to significant increases in carotenoid content, such as lycopene and β-carotene, through the manifestation of positive heterosis. These results are consistent with the observations in the present research, where positive heterosis for β-carotene was identified only in the 'Azur × 4/6' combination, showing an increase of 76.17%. The other analysed combinations generally exhibited negative heterosis values, emphasising the importance of rigorous parent selection to obtain hybrids with superior carotenoid content.

The cross combination 'Azur × 4/6' showed a significant positive heterosis for β-carotene (76.17%), suggesting that this cross may have a significant advantage in terms of carotenoid content, especially β-carotene. Heterotic progress (hp) values were predominantly negative, especially for lycopene in combinations such as 'Azur × Northblue' (−19.22) and 'Delicia × Duke' (−68.70). This suggests that many hybrid combinations show a regression in carotenoid content compared to the best parent, especially for lycopene. The 'Delicia × 4/6' combination was identified as a particularly promising hybrid combination, showing significant increases in ΔG for both lycopene and β-carotene, accompanied by favourable heritability (H^2) and consistent heterotic progress (hp). 'Azur × 4/6' also appears to have potential for β-carotene improvement based on positive heterosis (76.17%) and positive hp (4.22), making it a strong candidate for selection. The presence of negative heterosis and negative hp in numerous combinations of hybrids indicates the need for more targeted breeding programmes to maximise carotenoid content. It is important to note that although some hybrid combinations showed negative heterosis and hp, this may be related to the stability of the traits. The results indicated that the hybrid combinations could not outperform their parental varieties in terms of carotenoid content. However, there was evidence that they showed consistency or resilience in the expression of lycopene and β-carotene content.

As shown in Table 12, the highest values of ΔG for ascorbic acid were found in 'Delicia × Duke' (15.32) and 'Azur × 6/38' (13.89), indicating a significant genetic improvement. 'Delicia × 4/6' and 'Azur × Northblue' also showed a comparatively high ΔG (11.94 and 10.75, respectively), indicating a good potential for increased ascorbic acid

content in the hybrid combinations progeny. For RSA%, the highest ΔG values were observed in 'Delicia × Northblue' (0.16) and 'Azur × Duke' (0.05), indicating a modest increase in antioxidant activity. The ΔG values for RSA% were very low (0.01) for both 'Simultan × Duke' and 'Delicia × Duke', indicating a minimal increase in antioxidant capacity due to these combinations.

Table 12. Statistical analysis of ascorbic acid content and DPPH radical scavenging activity (RSA%) in F1 blueberry hybrids in different combinations of hybrids according to parents.

Hybrid Combination (Parents ♀× ♂)	Ascorbic Acid Content, mg/100 g				RSA, %			
	ΔG	H^2	Heterosis, %	hp	ΔG	H^2	Heterosis, %	hp
Azur × 6/38	13.89	0.70	29.67	1.06	0.03	0.84	−0.54	−0.33
Azur × 4/6	8.98	0.72	19.68	2.98	0.04	0.96	−3.88	−9.61
Azur × Duke	7.20	0.53	−2.41	−0.04	0.05	1.00	−5.73	−2.11
Azur × Northblue	10.75	0.54	1.90	0.03	0.04	0.70	−4.81	20.39
Delicia × 4/6	11.94	0.96	132.39	27.11	0.02	0.82	10.52	0.56
Delicia × Duke	15.32	1.00	221.58	25.94	0.01	0.91	−23.86	−0.51
Delicia × Northblue	4.83	0.61	47.25	3.04	0.16	0.44	2.90	1.88
Simultan × Duke	6.54	0.86	448.99	75.84	0.01	0.86	−9.38	−2.23
Simultan × Northblue	7.71	1.00	142.25	8.55	0.04	0.96	−8.52	−5.20

Analysis of the ascorbic acid content of blueberry hybrids revealed significant positive heterosis potential, particularly in the 'Simultan × Duke' (448.99%), 'Delicia × Duke' (221.58%), and 'Delicia × 4/6' (132.39%) combinations. These results suggest that hybridisation favourably affects the concentration of vitamin C in fruits, which is important for their nutritional quality and resistance to oxidative stress [50,58]. However, most hybrids exhibited negative heterosis values for antioxidant activity as measured by DPPH radical scavenging activity (RSA%), except for the 'Azur × Northblue' combination, which showed a slight increase of +20.39%. This discrepancy suggests that total antioxidant activity is influenced by factors other than ascorbic acid content, such as the presence of other bioactive compounds, including polyphenols and anthocyanins, which may act synergistically [59]. High heritability indices for ascorbic acid (up to 1.00) confirm that this trait is genetically highly transmissible, offering favourable prospects for breeding programmes aimed at enhancing the fruit's nutritional value and antioxidant activity [53].

4. Conclusions

Analysis of F_1 hybrids derived from different blueberry parental combinations revealed significant genetic variability for bioactive compounds and fruit quality traits. From a morphological perspective, the hybrid combinations generally showed moderate variation in berry size (length and diameter), with 'Azur × Duke' and 'Simultan × Duke' producing the largest fruits, which may increase market appeal.

Among all combinations, hybrid combinations with 'Delicia' and 'Simultan' as female parents, especially when crossed with '4/6' or 'Northblue', consistently showed superior performance in several key parameters. In particular, 'Delicia × 4/6' stood out with the highest concentrations of polyphenols, flavonoids and carotenoids (both lycopene and β-carotene), as well as elevated sugar levels and relatively low acidity, indicating its strong potential for developing antioxidant-rich, sweet-tasting fruits suitable for fresh consumption. In addition, its low coefficients of variation for most traits suggest a high degree of stability and predictability, traits of great value for commercial cultivation and breeding programmes.

Hybrid combinations with 'Azur', especially 'Azur × 4/6' and 'Azur × Duke', showed strong potential for increasing anthocyanin and β-carotene content, although traits such as total acidity and DPPH radical scavenging activity showed more complex inheritance patterns with variable heterosis. The 'Azur × Northblue' hybrid combination also showed promising results, especially for total anthocyanin and tannin content, but with a less favourable profile for sugar and ascorbic acid. On the other hand, 'Simultan × Duke' was characterised by the highest firmness and weight of berries, together with high sugar content and a favourable balance between antioxidants and acidity, making it an excellent candidate for mechanical harvesting and long shelf-life applications. Interestingly, the analysis showed that DPPH radical scavenging activity does not always correlate directly with ascorbic acid content, as seen in 'Delicia × Duke', which had high ascorbic acid levels but low antioxidant capacity. This suggests that other bioactive compounds may play a more important role in contributing to the overall antioxidant potential of the fruit.

In general, hybrid combinations with lower CV% were preferred for traits such as polyphenol, flavonoid and carotenoid content, while other hybrid combinations with higher CV% showed more variability in important traits such as sugar content, acidity and fruit size.

In summary, 'Delicia × 4/6' emerges as a top candidate for breeding programmes aiming for higher polyphenol, flavonoid and carotenoid content with relatively consistent fruit characteristics. However, 'Simultan × Duke' also shows strong performance for fruit quality traits such as size and firmness, and 'Azur × Northblue' stands out for tannin and anthocyanin content. These results can help breeders to select hybrid combinations that meet specific nutritional and commercial objectives, underline the importance of parental selection in breeding programmes and suggest that specific combinations, such as 'Delicia × 4/6' and 'Simultan × Duke', can be effectively used to develop superior cultivars that combine high nutritional value with fruit firmness. In conclusion, the study highlights the importance of targeted hybridisation strategies in blueberry breeding programmes by identifying and combining parent lines with complementary traits to improve both the nutritional quality and agronomic performance of future cultivars.

The comparative results highlight the strong potential of the tested hybrid combinations to produce fruit of a quality comparable to or superior to that of the parent varieties and other commercial cultivars. Hybrid combinations that recorded high values for biochemical and morphometric parameters, such as 'Delicia × 4/6' and 'Simultan × Duke', show great promise as candidates for the development of highly nutritious, commercially valuable crops.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/horticulturae11060630/s1, Figure S1: Location of the hybrid field from Research Institute for Fruit Growing Pitesti-Maracineni; Figure S2: Total soluble solids (a), pH (b), total sugar content (c) and total acidity (d) in F1 blueberry hybrids in different combinations of hybrids according to parents; Figure S3: Total polyphenol content (a), total flavonoid content (b), total tannin content (c) and total anthocyanin content (d) in F1 blueberry hybrids in different combinations of hybrids according to parents; Figure S4: Lycopene content (a), β-carotene content (b), acorbic acid content (c), and DPPH radical scavenging activity (d) in F1 blueberry hybrids in different combinations of hybrids according to parents.

Author Contributions: Conceptualisation, O.H. and M.S.; methodology, O.H. and L.E.V.; software, O.H. and L.E.V.; validation, O.H., M.S. and L.E.V.; formal analysis, O.H.; investigation, O.H. and L.E.V.; resources, O.H. and L.E.V.; data curation, M.S. and L.E.V.; writing—original draft preparation, O.H.; writing—review and editing, O.H., M.S. and L.E.V.; visualisation, O.H., M.S. and L.E.V.; supervision, M.S. and L.E.V.; project administration, O.H. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

Conflicts of Interest: The authors declare no conflicts of interest.

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