Study on the impact of ploidy levels in shaping morphological and biochemical traits in haskap (*Lonicera caerulea* L. subsp. *edulis*)

（倍数性レベルがハスカップ（*Lonicera caerulea* L. subsp. *edulis*）の
形態学的および生化学的形質に及ぼす影響に関する研究）

By LI Jixiao

A thesis submitted in partial fulfillment of the requirements for the degree of
Doctor of Philosophy in the subject of biosphere science

The Graduate School of Environmental Science Hokkaido University
September 2024

Table of contents

List of tables vi
List of figures vii
List of abbreviations viii
Chapter 1 General introduction 9
1.1 Overview of haskap 9
1.2 Importance of ploidy in plant biology 10
1.3 Ploidy variation in haskap and its morphological effects 11
1.4 Biochemical characteristics of haskap 12
1.5 Research gap and objectives 13
1.6 Methodological overview 15
1.7 Research significance 16
Chapter 2 Morphological and general quality oarameters of different polyploid haskap fruits 21
2.1 Introduction to morphological studies 21
2.2 Materials and methods 22
2.2.1 Plant material and fruit collection 22
2.2.2 Morphological measurement of fruits 23
2.2.3 Measurement of fruit quality parameters 25
2.2.4 Statistical analysis 26
2.3 Results 27
2.3.1 General analysis of phenotypic traits and quality variability 27
2.3.2 Comparative analysis of morphological and quality characteristics 28
2.3.3 Correlation analysis of traits and quality across different ploidy levels of haskap 29
2.3.4 Principal component analysis 31
2.4 Discussion 32
Chapter 3 The impact of ploidy level on biochemical content accumulation in haskap 41
3.1 Introduction to biochemical studies 41

3.2 Materials and methods 46
3.2.1 Plant material and fruit collection methods 46
3.2.2 Chemicals and reagents overview 47
3.2.3 Sample preparation and extraction methodology 48
3.2.4 Derivatization process 49
3.2.5 Gas chromatography and mass spectrometry analysis 50
3.2.6 Statistical analysis 51
3.3 Results 52
3.3.1 Identification of bioactive compounds in haskap berries 52
3.3.2 Analysis of sugar and sugar alcohol composition in haskap berries across different ploidy levels 52
3.3.3 Detailed examination of organic acid composition in haskap berries 54
3.3.4 Exploring the relationship between ploidy levels and biochemical profiles 55
3.4 Discussion 56
3.4.1 Influence of ploidy level on sugar and sugar alcohol profiles 56
3.4.2 Influence of ploidy on organic acid profiles 59
3.4.3 Exploring the dynamics between ploidy levels and biochemical profiles 61
Chapter 4 General discussion and future outlook 68
4.1 Impact of ploidy on morphological traits 68
4.2 Influence of ploidy on sugar biosynthesis 69
4.3 Relationship between ploidy and organic acid content 71
4.4 Biochemical composition of haskap and comparison with other fruits 72
4.5 Implications for breeding and cultivation 74
4.6 Expanding the implications of ploidy research in haskap cultivation and beyond 75
References 78
Summary 90
Appendices 93
Appendix A 93

Appendix B 95
Appendix C 95
Appendix D 96
Appendix E 98
Appendix F 100
Acknowledgements 101

List of tables

Table 1. Heteromorphosis of fruit phenotypic and quality characters for ploidy haskap. 37
Table 2. Measurement of morphological and mass characteristics of haskap with different ploidy. 38

List of Figures

Fig. 1. Haskap trees (flowering period) growing in the field at Hokkaido University. 18
Fig. 2. Close-up of haskap fruits on the trees at Hokkaido University. 19
Fig. 3. Morphology of haskap leaves (up) and fruits (down) with different ploidy. 20
Fig. 4. Correlation matrix diagram for 12 haskap fruit trait analysis. 39
Fig. 5. PCA loading plots (up) and scores (down) for phenotypic and quality parameters of different haskap fruits. 40
Fig. 6. Gas chromatogram and mass spectra for sample P4-37. Gas chromatogram (A), and mass spectra of fructose (B), glucose (C), mannose (D), sucrose (E), galactose (F), sorbitol (G), malic acid (H), citric acid (I), and quinic acid (J) are shown. 63
Fig. 7. Content of the mannose (A, B), fructose (C, D), and glucose (E, F) in different ploidy haskap samples collected in 2021 and 2022 in unit of FW (A, C, E) and CPF (B, D, F). 64
Fig. 8. Content of the galactose (A, B), sucrose (C, D), and sorbitol (E, F) in different ploidy haskap samples collected in 2021 and 2022 in unit of FW (A, C, E) and CPF (B, D, F). 65
Fig. 9. Content of malic acid (A, B), citric acid (C, D), and quinic acid (E, F) in different ploidy haskap samples collected in 2021 and 2022 in unit of FW (A, C, E) and CPF (B, D, F). 66
Fig. 10. Analysis of the linear regression between chromosome number and biochemical substance. (A) mannose, (B) fructose, (C) glucose, (D) galactose, (E) sucrose, (F) sorbitol, (G) malic acid, (H) citric acid, and (I) quinic acid. 67

List of Abbreviations

ANOVA	One-way analysis of variance
CPF	Content per individual fruit
CV	Coefficient of variation
EI-MS	Electron ionization mass spectrometry
FW	Fresh weight
GC-MS	Gas Chromatography-Mass Spectrometry
HPLC	High-performance liquid chromatography
MSTFA	N-methyl-N-(trimethylsilyl) trifluoroacetamide
PC	Principal component
PCA	Principal component analysis
RI	Retention indices
SI	Shape Index

Chapter 1
General introduction

1.1 Overview of haskap

Haskap (*Lonicera caerulea* L. subsp. *edulis*), commonly referred to as blue honeysuckle, is a member of the Caprifoliaceae family. This deciduous shrub is celebrated for its resilience and the distinctive elongated fruits it produces, which resemble blueberries but with unique characteristics. Typically reaching heights of 1.5 to 2 m, the plant is adorned with oval leaves and striking yellow-white flowers that bloom profusely (Fig. 1). The berries are particularly noted for their deep blue-purple color, adding a visual appeal that complements their unique taste—a perfect blend of tangy and sweet flavors that tantalize the palate (Fig. 2). Beyond their ornamental value, these berries are laden with essential nutrients, including a rich array of vitamins, minerals, and antioxidants, which underscore their significant health benefits, enhancing their appeal in nutritional and health-focused circles (Celli et al., 2014; Rupasinghe et al., 2018).

Originating from the cool temperate zones of the Northern Hemisphere, specifically from regions in Canada, Russia, and Japan, the haskap is perfectly adapted to endure cold winters and short growing seasons. Its cultivation has now spread extensively across the United States, Canada, and parts of Europe, driven by its hardiness and the health benefits of its fruits. This expansion is supported by the ability of the plant to adapt to various environmental conditions, thus broadening its agricultural and nutritional footprint globally (Zhu et al., 2023). In terms of cultivation, haskap prospers in well-drained, fertile soils with a pH ranging from slightly acidic to neutral. It requires a good amount of sunlight, although it can grow in partial shade, which makes it versatile for various farming setups. The propagation of this shrub is commonly achieved through cuttings or seeds. In a commercial context, the plant requires minimal maintenance once established and is frequently arranged in rows to enhance production efficiency. The berries ripen early in the summer, providing a timely harvest especially beneficial in regions where the growing season is notably brief. The choice of cultivar, local growing conditions, and the maturity of the plants are pivotal in achieving optimal yields (Fujita et al., 2020).

From an agricultural perspective, haskap is prized for its early ripening, ease of

cultivation, and natural resistance to many pests and diseases. Nutritionally, the berries are remarkably rich in vitamin C and boast various antioxidants and phytochemicals, contributing to their anti-inflammatory and cardioprotective properties. These traits have led to their inclusion in a diverse array of culinary products, ranging from fresh consumption to their use in jams, juices, and desserts (Gołba et al., 2020).

Extensive scientific research has been devoted to exploring the genetic diversity of the haskap, which is crucial for developing breeding programs and conservation efforts. Detailed studies on the phytochemical makeup of the berries highlight their significant antioxidant capacity and nutritional value. Current breeding efforts are focused on producing new cultivars with enhanced features such as a larger berry size, improved flavors, and increased yields, signaling a promising future for haskap in both residential gardens and large-scale agricultural operations (Bieniek, 2021).

1.2 Importance of ploidy in plant biology

Ploidy refers to the number of complete sets of chromosomes within a cell nucleus, a crucial factor in defining the genetic framework of plants. This genetic attribute is not merely foundational to their biology but also plays a fundamental role in their evolutionary processes and biodiversity. In the plant kingdom, ploidy levels range from diploidy, involving two sets of chromosomes, to polyploidy, which includes multiple sets. Polyploidy, in particular, has been a dynamic force in the evolution of plants, driving the emergence of new species and facilitating adaptation to various ecological niches. Both natural evolutionary processes and deliberate human interventions in breeding programs can induce polyploidy, enhancing the variability of traits and increasing agricultural value (Otto & Whitton, 2000).

The exploration of ploidy began in earnest in the early 20th century and quickly established itself as a cornerstone of plant genetics. Renowned scientists such as Barbara McClintock (1939) pioneered studies on chromosome duplication, significantly advancing the understanding of genetic variability and its practical applications. This groundbreaking work has propelled ongoing research and innovation in the field of plant breeding, deepening our understanding of plant evolution, genetics, and cultivation techniques, and continually expanding the potential for agricultural development (Soltis & Soltis, 2000). Alterations in ploidy levels can have profound effects on plant

morphology, affecting the size, shape, and color of leaves, flowers, and fruits. Typically, an increase in ploidy leads to larger cell sizes, which may contribute to more robust and vigorous plant structures. These morphological changes are not only of academic interest but also have substantial practical implications, particularly in enhancing the adaptability of a plant to diverse environmental conditions and improving its agricultural productivity (Levin, 1983). Beyond the visible traits, variations in ploidy affect internal biochemical processes, altering the synthesis and accumulation of various compounds critical for plant growth, defense, and development. These biochemical changes are especially significant from nutritional and medicinal viewpoints, as they influence the health benefits of plants and their capacity for disease prevention, ultimately affecting their overall utility to humans (Chen, 2007). In the realm of plant breeding, strategic manipulation of ploidy levels is used to develop novel plant varieties endowed with desired traits such as increased size, enhanced nutritional values, and superior resistance to diseases and pests. The introduction of advanced genetic engineering and biotechnological tools has facilitated precise control over ploidy adjustments, paving the way for novel innovations in agricultural practices and plant research (Yirga, 2021).

The study of ploidy and its manipulation offers profound insights into plant biology, affecting everything from evolutionary biology to practical agricultural applications. It underscores the importance of genetic diversity and adaptability, showcasing the pivotal role of polyploidy in enhancing plant traits and biodiversity in ecosystems across the globe.

1.3 Ploidy variation in haskap and its morphological effects

Ploidy is a critical genetic characteristic in haskap cultivation and research. This plant exhibits a range of ploidy levels, including diploid, triploid, and tetraploid forms, each influencing its genetic makeup and phenotypic traits. The prevalence of each ploidy level varies, with certain levels more common in specific geographic areas or among different cultivars. Understanding the distribution and frequency of these ploidy levels is crucial for targeted breeding and conservation efforts (Miyashita & Hoshino, 2014).

Ploidy variation in haskap can occur naturally through mechanisms such as chromosomal duplication during cell division. Additionally, breeding techniques can induce changes in ploidy, with studies revealing the complex interplay between genetic

factors that lead to these variations. Such an understanding is essential for manipulating these variations to develop new cultivars with desired traits, enhancing the commercial and ecological value of the plant. Variations in ploidy levels manifest in several physical characteristics of haskap, notably affecting fruit size, shape, and the robustness of the plant. For instance, increased ploidy levels often lead to larger cell sizes, which can result in larger and more robust fruits and plants (Fig. 3).

These changes are not merely of academic interest but have significant practical implications, influencing the marketability of the fruit and the suitability of the plant for different climates and soils. Beyond physical traits, ploidy levels in haskap also influence the biochemical composition of the fruits, impacting the concentration of critical nutrients and phytochemicals. These biochemical variations affect the nutritional value and health-promoting properties of the fruit, making ploidy an essential factor in the commercial cultivation of nutrient-rich cultivars. A deep understanding of ploidy variations is indispensable for effective breeding strategies in haskap cultivation. Selective breeding for specific ploidy levels can produce cultivars with enhanced fruit quality, increased disease resistance, and better environmental adaptability. Furthermore, knowledge of ploidy impacts cultivation practices, influencing decisions related to planting density, fertilization, and harvesting schedules, thereby optimizing yield and quality.

The study and manipulation of ploidy levels in haskap offer profound insights into its biology and provide practical benefits in agriculture. By selecting for specific ploidy levels, breeders can develop haskap cultivars that are not only more robust and nutritious but also better adapted to diverse environmental conditions and market demands. This genetic characteristic thus plays a pivotal role in the ongoing development and improvement of haskap as a commercially valuable crop.

1.4 Biochemical characteristics of haskap

Haskap berries are increasingly becoming recognized for their exceptional nutritional and health-promoting properties. These berries are distinguished by their high concentrations of antioxidants, vitamins, and minerals, surpassing many commonly consumed berries in these aspects. Their rich phytochemical profile not only defines their unique taste but also contributes to their broad spectrum of health benefits, making them a superior choice for nutritional enhancement in diets (Celli et al., 2014).

The antioxidant content of haskap is particularly remarkable for its high levels of anthocyanins and flavonoids (Rupasinghe et al., 2012). These compounds are crucial for their ability to neutralize free radicals and reduce oxidative stress, offering protective effects against various chronic diseases. The rich content of vitamin C and a well-balanced suite of essential minerals of the berries further enhance their health benefits, supporting overall wellness and preventing nutritional deficiencies. Compared to other berries such as blueberries or strawberries, haskap berries exhibit superior levels of these beneficial antioxidants and vitamins, which highlights their potential as a superfood in health-conscious diets (Rupasinghe et al., 2012). Research has shown significant variability in the biochemical composition of haskap berries across different cultivars and growing conditions, such as soil type and climate (De Silva & Rupasinghe, 2020). This variability affects the concentration and profile of phytochemicals, including phenolics and anthocyanins, which are critical for the nutritional properties of the berries. Understanding these variations is vital for breeding programs aimed at enhancing specific nutritional traits, thereby maximizing the health benefits provided by different haskap cultivars (Gawroński et al., 2020). Advanced techniques such as high-performance liquid chromatography (HPLC) have been used to delve deeper into the phenolic profiles of haskap berries, identifying dominant anthocyanins such as cyanidin-3-glucoside, which comprises a significant portion of the total anthocyanin content. These analytical methods provide a detailed understanding of the variations in phenolic content among different haskap cultivars, contributing to targeted nutritional and therapeutic applications of this berry (Khattab et al., 2016).

Haskap berries stand out in the realm of superfruits due to their robust antioxidant capacities and rich nutritional profile. Their diverse phytochemical composition, influenced by genetic and environmental factors, offers numerous health benefits and makes them a promising candidate for both dietary supplementation and potential functional food products. Ongoing research and breeding efforts continue to explore and enhance the health-promoting qualities of haskap berries, solidifying their status as a valuable addition to health-oriented diets.

1.5 Research gap and objectives

The expanding body of literature on haskap provides significant insights into its

genetic and biochemical composition. Despite this, several critical gaps remain, particularly concerning the detailed implications of ploidy variation and the environmental influences on the genetic and biochemical traits of haskap. Notably, while foundational knowledge about ploidy levels exists, there is a lack of comprehensive research into how these genetic variations manifest in observable morphological, and biochemical differences. This gap is crucial for understanding the full potential of haskap in agriculture and health sciences.

The specific effects of ploidy variation on phenotypic expressions, such as fruit size, shape, and the overall robustness of the plant, are poorly understood. These traits directly influence the physiology of the plant and its adaptation to environmental stresses, which are vital for developing resilient cultivars. Moreover, the role of environmental factors in influencing these genetic expressions offers another layer of complexity and potential research focus, which has not been thoroughly explored (Miyashita & Hoshino, 2015). While some studies have begun to address the phytochemical profiles of haskap, the extent of biochemical variability across different cultivars, especially under varied climatic and soil conditions, remains underexplored. Such studies are essential for identifying cultivars with specific desirable traits, such as higher nutritional values or better adaptability to adverse growing conditions. Understanding these variations can lead to more targeted and efficient breeding programs (Fujita et al., 2020a).

The research aims to conduct a thorough investigation into the genetic foundations of ploidy variation in haskap plants, delving into how these variations influence the morphological and biochemical traits of the plant. A key objective involves a meticulous exploration of the diverse genetic profiles and their impact on the physical structure and chemical makeup of the plants. Additionally, this study performed an extensive biochemical analysis of haskap fruits, emphasizing the comparative study across different ploidy levels and cultivars. This comprehensive analysis seeks not only to elucidate the biochemical diversity inherent in these variations but also to enhance understanding of how genetic differences can affect fruit composition, potentially influencing both agronomic viability and market value. Through this in-depth examination, the project aspires to contribute significantly to the optimization of breeding and cultivation practices tailored to the unique properties of haskap fruits.

Addressing these research objectives will fill the current knowledge gaps,

providing valuable insights for plant geneticists, breeders, and agriculturists. This will pave the way for more informed and efficient cultivation and utilization of haskap, enhancing its role as a fruit crop with significant nutritional and commercial value. The study will also contribute to the broader field of plant biology by detailing the interactions between genetic makeup and environmental factors in shaping plant traits.

1.6 Methodological overview

This research uses a comprehensive methodological framework to investigate the morphological and biochemical characteristics of haskap. Various cultivars are selected to ensure a representative sample of genetic diversity, and fruits are harvested at peak maturity to capture their optimal biochemical profiles. Special attention is given to the diversity of geographical origins and cultivation conditions to include a wide range of environmental influences, which are crucial for understanding the adaptability and nutritional potential of the plant.

Morphological traits of the haskap fruits, such as weight and volume, are systematically evaluated. This step is essential in linking genetic diversity to observable physical characteristics, providing insights into how genetic variations manifest in fruit morphology. Such data are vital for breeders and researchers focusing on the enhancement of fruit quality and yield. The core of the study involves detailed biochemical analyses using high-precision techniques such as gas chromatography-mass spectrometry (GC-MS). This method is highly regarded for its accuracy in quantifying phytochemicals and is used to profile key compounds such as sugars, sugar alcohols, and organic acids. These compounds play a crucial role in defining the nutritional value and health benefits of the haskap fruits. Such analyses not only enhance our understanding of the biochemical diversity among the cultivars but also contribute to the development of value-added products (Kännaste et al., 2014). Data obtained from the biochemical and morphological assessments undergo rigorous statistical analysis. This phase involves comparing variations across different cultivars to identify significant patterns and correlations. The goal is to elucidate relationships between genetic diversity, environmental factors, and plant traits, providing a robust basis for further research and practical applications in plant breeding and cultivation (Allwood et al., 2009). The research is conducted with strict adherence to ethical standards and sustainability

principles, ensuring that the practices are responsible and minimize environmental impact. This commitment is essential for maintaining the integrity of the research and its applicability in real-world agricultural and environmental settings.

This methodological framework is designed to provide a deep and nuanced understanding of the complex traits of haskap. By using advanced analytical techniques and rigorous statistical methods, this study aims to deliver robust and meaningful contributions to the field of plant genetics and horticulture, enhancing the knowledge base necessary for the effective cultivation and utilization of haskap.

1.7 Research significance

This research holds significance for the field of plant genetics and horticulture, particularly in the cultivation and improvement of haskap. The findings of this study are poised to contribute to several key areas:

- **Genetic breeding advancements:** By elucidating the impact of ploidy variations on both morphological and biochemical traits, this research provides critical insights that can guide future breeding programs. Selective breeding practices can leverage these insights to develop haskap cultivars with optimized traits such as increased fruit size, improved nutritional profiles, and enhanced sensory qualities.
- **Agricultural practices and crop management:** Understanding the genetic underpinnings of phenotypic traits allows for more informed decisions in agricultural practices. This knowledge aids in the selection of suitable cultivars for specific climates and soil conditions, potentially leading to more resilient agricultural systems and increased productivity.
- **Nutritional enhancement and health benefits:** The exploration of this study of how genetic factors influence biochemical compositions, such as antioxidant levels, directly contributes to the nutritional and medicinal value of haskap fruits. This has implications for food science and health industries, providing a foundation for using haskap as a functional food ingredient.
- **Conservation and biodiversity:** By characterizing the genetic diversity within haskap species, the research also supports conservation efforts. Preserving genetic diversity is crucial for the resilience of species, allowing them to adapt to changing environmental conditions and resist pests and diseases.

- **Economic impact:** With the commercial cultivation and breeding implications, the findings of this study can also impact the economic aspects of haskap production. Improved cultivars can attract a broader market due to better taste profiles and health benefits, enhancing economic returns for farmers and producers.

In summary, this research not only fills a critical gap in the current scientific understanding of the genetic and phenotypic dynamics of haskap but also sets the stage for practical applications that extend beyond academia into the commercial and environmental realms.

Fig. 1. Haskap trees (flowering period) growing in the field at Hokkaido University.

Fig. 2. Close-up of haskap fruits on the trees at Hokkaido University.

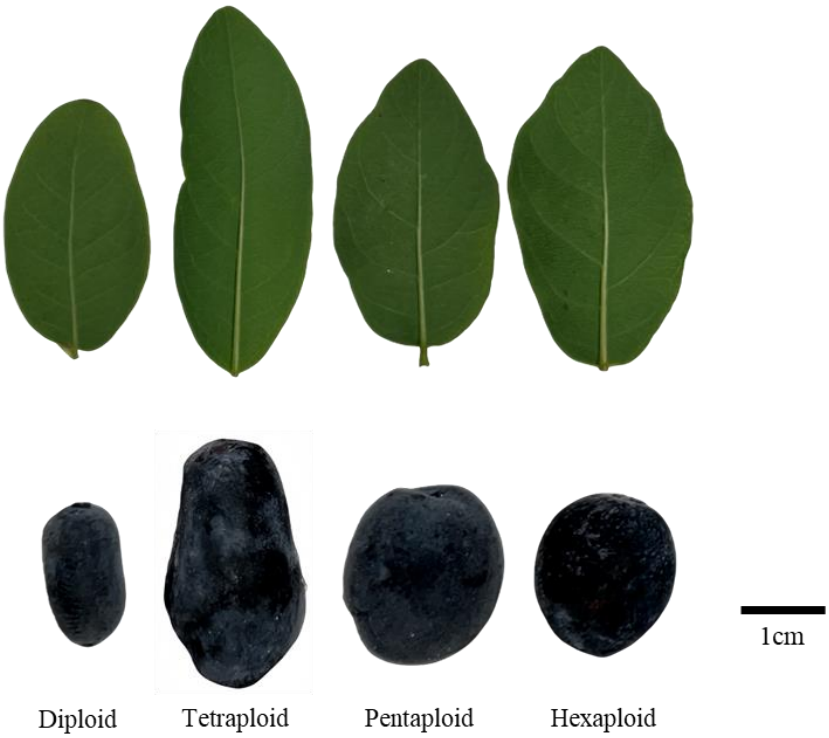


Fig. 3. Morphology of haskap leaves (up) and fruits (down) with different ploidy levels

Chapter 2
Morphological and general quality parameters of different polyploid haskap fruits

2.1 Introduction to morphological studies

This investigation intricately examines the dynamic interrelationship between the morphological attributes and quality indices of haskap, asserting that dimensions such as fruit dimension, mass, and shape are the manifestations of intricate genetic, physiological, and environmental interactions. This study meticulously evaluated pivotal quality metrics, specifically pH, Brix, and moisture content, which are integral in assessing the palatability and longevity of the fruit post-harvest.

Recognizing the role of polyploidy, characterized by cells housing multiple paired chromosome sets, this research acknowledges its contribution to marked alterations in plant morphology, potentially influencing fruit dimension and pivotal quality metrics such as sweetness, acidity, and hydration level. In elucidating these morphological and qualitative discrepancies, the study offers vital insights pertinent to breeding programs aimed at the enhancement of fruit quality, yield, and environmental resilience. The process of interspecific hybridization involving haskap and its kin has given rise to novel phenotypes in hybrids, encompassing variations in fruit hue, attributed to the concentration and spatial distribution of anthocyanins, notably cyanidin 3-glucoside and peonidin 3,5-diglucoside (Fujita et al., 2020a; 2020b). Furthermore, polyploid haskap plants, emanating from both inter- and intraploid hybridization, exhibit a diverse array of ploidy levels, influencing not only their morphological attributes but also their fruit setting efficacy (Miyashita & Hoshino, 2015).

The influence of ploidy on plant growth and morphological structure is deemed critical in the realms of agricultural science and horticulture. Variations in ploidy substantially impact numerous plant characteristics, encompassing morphology, biomass generation, and resilience against biotic and abiotic stressors. Plants with polyploidy are frequently associated with enhanced attributes, such as increased biomass, elevated synthesis of secondary metabolites, and bolstered stress endurance, positioning them as valuable assets in agricultural and horticultural endeavors. For instance, induced

polyploidy alters the synthesis patterns of secondary metabolites in medicinal plants, thereby amplifying their pharmacological worth (Madani et al., 2021). Moreover, the artificial induction of polyploidy in plants can lead to significant anatomical and morphological transformations, alongside an increase in yield, vigor, and tolerance to stress, thereby presenting a potent tool in plant breeding initiatives (Chen et al., 2020).

Research further indicates that polyploidy enhances plant resilience against various stressors, thereby influencing growth and development under unfavorable environmental conditions (Tossi et al., 2022). This comprehensive exploration of the morphological features and quality metrics of haskap fruits across diverse ploidy levels, inclusive of diploid, tetraploid, pentaploid, hexaploid, and aneuploid, seeks to document and interpret these variations within the expansive context of plant science, breeding, and horticulture. By intricately examining and correlating physical attributes with quality metrics and ploidy degrees, this study aspires to contribute substantial insights to the prevailing knowledge corpus, thereby propelling the cultivation and application of haskap and potentially other fruit crops. The assimilation of this knowledge into agricultural methodologies and technologies is expected to optimize crop yield and quality, paving the way for an era of sustainable and resilient agricultural systems.

2.2 Materials and methods

2.2.1 Plant material and fruit collection

In the context of this investigation, the focal biological specimens were haskap trees. These specimens were meticulously cultivated within the expansive premises of the experiment farm, an integral part of the Field Science Center for Northern Biosphere at Hokkaido University, located in the vibrant city of Sapporo, Hokkaido, Japan. The agricultural management of these trees was rigorously upheld, adhering to the highest standards of agronomic practices, ensuring the integrity and reliability of the experimental outcomes. The genetic diversity of the sample set was robust, encompassing a wide range of ploidy levels. This assortment included a singular diploid ($2n = 2x = 18$), a trio of tetraploids ($2n = 4x = 36$), a collection of five pentaploids ($2n = 5x = 45$), a pair of hexaploids ($2n = 6x = 54$), and a unique aneuploid specimen ($2n = 6x-3 = 51$). Such genetic variety was instrumental in enriching the study with comprehensive insights into the biological nuances of these specimens. The harvesting of fruit samples was

strategically conducted over the summer months, extending from June through August, in the consecutive years of 2022 and 2023. The selection criteria for these fruits were stringent, with emphasis on optimal growth characteristics, the absence of any disease, avoidance of insect infestation, and freedom from mechanical damage. Additionally, a consistent ripening period was a key consideration in the selection process. Thirty fruits were selected from each independent plant as the test samples. Upon collection, these fruits were promptly subjected to precise data measurement protocols, ensuring that the captured data was as accurate and relevant as possible, enhancing the overall validity and reliability of the experimental findings.

2.2.2 Morphological measurement of fruits

The measurement of fresh fruit mass and its subsequent volumetric and shape analyses form a critical part of assessing fruit quality, conducted with a meticulous approach using sophisticated instrumentation to ensure high precision and accuracy. The evaluation began with the precise determination of the fruit mass using an ED224S electronic balance, which boasts an exceptional precision of 0.0001 g (Sartorius, Germany). This balance, known for its reliability and accuracy, was instrumental in ensuring that the weight measurements were free from any significant error, thereby laying a solid foundation for further analysis. Following the mass measurement, the volume of the fruit was calculated using the buoyancy method. This technique is based on the principle that the buoyant force exerted on a submerged object is equal to the weight of the fluid it displaces, which is directly proportional to the volume of the object. In practical terms, this involved recording the mass of the fruit before and after complete immersion in a fluid. The change in mass, coupled with the known density of the fluid, was used to compute the fruit volume through a formula that integrates these parameters. This method not only provides a highly accurate measure of volume but also avoids the physical intrusion of other volume-measuring techniques, preserving the integrity of the fruit. To complement the volumetric analysis, the linear dimensions of each fruit—specifically, the longitudinal (length) and equatorial (width) diameters—were measured using a CD-S15C caliper (Mitutoyo, Japan). This caliper, noted for its precision of 0.01 mm, enabled the exact measurement of these dimensions, which are crucial for calculating the Fruit Shape Index. This index, which is calculated by dividing the longitudinal diameter by the equatorial diameter, serves as an essential metric in

categorizing the geometric characteristics of the fruit, providing insights into its growth patterns and potential quality. The suite of equipment used in these assessments also included a B-212 pH meter (Horiba, Japan) and a PAL-BXIACID F5 refractometer (Atago, Japan) for chemical analysis of the fruit juices, assessing properties such as acidity and soluble solid content. Moreover, an FDU-2200 freeze dryer (Tokyo Rikakikai, Japan) was used to measure the moisture content of the fruit by comparing its mass before and after freeze-drying. This measurement is vital for understanding the water content of the fruit, which can significantly affect both its freshness and shelf life. The fruit shape index (SI) is an analytical measure used to characterize the geometric form of fruits. It is computed by dividing the longitudinal diameter (length) of the fruit by its equatorial diameter (width). This ratio provides a numerical expression that helps in categorizing the fruit based on its shape, whether it is more elongated or rounded. This index is particularly useful in studies related to fruit development and quality, as different shapes can influence not only the aesthetic appeal and marketability of the fruits but also their packing, processing, and storage efficiencies. Accurate measurement of these dimensions, facilitated by precision instruments such as the CD-S15C caliper, ensures that the SI reflects true physical characteristics, allowing researchers and producers to make informed decisions about crop management and marketing strategies. This calculation is a pivotal component of the comprehensive fruit quality assessment process, linking physical attributes to potential consumer preferences and commercial value.

$$SI_{approx} = \frac{1}{2}\left(\frac{Fl_{mean}+Fl_{SD}}{Fw_{mean}-Fw_{SD}} + \frac{Fl_{mean}-Fl_{SD}}{Fw_{mean}+Fw_{SD}}\right)$$

- Fl_{mean}: The mean of fruit length, representing the average of every single longitudinal diameter measurement.
- Fw_{mean}: The mean of fruit width, representing the average of every single transverse diameter measurement.
- Fl_{SD}: The standard deviation of the longitudinal diameter, indicating the dispersion of every single longitudinal diameter measurement.
- Fw_{SD}: The standard deviation of the transverse diameter, indicating the dispersion of every single transverse diameter measurement.
- SI_{approx}: An approximate value of the SI, the average of SImax and SImin, providing a simplified estimate of the SI when considering the uncertainty in both

longitudinal and transverse diameters.

Together, these instruments and methodologies represent a comprehensive approach to fruit quality assessment, combining mass, volume, dimension measurement, and chemical analysis to provide a thorough understanding of fruit characteristics. This rigorous analysis ensures that the data collected is both precise and reflective of the true nature of the fruits under study, offering valuable insights into their potential quality and marketability.

2.2.3 Measurement of fruit quality parameters

In the meticulous procedure of selecting fruits for phenotypic trait analysis, a rigorous standard was employed. This ensured that all fruits included in the study were consistently sized and free from any blemishes caused by pests or diseases, which is critical for preserving the accuracy of the experimental results. The individual weights of the fruits and their peels were precisely determined using an analytical balance known for its high precision. The volume measurement of the fruits used the water displacement technique, where the increased water level in a cylindrical container, multiplied by the base area of the container, provided the necessary data. The dimensions of each fruit—length, width, and the thickness of the peel—were accurately gauged with calipers, tools known for their precision in measuring small distances. An index describing the shape of the fruit was calculated by dividing the length of the fruit by its width, providing insights into the physical form of the fruit. Only the healthiest seeds, those that were normal in appearance and development, were kept for further examination. Any seeds that appeared shriveled or underdeveloped were discarded. This selection was crucial for accurate assessments of seed mass and for counting the number of seeds each fruit contained. The chemical properties of the fruit, such as acidity levels (pH) and the concentration of soluble solids (measured in degrees Brix), were assessed using a pH meter and a digital refractometer, respectively. These instruments are essential in analyzing the chemical composition of the fruit juices. Lastly, the moisture content of the fruit samples was meticulously calculated. This was performed by noting the weight differences before and after the freeze-drying process. Freeze-drying is a dehydration process that preserves perishable materials, making it an ideal method for determining the water content within the fruits. This final measurement provided critical data that contributes to understanding the water retention properties and overall quality of the fruits under study.

2.2.4 Statistical analysis

Statistical analysis played a pivotal role in the research, leveraging the capabilities of the SPSS 26 software (SPSS Inc., IBM, Armonk, NY, USA) to compute a range of essential statistical metrics. This initial phase included the computation of minimum, maximum, and average values for each fruit quality trait being examined. Additionally, measures of variability such as the standard deviation, the range of variation, and the coefficient of variation (CV), which is calculated as the standard deviation divided by the mean and then multiplied by 100%, were determined. These basic statistical measures provided the necessary foundation for conducting more advanced analyses.

As the study progressed, more sophisticated statistical techniques were used to delve deeper into the data. Correlation analysis and multiple comparisons were conducted to unearth the complex relationships and variations among the data sets. One of the key methods used was a one-way analysis of variance (ANOVA), which was complemented by Duncan's multiple range test for post-hoc analysis, with a significance threshold set at $p < 0.05$. This thorough approach was crucial for identifying significant differences between the groups being studied, thereby illuminating how different ploidy levels affect various fruit quality traits.

Additionally, a principal component analysis (PCA) was used to simplify the complex, multidimensional data into principal components. This technique was instrumental in uncovering the underlying patterns and relationships within the data, providing critical insights into the primary factors that influence fruit quality traits. Such insights are essential for a comprehensive understanding of the complex interactions that influence these traits. To ensure the statistical robustness of the analysis, particularly with variables that tend to skew such as Brix and moisture content, a square root arcsine transformation was applied. This transformation helped normalize the data distribution, thus reinforcing the validity of the analysis performed.

The findings from these statistical analyses were not only documented in the numerical outputs of SPSS 26 but also visually represented through the GraphPad Prism 9 software (GraphPad Software Inc., CA, USA). This dual-software approach enabled the creation of clear and detailed graphs that effectively illustrated the intricate data nuances. These visualizations were critical for interpreting the results, making complex statistical information accessible and understandable. They highlighted the distinct impacts of

different ploidy levels on the morphological and quality attributes of the fruits, offering valuable perspectives on how these genetic variations manifest in physical and chemical traits. This comprehensive statistical and visual analysis thus provided a deep dive into the data, enhancing the overall understanding and dissemination of the research findings.

2.3 Results

2.3.1 General analysis of phenotypic traits and quality variability

In an extensive evaluation of the morphological and quality attributes of 360 haskap fruit samples with varying ploidy levels at maturity, there was a notable observation of substantial variability in most assessed parameters. This variance underscores a significant diversity in the phenotypic traits of the haskap fruits being investigated (Table 1).

As shown in the results in Table 1, the average fruit mass was recorded at 0.6706 g. This parameter exhibited notable fluctuation, with a standard deviation of 0.4067 g and a range from 0.141 to 1.6307 g, resulting in a CV of 60.65%. Similarly, the average fruit volume was measured at 0.77 cm^3, with an even greater variability reflected by a CV of 69.74% and spanned from 0.17 to 2.26 cm^3. These statistics are indicative of a pronounced diversity in both the mass and volume of the fruits across different ploidy levels. In terms of physical dimensions, the fruits displayed an average vertical diameter of 13.39 mm and an average transverse diameter of 10.12 mm, with CVs of 28.51 and 20.15%, respectively. This variation suggests moderate diversity in these dimensions among the samples. Peel thickness and peel mass also demonstrated variability with CVs of 31.94 and 34.39%, respectively, highlighting differences in these traits among the fruits of different ploidy levels. Seed-related traits were also diverse, with an average seed number per fruit of 19 and an average single seed mass of 0.0017 g. These traits showed significant variability with CVs of 37.14 and 35.17%, respectively, suggesting substantial fluctuation in seed characteristics across the various ploidy levels. Chemically, the fruits were more consistent. The average pH value stood at 2.8 with a relatively stable CV of 10.30%. Similarly, the average sugar content (Brix) was measured at 13.4% with a CV of 14.45%, indicating a relative stability in sugar content across the samples. The water content exhibited very high stability, with an average of 84.65% and a CV of only 3.32%, highlighting uniformity in this trait among the different ploidy levels.

From this comprehensive analysis, it is clear that various ploidy levels in haskap fruits demonstrate considerable diversity in terms of mass, volume, seed number, and seed mass. In contrast, the diversity in physical dimensions such as vertical and transverse diameters, peel thickness, and peel mass is less marked. The chemical properties, particularly pH and sugar content, showed greater stability, with water content exhibiting the highest level of consistency. These insights are invaluable in understanding how ploidy affects the morphological and quality characteristics of haskap fruits, offering a nuanced view of their developmental and commercial potential.

2.3.2 Comparative analysis of morphological and quality characteristics

An in-depth comparative analysis was performed to examine the morphological and quality characteristics of haskap fruits at various ploidy levels. This examination highlighted notable differences, reflecting the influence of genetic variability on these traits (Table 2, Appendix A).

Diploid fruits exhibited average characteristics with a mass of 0.3487 g and a volume of 0.41 cm^3, alongside an average seed count of 19. These fruits maintained a pH of 2.7 and offered a sugar content of 14%, coupled with a water content of 85.80%. Morphologically, they had lengths and widths of 9.53 and 9.64 mm, respectively, with a shape index of 0.99, suggesting nearly spherical shapes. The peel thickness and peel mass were relatively modest at 0.29 mm and 0.0284 g, respectively, while the single seed mass stood at 0.0006 g. Meanwhile, tetraploid fruits exhibited a substantial increase in size with an average mass of 1.2934 g and a volume of 1.62 cm^3. The average seed count rose to 28. These fruits experienced a slight increase in pH to 2.8 but a decrease in sugar content to 13.1%. The water content was slightly lower at 84.82%. Significantly, the lengths and widths were 19.44 and 12.55 mm, respectively, yielding a shape index of 1.55, indicating more elongated forms. Both peel thickness and peel mass also saw increases to 0.39 mm and 0.0850 g, respectively, and the single seed mass escalated to 0.0021 g. Pentaploid fruits exhibited an average mass of 0.5312 g and a volume of 0.56 cm^3, with an average seed count decreasing to 14. These fruits maintained a stable pH of 2.8 and a sugar content of 13.8%, with a water content of 84.20%. Their lengths and widths measured 11.47 and 9.21 mm, respectively, giving them a shape index of 1.24, which indicated slightly elongated shapes. The peel thickness and peel mass were recorded at 0.48 mm and 0.0623 g, respectively, with a single seed mass of 0.0013 g. Aneuploid fruits

exhibited a unique profile with an average mass of 0.3977 grams and a volume of 0.42 cm^3. The average seed count was reduced to 11, and the pH increased to 3.0. These fruits had a notably higher sugar content of 17.1% and a reduced water content of 82.13%. The lengths and widths were 11.11 and 7.53 mm, respectively, resulting in a shape index of 1.48, indicating longer fruits. Peel thickness increased to 0.64 mm, the peel mass increased to 0.0514 g, and the single seed mass was 0.0014 g. Lastly, hexaploid fruits demonstrated characteristics with an average mass of 0.3822 g and a volume of 0.42 cm^3, and a seed count of 21. These fruits had a pH of 2.7 and the lowest sugar content of 11.0%, yet they had the highest water content at 86.22%. The lengths and widths of these fruits were 12.20 and 10.32 mm, respectively, yielding a shape index of 1.18, which slightly trends toward spherical. Notably, they had the thickest peels at an average of 0.73 mm, a peel mass of 0.0584 g, and the heaviest single seed mass at 0.0025 g.

This detailed assessment of the morphological and quality parameters across different ploidy levels of haskap fruits reveals significant variability. Specifically, tetraploid fruits exhibited the most considerable increase in size, hexaploid fruits had the thickest peels, and aneuploid fruits displayed the highest sugar content. These findings not only illuminate the profound impact of ploidy on the characteristics of haskap fruits but also offer crucial insights for future breeding programs and agricultural practices aimed at optimizing fruit production and quality.

2.3.3 Correlation analysis of traits and quality across different ploidy levels of haskap

In the comprehensive correlation analysis conducted to explore the relationships between haskap fruit characteristics and chromosome counts, several meaningful correlation indices emerged, highlighting intricate interactions between morphological and quality traits across varying ploidy levels (Fig. 4, Appendix B).

The chromosome count had a moderately weak negative correlation with key fruit characteristics such as fruit mass (-0.32), fruit volume (-0.35), and seed number (-0.32). This suggests that an increase in chromosome number may generally result in a reduction in these specific traits. Conversely, there was a substantial positive correlation observed between chromosome number and peel thickness (0.75) as well as single seed mass (0.44), indicating that higher chromosome numbers are associated with an enhancement in these particular traits. Notably, there was an exceptionally strong positive correlation between fruit mass and fruit volume (0.98), fruit length (0.93), and fruit width (0.82), illustrating

a significant link between these dimensional traits and suggesting that larger fruits tend to be not only heavier but also larger in both length and width. However, fruit mass inversely correlated with peel thickness (-0.46), suggesting that as fruits become heavier and larger, peel thickness may not necessarily increase in proportion. Similarly, fruit volume also exhibited a very strong positive correlation with fruit length (0.93) and width (0.81), further supporting the idea of size consistency in larger fruits. Yet, similar to fruit mass, fruit volume also negatively correlated with peel thickness (-0.46), reinforcing the observation of disproportionate growth between fruit size and peel thickness.

The relationship between seed number and fruit dimensional traits was relatively weak. However, a positive correlation (0.47) with single seed mass was observed, indicating that fruits with a greater number of seeds tend to have heavier individual seeds. Additionally, pH levels displayed a moderate positive correlation (0.54) with sugar content (Brix), suggesting a linkage between the acidity and sweetness of the fruits, whereas the correlation with most morphological traits remained insignificant. A negative correlation (-0.53) between sugar content (Brix) and single seed mass was detected, implying that fruits with higher sugar content may possess lighter seeds. This observation suggests an interesting trade-off between sweetness and seed weight within the fruit. The water content of the fruits exhibited a low correlation with most morphological traits, suggesting that it is relatively independent of the other observed characteristics of the fruits. Meanwhile, a strong positive correlation (0.83) between fruit length and width as well as positive correlations of the fruit shape index with several morphological traits, particularly fruit volume and length, indicate that the shape index is a reliable indicator of the overall size and form of the fruit. The strongest positive correlation observed between peel thickness and chromosome number, coupled with negative correlations with most morphological traits, particularly fruit volume and mass, suggests that higher chromosome counts may lead to the development of disproportionately thicker peels compared to overall fruit size. A moderate positive correlation was also observed between peel mass and both fruit volume and length, indicating that as fruits grow larger, their peel weight increases proportionally, potentially contributing to the overall fruit mass.

These detailed analytical results not only reveal the complex interdependencies among the morphological and quality traits of haskap fruits but also provide deep insights into how variations in ploidy levels influence fruit development and maturity. These

findings offer essential guidance for future breeding programs and agricultural practices aimed at optimizing fruit quality and understanding the genetic influences on fruit characteristics.

2.3.4 Principal component analysis

In this research, PCA was employed to investigate the complex correlations among the morphological and quality traits of haskap fruits across various ploidy levels. This statistical technique helped elucidate the primary factors that influence the traits of the fruit, serving as a basis for understanding their genetic and phenotypic variability (Fig. 5).

The results of the PCA demonstrated that the first principal component (PC1) was responsible for a significant proportion of the total variance, capturing 54.60%. PC1 was strongly associated with traits such as fruit mass, volume, length, and width. This finding indicates that PC1 predominantly reflects dimensions that are directly linked to the yield and market value of the fruits, suggesting that larger fruits are typically more desirable in commercial markets due to their size. Therefore, PC1 essentially represents the size-related traits that have a substantial impact on fruit size. The second principal component (PC2) accounted for a relatively significant portion of the variance as well, with 29.83%. It exhibited the strongest positive correlation with chromosome number and also demonstrated significant positive correlations with traits such as peel thickness, peel mass, and single seed mass. These correlations suggest that PC2 is likely associated with the structural characteristics of the fruits, indicating variations in the protective features and reproductive components of the fruits. A closer look at the loading plot highlighted a significant emphasis on chromosome number within PC2, suggesting that a higher chromosome number may contribute to more robust protective features such as thicker peels and larger seed masses. Additionally, PC2 exhibited strong associations with fruit water content and peel mass, hinting that as fruits increase in size and chromosome number, they may also exhibit enhanced water retention and heavier peels. This could be indicative of an evolutionary adaptation aimed at improving fruit viability and seed protection. Interestingly, traits such as pH and sugar content (Brix) displayed relatively low loadings on both principal components. This observation suggests that these chemical properties, while crucial for assessing fruit quality, have a less direct relationship with the morphological traits and ploidy levels of the fruits. Similarly, the fruit shape index also

showed low loadings on both PC1 and PC2, implying that the shape of the fruit may be influenced by a range of factors beyond just morphological or genetic determinants.

The PCA results effectively highlighted the intricate relationships and significant correlations between the morphological and quality traits of haskap fruits at different ploidy levels. It also pointed out some traits that appear to be relatively independent of these principal components. The traits related to fruit size were primarily encapsulated by PC1, while PC2 captured more of the structural and protective characteristics of the fruit. These insights are instrumental for advancing our understanding and prediction of the phenotypic expressions associated with different ploidy levels. They provide crucial guidance for targeted breeding programs, variety selection, and the optimization of agricultural practices focused on enhancing both the yield and quality of haskap fruits.

2.4 Discussion

This study provides a methodical exploration of the morphological characteristics and basic quality traits of haskap fruits, underscoring the profound impact of ploidy levels on these parameters. By performing an exhaustive analysis, it can be observed that ploidy levels significantly influence the structural features and critical quality indicators of haskap fruits, offering robust empirical support for the substantial role ploidy plays in the developmental and qualitative dynamics of these fruits.

The research detailed in this study highlights the considerable variability in physical form and the differences in quality observed across different ploidy levels, emphasizing the crucial influence of ploidy on the developmental processes and quality attributes of the fruit. Notably, fruits with tetraploid configurations demonstrated marked increases in mass, volume, peel thickness, and seed mass when compared to their diploid counterparts. This enhancement in morphological traits among tetraploid fruits mirrors trends observed in studies of other fruit species, such as yellow-fleshed kiwifruits. In these kiwifruits, attributes such as fruit weight and firmness were significantly impacted by the ploidy level of the pollen donor (Oh et al., 2021). These findings not only validate the significant effects of ploidy on fruit development and quality but also align with broader patterns observed in the botanical sciences, where genetic variations often translate into notable phenotypic differences.

The findings from this study reinforce those of earlier research, particularly

highlighting the significant genetic impact of ploidy on the biosynthesis and accumulation of sugars in fruits exhibiting varied ploidy levels, as reported by Li et al. (2023). This research specifically points to aneuploid fruits, which are noted for their elevated sugar content—a trait that is attributed to a sophisticated genetic predisposition that encourages sugar accumulation in fruits with specific chromosomal configurations. This investigation delves deeper into the nuances of how such superior sugar levels in aneuploid fruits are the result of complex genetic influences that affect the pathways of sugar biosynthesis, including those of key sugars such as fructose and glucose.

Moreover, the study brings to light that the behavior of other sugars such as mannose and sorbitol displays marked variations across different ploidy levels (Li & Hoshino, 2024). These observations underline the intricate genetic interplays that govern sugar metabolism in fruits, reflecting how chromosomal variations can lead to significant differences in fruit composition and taste profiles. Such insights are invaluable for understanding the genetic foundations of fruit quality traits, potentially guiding breeding programs aimed at enhancing fruit sweetness through targeted genetic manipulation. This deepened understanding not only enriches the scientific discourse on plant genetics and fruit biochemistry but also holds practical implications for agricultural practices and the improvement of fruit crops for commercial consumption.

The increased sugar content observed in aneuploid fruits can be traced back to modifications in metabolic pathways directly influenced by their unique genetic constitution. These genetic alterations facilitate an uptick in both the biosynthesis and accumulation of sugars, enhancing the taste of the fruit and augmenting its nutritional profile. Furthermore, the variability observed in peel thickness and seed mass among fruits of different ploidy levels could reflect underlying differences in defensive mechanisms and reproductive strategies, which are shaped by the genetic diversity induced by variations in ploidy. This concept is supported by findings that suggest polyploidy may offer certain advantages under specific environmental conditions, potentially bolstering the resilience of a plant to various stresses and improving its chances for survival and reproductive success (Adams & Wendel, 2005). The link between ploidy levels and the heightened accumulation of sugars sheds light on genetic predispositions that promote sugar biosynthesis in fruits characterized by particular ploidy configurations. These findings emphasize the critical role of ploidy levels in the

breeding and cultivation strategies of haskap fruits, aiming to optimize both fruit quality and yield. The genetic factors influencing sugar content and morphological traits across different ploidy levels exert a multifaceted and profound impact on fruit development and quality. This highlights the importance of integrating knowledge about ploidy into genetic improvement strategies and agricultural practices, reinforcing the role of ploidy in the cultivation and enhancement of haskap fruits. A comprehensive understanding of how genetic factors tied to ploidy levels influence fruit characteristics is vital for developing targeted breeding programs that enhance desirable traits such as sweetness, size, and overall fruit quality, thus ensuring the production of superior fruit cultivars.

Polyploidization significantly influences not only the size of individual plant cells but also extends to impact the broader morphological, physiological, and biochemical characteristics of plants. Although an increase in cell size resulting from polyploidization does not invariably lead to a larger overall organism size, often due to a reduction in the number of cell divisions, this process profoundly affects a wide array of plant traits. These traits include plant height, growth habits, the number of roots and shoots, characteristics of leaves, as well as the quantity and dimensions of flowers, seeds, and pollen (Neenu et al., 2024). Additionally, even the composition of cell walls is altered. Polyploids frequently exhibit improved morphological traits compared to their diploid counterparts, which often endow them with superior characteristics. These enhancements not only contribute to the aesthetic and structural aspects of the plants but may also enhance their survival and adaptation capabilities under various environmental conditions (Balao et al., 2011; Stebbins, 1971; Trojak-Goluch et al., 2021).

This study delves into the complex interactions between the morphological traits and quality characteristics of fruits, highlighting the profound interconnections among these attributes. The analysis reveals a strong positive correlation between fruit mass and volume, suggesting that as fruits increase in size, their overall mass also increases proportionally. In contrast, a significant negative correlation with peel thickness indicates that as fruits become larger, their peel does not necessarily thicken at the same rate. This points to an intricate relationship where enhancements in one trait may lead to compensatory or opposing changes in others, reflecting the nuanced influence of polyploidization on plant development and performance.

The observed positive correlation between chromosome number and specific

structural characteristics of the fruit, such as peel thickness and single seed mass, emphasizes the critical role that ploidy levels play in shaping plant traits. This relationship highlights how genetic factors at the chromosomal level can significantly influence physical attributes that may relate to the adaptive strategies of a plant. This is corroborated by research on grapevines, where tetraploid plants exhibited noticeable morphological changes when compared to their diploid counterparts, potentially as adaptations to environmental stresses (Catalano et al., 2021). Similar to findings in other studies, the interactions between morphological traits and quality characteristics in haskap fruits reveal a complex web of relationships, as observed in studies on blackberries (Sabooni & Gharaghani, 2022). These studies illustrate that polyploidy does not only alter physical features but can also impact physiological and phytohormonal characteristics, thereby affecting the overall plant physiology and its response to environmental cues. The insights gained from this research enrich our understanding of haskap fruit characteristics and provide valuable guidance for future breeding programs and agricultural practices. By examining the intricate relationships among these traits, breeders and farmers can better strategize on selecting plant varieties with desirable traits that cater to specific market needs or environmental conditions.

Furthermore, the PCA conducted in this study sheds light on the importance of traits that influence fruit size, which are directly associated with agricultural yield and market value (Sharma et al., 2018). The size and appearance of fruits are significant factors in consumers' purchasing decisions, highlighting the practical implications of these findings for agricultural production and marketing (Olfati et al., 2010). By understanding these dynamics, growers are equipped with a scientific foundation to select plants with optimal ploidy levels, ultimately aiming to enhance both yield and fruit quality to meet market demand effectively. This study not only advances the scientific knowledge of plant trait formation at various ploidy levels but also serves as a cornerstone for applied agricultural strategies, ensuring that the cultivation of haskap fruits aligns with both commercial objectives and ecological sustainability. The notable correlation observed between chromosome number and key structural features of fruits, such as peel thickness, underscores potential adaptations related to the reproductive strategies and protective mechanisms of a plant. This relationship could provide valuable insights into how plants enhance their natural defense capabilities, which is critical for optimizing fruit quality

and ensuring the viability of offspring. Understanding these genetic interactions opens avenues for improving the inherent defenses of the plant against environmental stressors while maintaining or even enhancing the quality of the fruit (Wang et al., 2020). Looking ahead, future research could expand on these findings by exploring how variations in ploidy levels influence other bioactive components of haskap fruits, such as antioxidants and phytonutrients, and assessing their potential health benefits. Such studies could offer a more comprehensive understanding of how genetic factors contribute to the nutritional value of fruits and support sustainable agricultural practices by aligning crop development with health and environmental benefits.

In conclusion, this study not only provides crucial insights into the cultivation, breeding, and marketing strategies of haskap fruits but also broadens the scope of ploidy research, suggesting its application in other agricultural contexts. By effectively controlling and harnessing ploidy variations, there is potential to further enhance crop yield, quality, and adaptability. This approach aims to meet evolving market demands and environmental challenges, positioning ploidy management as a pivotal strategy in the future of agricultural science and production. This research marks a significant step forward in linking genetic research with practical agricultural applications, offering a blueprint for leveraging genetic diversity to improve crop performance across various agricultural sectors.

Table 1. Heteromorphosis of fruit phenotypic and quality characters for ploidy of haskap.

Item	Mean	Standard deviation	Minimum	Maximum	Range	Coefficient of variation %
Fruit mass/g	0.6706	0.4067	0.141	1.6307	1.4894	60.65
Fruit volume/cm^{-3}	0.77	0.54	0.17	2.26	2.09	69.74
Vertical diameter/mm	13.39	3.82	8.95	22.77	13.82	28.51
Transverse diameter/mm	10.12	2.04	6.51	13.88	7.37	20.15
Peel thickness/mm	0.50	0.16	0.28	0.78	0.50	31.94
Peel mass/g	0.0636	0.0219	0.0267	0.1222	0.0955	34.39
Number of seeds per fruit	19	7	6	32	26	37.14
Single seed mass/g	0.0017	0.0006	0.0005	0.0027	0.0022	35.17
pH	2.8	0.3	1.4	3.7	2.3	10.30
Brix /%	13.4	1.9	9.5	17.5	8.1	14.45
Moisture /%	84.65	2.81	76.23	88.02	11.79	3.32

Table 2. Measurement of morphological and mass characteristics of haskap with different ploidy levels.

Ploidy /Chromosome count	Fruit mass (g)	Fruit volume (cm^{-3})	Fruit length (mm)	Fruit width (mm)	Shape index	Peel thickness (mm)	Peel mass （g)	Seed number	Single seed mass (g)	pH	Brix (%)	Fruit moisture (%)
Diploid /18	0.3487 ± 0.1101c	0.41 ± 0.04c	9.53 ± 0.33d	9.64 ± 0.23c	0.99 ± 0.01c	0.29 ± 0.01e	0.0284 ± 0.0008d	19 ± 2c	0.0006 ± 0.0001d	2.7 ± 0.1bc	14.0 ± 0.1b	85.80 ± 1.94ab
Tetraploid /36	1.2934 ± 0.2181a	1.62 ± 0.33a	19.44 ±1.20a	12.55 ± 1.08a	1.55 ± 0.08a	0.39 ± 0.03d	0.0850 ± 0.0305a	28 ± 2a	0.0021 ± 0.0002b	2.8 ± 0.2b	13.1 ± 0.8c	84.82 ± 1.88bc
Pentaploid /45	0.5312 ± 0.1581b	0.56 ± 0.18b	11.47 ± 1.14c	9.21 ± 1.21c	1.24 ± 0.13b	0.48 ± 0.13c	0.0623 ± 0.0051b	14 ± 4d	0.0013 ± 0.0003c	2.8 ± 0.3b	13.8 ± 1.6b	84.20 ± 3.41b
Aneuploid /51	0.3977 ± 0.0237c	0.42 ± 0.01c	11.11 ± 0.17c	7.53 ± 0.24d	1.48 ± 0.01a	0.64 ± 0.01b	0.0514 ± 0.0009c	11 ± 3e	0.0014 ± 0.0001c	3.0 ± 0.3a	17.1 ± 0.2a	82.13 ± 1.66d
Hexaploid /54	0.3822 ± 0.2216c	0.42 ± 0.22c	12.20 ± 2.53b	10.32 ± 2.26b	1.18 ± 0.25b	0.73 ± 0.03a	0.0584 ± 0.0027b	21 ± 3b	0.0025 ± 0.0001a	2.7 ± 0.2c	11.0 ± 1.4d	86.22 ± 1.77a

*Different lowercase letters in the same column indicate significant differences ($p < 0.05$)

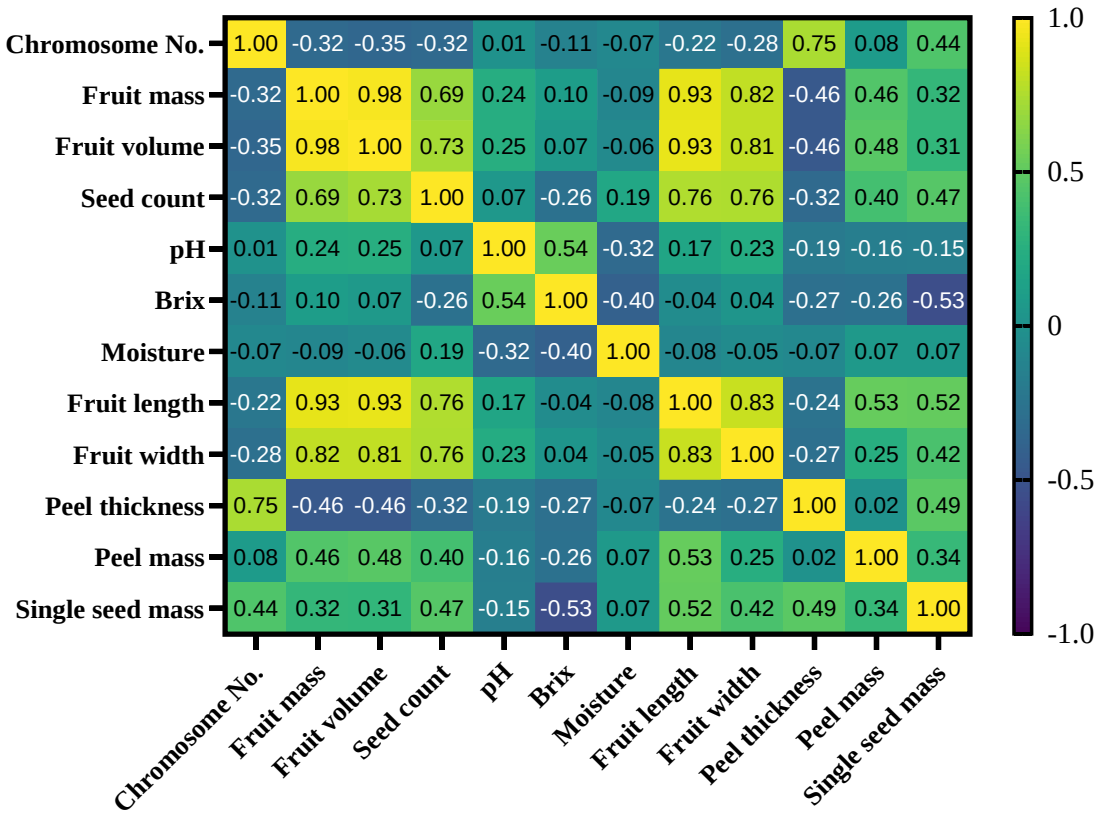


Fig. 4. Correlation matrix diagram for the analysis of 12 traits of haskap fruit.

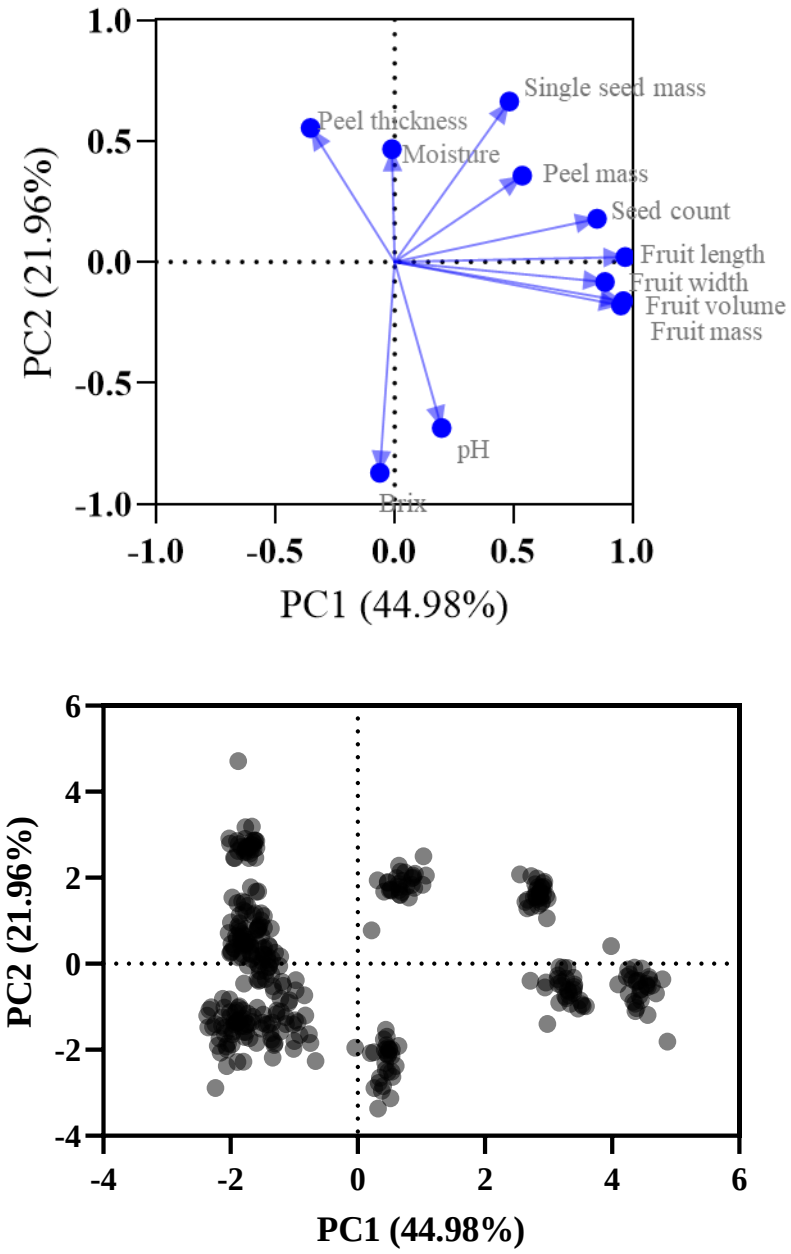


Fig. 5. PCA loading plots (up) and scores (down) for phenotypic and quality parameters of different haskap fruits.

Chapter 3 The impact of ploidy level on biochemical content accumulation in haskap

This chapter is rewritten based on the research article "Elucidating the impact of ploidy level on biochemical content accumulation in haskap (*Lonicera caerulea* L. subsp. *edulis* (Turcz. ex Herder) Hultén) fruits: A comprehensive approach for fruit assessment." (https://doi.org/10.1016/j.scienta.2023.112831) published in Scientia Horticulturae 327: 112831 (2024).

3.1 Introduction to biochemical studies

Polyploidy represents a biological condition characterized by the occurrence of three or more complete sets of chromosomes within the somatic cells of an organism. This phenomenon is widely observed across the natural world, especially prevalent among higher plant species (Hegarty & Hiscock, 2008). Depending on the source of the chromosome sets, polyploidy may be classified as either homologous or heterologous. It is interesting to note that the distribution of polyploidy varies among different types of woody plants; for instance, gymnosperms tend to exhibit fewer polyploid species compared to angiosperms (Ahuja, 2005). Polyploids are often associated with numerous beneficial traits, including faster growth rates, improved quality, and increased concentrations of metabolites. These advantages can lead to various phenotypic changes such as plant gigantism, enhanced resistance to environmental stressors, reduced fertility, and heightened production of essential metabolites (Van de Peer et al., 2017). In the realm of plant breeding, there is significant interest in both naturally occurring and artificially induced polyploids. Naturally occurring polyploids that have undergone extensive natural selection and adaptation tend to exhibit superior growth characteristics when compared to their artificially bred counterparts, thus they are often more sought after in agricultural practices (Osborn et al., 2003). However, the relative rarity of natural polyploids within populations poses a challenge in developing large and genetically diverse populations necessary for effective polyploid breeding programs (Husband et al., 2013). Polyploidization serves as a crucial driver in the evolution and diversification of plant species, playing a pivotal role in the emergence of new plant forms within various groups (Soltis & Soltis, 2000). This process often involves mechanisms such as cell fusion and chromosome duplication, which accelerate the emergence of novel phenotypes. As a result, species with higher levels of ploidy often exhibit greater adaptability compared to their lower-ploidy progenitors, displaying enhanced resilience and variability in their traits (Prentis et al., 2008). Woody plants, characterized by their extended maturation

periods, substantial physical dimensions, and modes of dispersion, present unique challenges for cytogenetic analysis. These challenges have shifted the focus of recent scientific inquiries toward exploring intraspecific polyploidy within woody plant populations (Stebbins, 1940).

Moreover, the rarity of woody plant species and the largely unexplored chromosomal variations among them add layers of complexity to the efforts of artificially inducing polyploidy. Xu et al. (2019) and De Kort et al. (2021) have highlighted these difficulties, noting the significant gaps in our understanding of chromosomal behavior in these plants. The intricate genetic frameworks and ecological contributions of woody plants underscore the need for advanced genetic tools and methodologies to better understand and utilize their polyploidy traits.

To further elaborate, the evolutionary advantages of polyploidy in plants are not only confined to adaptability and phenotype variation. Polyploid plants are often capable of occupying ecological niches that their diploid counterparts cannot, potentially leading to increased species richness and ecosystem stability (Ramsey & Schemske, 2002). Additionally, polyploidy may confer advantages under specific environmental stresses, offering insights into plant survival strategies under changing climatic conditions (Te Beest et al., 2012). These aspects make polyploidy a valuable subject of study in understanding plant resilience and adaptability. Efforts to harness the benefits of polyploidy in woody plants, however, must navigate the inherent complexities of these species' reproductive strategies and genetic makeup. The artificial induction of polyploidy requires precise scientific intervention, often necessitating a deeper understanding of the genetic and epigenetic factors influencing chromosome doubling (Comai, 2005). The potential of using polyploidy to improve woody plant species for commercial and environmental purposes remains an enticing but challenging frontier in plant sciences.

Extensive research on the Caprifoliaceae family has shown that during the fruit development phase, there is a consistent build-up of nutrients, which peaks at the stage of maturation (Dashbaldan et al., 2019; Qi et al., 2021). In particular, studies focusing on *Lonicera japonica*, a species within this family, have observed that polyploid variants contain a higher concentration of metabolic components than their diploid counterparts, presenting both genetic and phenotypic superiority (Peterson & Moran, 2019; Wang et al., 2020). This enhancement in polyploids is believed to stem from gene dosage effects,

where an increase in the number of gene copies leads to a proportional rise in the production level of gene transcription products. This upsurge in gene expression can alter various plant traits (Chen, 2007; Lavania, 2005). For example, research indicates that the guard cells and stomata of polyploid *Lonicera edulis* are significantly larger than those in diploid plants (Yao et al., 2018), and the overall morphological dimensions, biochemical composition, and biological resilience of polyploid *Lonicera japonica* are considerably superior to those of its diploid form (Li et al., 2009; Yan et al., 2023). The phenomenon of polyploidy leading to enhanced plant features is not merely confined to enhanced metabolic components. Polyploids often exhibit broader ecological adaptability, which could be pivotal in environments subject to climate variability and stress factors. This robustness is attributed to their enhanced genetic makeup, which allows for greater flexibility in physiological and metabolic responses under stress (Soltis et al., 2016). Furthermore, the nutritional content improvements observed in polyploids may have significant implications for agriculture, particularly in the cultivation of fruit-bearing plants where nutrient richness is desirable for both market value and health benefits (Omere et al., 2023). Moreover, the larger cell size typical in polyploids can influence not only stomatal size but also photosynthetic capacity, potentially enhancing growth rates and biomass accumulation (Corneillie et al., 2018). Such traits make polyploid varieties particularly attractive for breeding programs aimed at improving agricultural productivity and resilience.

Haskap fruits, recognized for their numerous beneficial properties including biological activity, rich nutritional content, strong cold resistance, and straightforward cultivation practices, are increasingly being regarded as a "superfood." This growing acclaim is part of a broader trend of commercialization on a global scale, as more consumers and industries discover the potential of the plant (Celli et al., 2014; Lee & Sharma, 2020). In particular, the antioxidant capacity of haskap berries stands out, surpassing that of similar fruits within the *Lonicera*, *Rubus*, and *Vaccinium* genera, marking them as especially valuable for health-oriented diets (Celli et al., 2014; Fujita et al., 2020a; Kan et al., 2022). Extensive research has been dedicated to understanding the biochemical profile of haskap berries, focusing on extracting, isolating, and identifying their key chemical components, which primarily include anthocyanins, sugars, and organic acids (Celli et al., 2014; Cheng et al., 2022; Senica et al., 2019; Wojdyło et al.,

2013). These compounds contribute significantly to the health benefits of the berries, such as anti-inflammatory properties, cardiovascular health support, and potential anti-cancer effects (Joseph et al., 2014; Kalt et al., 2020). Despite the extensive research into the breeding, biological functions, and applications of haskap, there remains a notable gap in comparative studies examining the chemical compositions of haskap berries from plants of different ploidy levels. Such studies are crucial as variations in ploidy can influence the concentration and composition of bioactive compounds, potentially affecting the nutritional and medicinal qualities of the berries (Gołba et al., 2020; Grygorieva et al., 2021; Sharma & Lee, 2021; Zehfus et al., 2021).

Furthermore, the exploration of genetic diversity in haskap cultivation could provide insights into optimizing the production of desirable traits such as flavor, size, and phytochemical content, enhancing the berries' commercial and health value. This area of research could greatly benefit from advanced genetic and biotechnological methods, which would help in precisely identifying and enhancing the traits that contribute to the superfood status of the berries (Rakhmangulov et al., 2023; Scalzo et al., 2005). In haskap plants, notable differences in morphological characteristics such as leaf and fruit structures can be observed across plants with varying ploidy levels. However, these variations tend to diminish among plants with higher-order ploidy, suggesting a certain uniformity in morphological traits as ploidy increases (Li et al., 2023). In the natural habitats of Japan, both diploid and tetraploid haskap plants have been identified, with the latter showing a wider distribution across diverse environmental conditions, which points to their enhanced adaptability. Specifically, tetraploid haskap plants are more commonly observed in mountainous areas, indicating a possible preference or advantage in such environments, while diploids tend to occupy other distinct ecological niches (Miyashita et al., 2011).

It is hypothesized that polyploid haskap fruits possess higher concentrations of biochemical constituents compared to their diploid counterparts, potentially contributing to their robustness and adaptability. This relationship between the biochemical content and ploidy levels in haskap fruits raises intriguing questions about the impact of genetic factors on the nutritional and medicinal qualities of the fruits. The consistency of this correlation across different ploidy levels in haskap fruits suggests a pattern that could be critical for future breeding and cultivation strategies but requires further detailed

investigation. Expanding on this, research into the biochemical profiles of polyploid plants could uncover links between genetic diversity and phytochemical richness, offering insights into how polyploidy could be leveraged to enhance plant health benefits. Increased ploidy levels can lead to improved stress resistance and higher production levels of secondary metabolites, which are crucial for plant defense mechanisms and human health (Chandra & Dubey, 2010; Lavania, 2005; Sharma et al., 2018).

Moreover, understanding the adaptive mechanisms of tetraploid haskap in harsher, mountainous environments could inform ecological and agricultural practices, potentially guiding the development of crop varieties better suited to cope with climatic changes. Turner et al. (2019) suggest that polyploidy may confer ecological advantages that allow plants to thrive under varied and challenging conditions, making the study of these traits particularly relevant in the context of global biodiversity conservation and sustainable agriculture. Miyashita and Hoshino (2015) adeptly generated polyploid haskap plants by employing techniques of both interploid and intraploid hybridization, marking a significant advance in the cultivation of this species. The focus of their subsequent research was to conduct a detailed analysis of the biochemical composition—specifically sugar, organic acid, and sugar alcohol content—in both diploid and polyploid haskap fruits. The principal goal of this ongoing investigation is to thoroughly characterize these new polyploid variants of haskap. By doing so, the study aims to lay a robust theoretical foundation that will facilitate the integration of these polyploid germplasms into future breeding programs. This exploration into the biochemical profiles of polyploid haskap fruits is crucial because these components significantly influence the taste, nutritional value, and processing suitability of the fruit. For instance, the balance between sugars and organic acids can determine the palatability of the fruit, which in turn affects consumer preference and marketability (De Silva & Rupasinghe, 2020). Furthermore, the presence of sugar alcohols may impact the osmotic properties and preservation qualities of the fruit, which are vital for extending its shelf life and enhancing its commercial value (Moing, 2000).

The creation and characterization of these polyploid plants not only contribute to our understanding of genetic diversity and adaptation but also have practical implications. For example, polyploids often exhibit greater stress tolerance and robustness, traits that are highly desirable in the face of climate change and variable environmental conditions

(Sattler et al., 2016). Additionally, the enhanced genetic variability within polyploids could lead to fruits with improved resistance to diseases and pests, reducing the need for chemical interventions in agriculture (Tossi et al., 2022).

Moreover, the aim of this study to provide a theoretical framework for future breeding reflects a strategic approach to agricultural innovation. By understanding the genetic and biochemical nuances of these polyploid variants, breeders can more effectively select traits that enhance yield, quality, and adaptability. This could lead to the development of haskap varieties that are not only more resilient but also tailored to specific climates and soil types, thereby broadening their cultivation range and economic impact (Patel & Kumar, 2023).

3.2 Materials and methods

3.2.1 Plant material and fruit collection methods

In the context of this study, haskap trees were cultivated under controlled conditions at the Experiment Farm of the Field Science Center for Northern Biosphere at Hokkaido University, located in Sapporo, Hokkaido, Japan. The management of these trees adhered to established agronomic practices to ensure healthy growth and accurate experimental outcomes. The sample set included a diverse range of ploidy levels to facilitate a comprehensive analysis of genetic influence on fruit characteristics. Specifically, the collection comprised one diploid (2n = 2x = 18; accession name: P2-7), three tetraploids (2n = 4x = 36; accession names: P4-17, P4-27, P4-37), five pentaploids (2n = 5x = 45; accession names: P5-1, P5-3, P5-4, P5-5, P5-6), two hexaploids (2n = 6x = 54; accession names: P6-8, P6-9), and one aneuploid (2n = 6x−3 = 51; accession name: PA-10). Fruit samples were methodically collected during the peak growing seasons of June to August in both 2021 and 2022. The selection criteria for the fruits included optimal growth conditions, the absence of diseases, avoidance of insect infestations, a lack of mechanical damage, and consistency in the ripening period across the samples. These stringent selection criteria were crucial for minimizing variability and ensuring that any differences observed in the biochemical analyses could be attributed to genetic differences rather than external factors.

Post-harvest, the fruits were carefully cleaned to remove any extraneous materials, ensuring that only the fruit itself would be analyzed. They were then sealed in

self-closing bags to prevent contamination and immediately frozen. Storage was in a freezer set to a stringent temperature of −80°C. This ultra-low temperature storage was critical for halting any biochemical processes, thus preserving the integrity of the original biochemical state of the fruits, which is essential for ensuring that the analyses reflect the true nature of the fruit at the time of harvest. The meticulous approach to the collection and preservation of fruit samples in this study provides a robust foundation for subsequent biochemical analyses, ensuring that the data obtained are both reliable and reproducible. This careful handling is vital in experimental studies where the goal is to draw meaningful conclusions from the data, particularly in studies involving genetic and biochemical investigations where even minor contamination or degradation can skew results significantly. Such practices are aligned with those recommended in the literature for handling plant specimens for scientific research, where maintaining the integrity of the samples post-collection is paramount (Smith et al., 2020). The thorough and careful approach adopted in this study sets a standard for future research in plant genetics and biochemistry, ensuring that results are based on the best possible sample integrity.

3.2.2 Overview of chemicals and reagents

For the identification and quantification of compounds in this study, chemical standards of exceptionally high purity, exceeding 98%, were used. These standards were procured from reputable suppliers such as Fujifilm Wako (Japan) and Alfa Aesar (USA), ensuring the reliability and accuracy of analytical results. Solvents such as methanol, acetone, and hexane, critical for HPLC, were sourced exclusively from Fujifilm Wako and Sigma-Aldrich (USA). These solvents are specifically designated as HPLC grade, which is essential for achieving precise and reproducible results in chromatographic separations. Derivatization, a key process in enhancing the detectability and separability of compounds in HPLC, used reagents such as N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) and O-methylhydroxylamine hydrochloride, which were also obtained from Fujifilm Wako. These reagents are crucial for preparing samples in a form that is more amenable to analysis by making them volatile or improving their chromatographic properties. An internal standard, ribitol, was incorporated into each sample to provide a consistent reference point for quantification, sourced from Fujifilm Wako. Using an internal standard is a fundamental practice in analytical chemistry, particularly in quantitative analyses, as it helps to correct for any variations in sample

preparation and instrument response, leading to more accurate results. For all experiments, ultrapure water was used, which was purified using a Millipore Milli-Q laboratory water system equipped with a 0.35 μm filter membrane. Using such highly purified water is critical to avoid any contaminants that may interfere with the detection and quantification of the target analytes.

This rigorous selection and use of high-quality chemicals and reagents are aligned with the best practices in analytical chemistry. The meticulous attention to these details ensures that the findings of this study are based on precise and reliable analytical methods, contributing valuable insights to the field of chemical analysis of biological samples. Such practices are not only crucial for the reproducibility of the results but also enhance the contribution of this study to ongoing research in analytical methodologies.

3.2.3 Sample preparation and extraction methodology

For the biochemical analysis of the fruit samples, an initial step involved the dehydration of the samples using a lyophilizer, converting the fruits into fine powder. This process of freeze-drying is critical as it preserves the integrity of volatile compounds and prevents the degradation of heat-sensitive components, ensuring that the natural chemical profile of the fruits is maintained.

Following the dehydration process, the powdered samples were subjected to ultrasonic extraction using a 50% acidified methanol solution. This extraction was performed at a solid-to-liquid ratio of 1:2 (mg/mL), which facilitated the efficient solubilization of small-molecule polar compounds, including phenols, acids, and sugars. The choice of acidified methanol as an extracting solvent is particularly advantageous for polar compounds due to its ability to disrupt molecular interactions and enhance solute recovery. The extraction was conducted at 70°C for 20 min with an ultrasonic frequency of 40 kHz, a method known for its efficiency in disrupting cell walls and facilitating the rapid release of cellular contents. Post-extraction, the samples were centrifuged at 2200 × g for 20 min. This high-speed centrifugation step is essential in separating the solid residues from the liquid extract, allowing for the collection of a clear supernatant containing the target analytes. The final step in the sample preparation involved filtering the supernatant through a 0.2 μm membrane filter. This filtration process is crucial to remove any remaining particulate matter, ensuring the purity and clarity of the samples for subsequent analytical assessments. Using ultrasonic extraction combined with high-

speed centrifugation and meticulous filtration underscores a thorough approach to sample preparation, aimed at maximizing the recovery and purity of biochemical analytes.

Moreover, this detailed extraction and preparation protocol enables the effective study of the biochemical composition of fruits, providing insights into their nutritional and medicinal properties. Such methodologies are critical in advancing our understanding of plant biochemistry and the potential health benefits of fruit-derived compounds, particularly in the context of natural product research and functional food development.

3.2.4 Derivatization process

The derivatization procedure used in this study was adapted from the method previously described by Ruiz-Matute et al. (2011), incorporating several modifications to optimize the reaction for the specific analysis of fruit extracts. Initially, a 20 μL aliquot of the fruit filtrate was subjected to vacuum centrifugation. This step is crucial as it ensures the complete evaporation of the solvent, leaving behind only the dry residue of the target analytes, which is necessary for effective derivatization. Following the drying process, 150 μL of a 20 mg/mL O-methylhydroxylamine hydrochloride in pyridine solution was added to the residue. This reagent is used to chemically modify the carbonyl groups in the sample, converting them into their oxime derivatives, thus stabilizing them and enhancing their volatility and detectability in subsequent analytical methods. The mixture was then incubated at 40°C with shaking at 150 × g in an incubator shaker for 90 min. This controlled environment facilitates the reaction by providing the necessary heat and agitation to promote efficient chemical conversion.

After the initial reaction phase, the sample temperature was lowered to halt the oximation process. Subsequently, 75 μL of MSTFA was added for silylation, a process that introduces silyl groups to replace labile hydrogen atoms on other functional groups such as hydroxyls, thereby making the molecules more volatile and amenable to gas chromatography. This mixture was then incubated at 37°C with shaking at 150 × g for an additional 30 min, allowing thorough silylation of the analytes. Upon completion of the derivatization process, the samples were cooled to room temperature to stabilize the newly formed derivatives and then transferred into vials for further analysis. This step is critical as it prevents any potential degradation or reversion of the derivatives back to their original forms.

This detailed and carefully controlled derivatization process is pivotal for the

precise analysis of fruit bioactives, particularly when using techniques such as mass spectrometry, where the volatility and stability of the compounds significantly impact the accuracy and reliability of the results.

3.2.5 Gas chromatography and mass spectrometry analysis

GC-MS is a sophisticated analytical technique used extensively to separate and identify volatile compounds. In the study in question, a small volume of the sample (1.0 μL) that had undergone derivatization was introduced into a state-of-the-art Thermo FOCUS GC system, which was integrated with a Thermo ITQ 700 MS detector. This setup, located in Austin, Texas, operates in a splitless mode to maximize sensitivity by ensuring that the entirety of the sample is vaporized and analyzed. The process of compound separation was facilitated by a TR-5 MS fused-silica capillary column, notable for its dimensions (30 meters in length, 0.25 mm in inner diameter) and a film thickness of 0.25 μm, provided by Thermo Scientific, Australia. The initial temperature of the oven was meticulously maintained at 70°C for five minutes, thereafter undergoing a precise temperature ramp at a rate of 5°C per minute up to a final temperature of 300°C, at which it was held steady for an additional five minutes. Helium, chosen for its inert properties and suitability as a carrier gas, was propelled through the system at a consistent flow rate of 1 mL/min. The operational parameters of the GC-MS were optimized for the accuracy and reproducibility of the results. The inlet temperature was carefully fixed at 230°C. Detection of the separated compounds was achieved through mass spectrometry, where mass spectra were recorded across a broad range of 40 to 650 m/z. This was performed in full scan monitoring mode, with a swift scan event time of 0.59 s. The ionization of samples was accomplished via electron impact, set at an energy level of 70 eV, enhancing the detection sensitivity. Furthermore, the detector voltage was regulated at 1.0 kV to achieve optimal ionization efficiency. Critical to the functioning of the mass spectrometer were the temperatures of the ion source, transfer line, and interface, each meticulously controlled at 210, 300, and 300°C, respectively, to ensure stable and consistent analyte detection. The calibration and fine-tuning of the mass spectrometer were conducted using perfluorotributylamine following the stringent specifications recommended by the manufacturer.

Such detailed parameter settings and methodological rigor underscore the reliability and precision of GC-MS in analytical studies, reflecting its indispensable role

in the field of chemical analysis. The ability of this technique to provide detailed compositional insights makes it invaluable in a wide range of scientific investigations, from environmental monitoring to forensic applications.

3.2.6 Statistical analysis

Statistical analysis techniques played a crucial role in the processing and interpretation of data derived from GC-MS outputs in the referenced study. Specifically, the peak extraction task was meticulously executed using the Xcalibur 2.0.7 software, a product of Thermo Fisher Scientific based in San Jose, California. This software was utilized to identify peaks that exhibited a minimum slope of 2000 min−1 and a width that exceeded three seconds, ensuring that only significant chromatographic peaks were considered for further analysis.

For the identification of target compounds, the study employed a dual approach that integrated the use of retention indices and electron ionization mass spectrometry library matches. This involved leveraging both the widely recognized NIST 2.0 commercial library and a specialized in-house database tailored for triterpene analysis. The process of matching involved comparing the original mass spectra from the extracted peaks with those available in the databases, which facilitated the accurate identification of compounds. Quantitative assessments were presented in two formats: milligrams per 100 grams of fresh weight (FW) and milligram content per individual fruit (CPF). All measurements were performed in triplicate to ensure reliability, with results typically expressed as the mean ± standard deviation, adhering to standard scientific reporting practices. The statistical analysis of the data was rigorously conducted using a one-way ANOVA, a statistical method used to discern significant differences across multiple groups. Where significant variances were observed, post-hoc multiple mean comparisons were performed using Waller-Duncan's test, which is effective in identifying specific group differences within the dataset.

These statistical procedures were executed using SPSS 26.0, a sophisticated software package from SPSS Inc., IBM, based in Armonk, New York. The significance of the findings was determined based on a Pearson p-value threshold of less than 0.05, confirming the statistical relevance of observed correlations and differences.

Furthermore, data visualization played a pivotal role in the presentation and interpretation of the results, with the use of GraphPad PRISM 9 from GraphPad Software

Inc., California. This tool allowed for the clear and concise display of complex statistical data, enhancing the interpretability and accessibility of the findings of this study.

3.3 Results

3.3.1 Identification of bioactive compounds in haskap berries

The identification of key bioactive compounds in haskap berries was conducted through a detailed analysis using GC-MS, following a silylation procedure with MSTFA to enhance the detection of these compounds. A detailed chromatographic analysis of the sample P4-37 is illustrated in Fig. 6, showcasing the efficacy of this method.

The chromatogram obtained from the fruit extract of P4-37 revealed 14 distinct peaks, corresponding to the presence of ten specific target compounds, indicating a rich biochemical profile. The mass spectra derived from the methanolic extracts of the P4-37 fruit highlighted the presence of several essential sugars and organic acids, notably fructose (Fig. 6A), glucose (Fig. 6B), mannose (Fig. 6C), sucrose (Fig. 6D), galactose (Fig. 6E), sorbitol (Fig. 6F), malic acid (Fig. 6G), citric acid (Fig. 6H), and quinic acid (Fig. 6I). These findings suggest a complex and nutritionally beneficial composition. Each of the detected compounds was accurately identified through a rigorous process of mass matching, using the comprehensive spectra available in the NIST library. This comparison ensured high confidence in the identification of compounds, reflecting the precision and reliability of the analytical methods employed.

This extensive profiling of haskap berry compounds, enabled by advanced GC-MS technology, not only affirms the presence of these beneficial compounds but also contributes significantly to the scientific understanding of the nutritional and medicinal properties of haskap berries. The identification of such a diverse array of compounds underscores the potential health benefits these berries may offer, particularly due to their sugar and organic acid content, which are critical to both metabolic and antioxidant processes in the human body.

3.3.2 Analysis of sugar and sugar alcohol composition in haskap berries across different ploidy levels

This research aimed to conduct a comprehensive quantitative analysis of the sugar and sugar alcohol composition in haskap berries, focusing on various ploidy levels. The study, spanning two consecutive years (2021 and 2022), detailed its findings in Fig. 7 and

8 and Appendices C, D, and E.

The results revealed that fructose was the most abundant sugar across all samples analyzed, followed by mannose and glucose (Fig. 7). Other sugars, including galactose and sucrose, along with the sugar alcohol sorbitol, were present in lesser amounts. Distinct variations in the sugar content were observed depending on the ploidy level of the haskap plants, with the tetraploid samples exhibiting the highest levels of sugars and the pentaploid samples exhibiting the lowest. The difference in sugar content between the highest and lowest was notably significant, exceeding a six-fold variation.

In the study, five different ploidy categories were examined, ordered from highest to lowest sugar content as tetraploid, hexaploid, pentaploid, aneuploid, and diploid. The year 2021 featured slightly higher overall sugar and sorbitol levels compared to 2022 (Fig. 8). Specifically, diploid samples displayed the lowest levels of fructose and glucose, whereas tetraploid samples contained the highest concentrations of these sugars. Mannose levels were significantly higher in diploid and tetraploid samples, decreasing noticeably in the other ploidies such as pentaploid, hexaploid, and aneuploid. Galactose levels were minimal in pentaploid and aneuploid samples and remained relatively stable in other ploidies. The sucrose content was overall low, with a slight increase observed in tetraploid samples compared to others (Fig. 8). Sorbitol, as the sole sugar alcohol evaluated, exhibited an increasing trend in its concentration with higher ploidy levels (Fig. 8). The study also highlighted the importance of considering morphological differences among samples, expressing the substance content per fruit in milligrams CPF for a more detailed evaluation, as shown in Fig. 7 and 8 and Appendices C and E.

Moreover, when expressing the substance content relative to the weight of fresh fruits per 100 mg, the patterns of sugar and sorbitol content remained consistent. Notably, tetraploid samples demonstrated a significantly higher content per fruit compared to other ploidies, with fructose being almost twice as abundant in these samples compared to those from hexaploid plants, which ranked second in sugar content. The analytical results were further detailed in the visual representations provided in Fig. 7 and 8, illustrating that individual fruits from specific samples, such as P5-4 and P6-9, exhibited higher content values than other ploidy levels when analyzed per milligram CPF. This study thus underscores the substantial variations in sugar and sugar alcohol content across different ploidy levels, suggesting a complex interplay between genetic factors and biochemical

composition in haskap berries. These findings not only advance our understanding of the nutritional characteristics of haskap but also highlight the potential for selective breeding and agricultural practices aimed at enhancing specific desirable traits in the fruit.

3.3.3 Detailed examination of organic acid composition in haskap berries

This investigation aimed to provide an extensive evaluation of the organic acids present in haskap fruits, specifically focusing on malic acid, citric acid, and quinic acid, over the course of two years, 2021 and 2022. The data from this comprehensive study are showcased in Fig. 9 and Appendices C, D, and E.

The findings of this study revealed that while variations in organic acid content were observed among the tested samples, these variations were generally less pronounced than those observed in the sugar and sugar alcohol profiles previously discussed. To offer a more detailed analysis, the content of organic acids was measured in two distinct units, with results illustrated in Fig. 9. A notable trend was identified in the content of quinic acid, which increased consistently with higher ploidy levels. This pattern, consistent across different units of measurement, suggests a significant influence of ploidy on the accumulation of quinic acid within haskap fruits. In contrast, malic acid levels were predominantly higher in tetraploid and aneuploid samples compared to other ploidy levels, indicating a specific affinity of these ploidy levels for malic acid accumulation. The data indicated that malic acid concentrations were particularly elevated in tetraploid and hexaploid samples when expressed in milligrams per fruit (mg CPF), highlighting a unique biochemical profile. Citric acid, known for its pivotal role in metabolic processes, was the most abundant organic acid observed in the haskap berries. However, pentaploid samples exhibited a lower content of citric acid compared to other ploidy levels, suggesting a nuanced interaction between genetic makeup and acid synthesis in these fruits.

The findings from this study contribute significantly to our understanding of the biochemical dynamics of haskap berries, especially in how genetic variations associated with ploidy levels can influence the metabolic pathways leading to organic acid production. This research not only advances the scientific knowledge of plant physiology and biochemistry but also holds implications for breeding strategies aimed at enhancing specific desirable traits in haskap berries, such as improved taste and potential health benefits associated with their organic acid content. By understanding these complex

interactions, researchers and cultivators can better manipulate agricultural practices and breeding techniques to optimize the health-related properties and commercial value of haskap berries, potentially leading to varieties with tailored organic acid profiles suited for specific consumer preferences or health benefits.

3.3.4 Exploring the relationship between ploidy levels and biochemical profiles

This comprehensive study delved into the genetic and biochemical diversity of haskap fruits, with a focus on how variations in ploidy levels, ranging from diploid to hexaploid, correlate with the concentration of various biochemical substances. These relationships were quantitatively analyzed, and the results are detailed in Fig. 10 and Appendix F.

In assessing the correlation between ploidy levels and biochemical content, different trends were observed across various compounds. For malic acid, the coefficient of determination (R^2) values were 0.4359 (mg·100 g^{-1} FW) and 0.2930 (mg CPF), indicating a relatively weak linear correlation with ploidy levels. Citric acid displayed even lower R^2 values of 0.2504 (mg·100 g^{-1} FW) and a negligible 0.0017 (mg CPF), suggesting a very weak or virtually non-existent linear relationship with ploidy levels. In contrast, quinic acid demonstrated a more pronounced linear relationship with ploidy levels, evidenced by R^2 values of 0.8478 (mg·100 g^{-1} FW) and 0.3988 (mg CPF). This indicates a strong correlation between the amount of quinic acid and the ploidy of the haskap fruits, suggesting that higher ploidy levels may influence a greater accumulation of quinic acid. The sugar content analysis revealed varying degrees of correlation with ploidy levels. Mannose exhibited a moderate linear relationship, with R^2 values of 0.4855 (mg·100 g^{-1} FW) and 0.0693 (mg CPF). However, other sugars such as fructose, glucose, galactose, and sucrose exhibited weak to non-existent correlations, with R^2 values ranging from as low as 0.0142 to 0.3124 (mg·100 g^{-1} FW) and from 0.0065 to 0.0561 (mg CPF), highlighting a complex interaction between ploidy levels and sugar synthesis. A standout observation was made with sorbitol, which exhibited a strong linear correlation with ploidy levels when measured in mg·100 g^{-1} FW (R^2 value of 0.9803), though this correlation weakened significantly when calculated per individual fruit.

These findings underscore the intricate relationship between genetic makeup and biochemical composition in plants. The variation in correlation strength across different compounds suggests that ploidy levels may influence specific metabolic pathways

differently. This knowledge not only enhances our understanding of plant biochemistry in relation to genetics but can also inform breeding and cultivation strategies aimed at optimizing certain desirable traits in haskap berries. By targeting specific ploidy levels, growers and breeders may be able to enhance the nutritional and flavor profiles of haskap berries, tailoring them to consumer preferences and potentially improving their health benefits.

3.4 Discussion

3.4.1 Influence of ploidy level on sugar and sugar alcohol profiles

This comprehensive analysis delves into the pivotal role ploidy levels play in the metabolic pathways that regulate sugar biosynthesis in haskap fruits. The variations observed in sugar content across different ploidy levels are not only profound but also indicative of the underlying genetic mechanisms that drive sugar accumulation. These insights are crucial for developing future breeding and cultivation strategies that aim to harness the inherent sugar profiles of haskap fruits to improve both their quality and yield. The interconnection between ploidy levels and sugar content has been a subject of interest not only in haskap but also in other plant species. For instance, a study highlighted similar fluctuations in sugar content under varied environmental conditions in different plant species, suggesting a broader ecological and genetic basis for these variations (Lemoine et al., 2013). Particularly, the direct correlation observed between ploidy levels and the enhanced accumulation of sugars such as fructose and glucose underscores the potent genetic influence on the biosynthesis of these essential compounds. This phenomenon aligns with findings stating that higher ploidy levels in *Cenchrus* species were associated with increased sugar accumulation, thereby supporting the hypothesis that genetic factors significantly contribute to metabolic capacity in polyploid plants (Chandra and Dubey, 2009). Moreover, the distinctive behavior of mannose, which exhibits higher concentrations in diploid and tetraploid haskap samples, indicates a specific genetic predisposition that favors its synthesis in these ploidy levels. This observation is supported by a study on Rangpur lime (*Citrus limonia*), which also highlighted the complex and sometimes unpredictable effects of polyploidization on plant metabolism (Allario et al., 2011).

These findings contribute valuable knowledge to the scientific community,

enhancing our understanding of how genetic complexity associated with ploidy variations can affect plant biochemistry. The ability of polyploid plants to exhibit varied metabolic traits, especially in sugar biosynthesis, offers promising avenues for agricultural innovation. By exploiting the genetic diversity and metabolic capabilities of polyploids, breeders can potentially develop fruit varieties that not only meet consumer taste preferences but also adapt better to environmental challenges, thus improving agricultural sustainability. The insights of this study into the genetic regulation of sugar synthesis across different ploidy levels enrich the growing body of literature and provide a foundational understanding necessary for the strategic development of breeding programs aimed at maximizing the agricultural and nutritional value of polyploid crops such as haskap berries. By integrating these genetic insights with modern biotechnological approaches, the potential to create superior fruit cultivars with optimized sugar content and enhanced overall quality is markedly increased, paving the way for future advancements in plant breeding and biotechnology.

The observation of reduced galactose accumulation in odd and intermediate ploidy samples, specifically in pentaploid and aneuploid haskap berries, points to a significant influence of ploidy level on galactose biosynthesis. This pattern is not unique to haskap but reflects a broader biological phenomenon that can be observed across various fruit species. For example, similar trends were identified in cranberries (*Vaccinium macrocarpon* Ait.), which further supports the hypothesis that variations in ploidy levels may universally affect galactose biosynthesis across different types of fruits (Vorsa and Polashock, 2005). In contrast, the minimal variation in sucrose content across different ploidy levels, along with its status as the least abundant sugar in the studied samples, suggests that sucrose biosynthesis may be less dependent on genetic factors and more on external environmental influences. This notion is supported by studies that highlight the significant role of environmental conditions in influencing sucrose levels in plants, indicating that external factors such as temperature, water availability, and soil quality may play a more pivotal role in sucrose accumulation than genetic predispositions (Smith et al., 1979; Sun et al., 2011).

Additionally, the consistent increase in sorbitol content with ascending ploidy levels in haskap berries underscores the potential direct impact of polyploidy on the biosynthesis and accumulation of this sugar alcohol. This correlation suggests that as the

number of genome sets increases, so does the capacity for sorbitol production, reflecting a possible adaptive metabolic response to polyploidy. This trend is consistent with the substantial morphological differences observed among the various ploidy levels, highlighting the need for precise measurement units such as milligrams per fruit (mg CPF) to accurately assess and compare the substance content across different genetic backgrounds.

Expressing the content of sugars and sugar alcohol in terms of fresh fruit weight is a method that enhances the uniformity of data representation concerning fruit quality. This approach effectively accounts for the considerable morphological and qualitative differences that can occur among fruit samples with varying ploidy levels, as highlighted in recent studies such as those by Li et al. (2023). This metric helps normalize data, providing a more accurate comparison across fruits of different sizes and weights, which is essential for evaluating the impact of genetic factors on fruit composition.

Moreover, while the application of individual fruit mass as a unit of analysis remains less common, its use brings distinct advantages, particularly in the study of fruits with notable morphological variability. Using the weight of individual fruits as a measurement standard allows for a deeper, more detailed understanding of the biochemical characteristics of each fruit sample. This method can be incredibly beneficial when assessing fruits such as haskap berries, which may exhibit significant size variations between different ploidy levels. The advantages of using individual fruit mass include the ability to capture subtle differences in sugar and sugar alcohol concentrations that may be overlooked when using bulk measurements. For example, a study on apple varieties showed that individual fruit weight analysis provided critical insights into how specific genetic modifications affect sugar concentrations within single fruits, thus offering implications for flavor and nutritional quality (Zhang et al., 2019). Furthermore, adopting this nuanced approach can also inform more effective breeding and cultivation strategies. By understanding how individual fruit characteristics correlate with consumer preferences for sweetness and fruit texture, breeders can selectively enhance desirable traits. This tailored approach can lead to the development of fruit varieties that not only meet but exceed market standards for taste and healthfulness. Incorporating these metrics into routine quality assessments could revolutionize how fruit quality is quantified, moving toward more precise and meaningful evaluations that reflect the true variability

within fruit populations. This shift could significantly impact agricultural practices and consumer satisfaction by ensuring that the fruits brought to market consistently meet high standards of quality and taste.

Overall, the employment of both fresh fruit weight and individual fruit mass as units for expressing biochemical contents is instrumental in advancing our understanding of fruit quality. These methods provide a comprehensive picture of how genetic and environmental factors interplay to shape the nutritional and sensory profiles of fruits, thereby guiding future research and development in the field of fruit production.

3.4.2 Influence of ploidy on organic acid profiles

The relationship between ploidy levels and the accumulation of organic acids in haskap fruits underscores the complex interplay between plant genetics and metabolic function. This connection is vividly illustrated through variations in organic acid composition, particularly with a noticeable increase in quinic acid concentrations associated with higher ploidy levels. Such findings reinforce the concept that genetic factors, including ploidy variations, significantly influence plant metabolic pathways.

The correlation of increased quinic acid content with higher ploidy levels aligns with broader botanical research, suggesting a generalized ploidy effect on the biosynthesis of certain organic acids. For example, research conducted on *Lonicera japonica*, a plant species unrelated to haskap, also demonstrated that higher ploidy levels could enhance the levels of specific phytochemicals, including quinic acid (Liang et al., 2011). This parallel suggests a potentially universal mechanism in polyploid plants that favors the accumulation of particular organic compounds. The underlying mechanisms for these observations may involve alterations in enzyme activities responsible for organic acid synthesis, which are affected by the gene dosage effects of polyploidy. Higher copies of genes in polyploid plants could lead to increased enzyme concentrations, thus upregulating the metabolic pathways that produce organic acids such as quinic acid. Additionally, the enhanced ability of polyploid plants to absorb and assimilate nutrients from the soil due to increased cell size and metabolic capacity may also contribute to higher organic acid levels. The distribution of malic acid in haskap berries exhibits a fascinating pattern where tetraploid and aneuploid samples exhibit elevated levels of this organic acid. Such a pattern suggests that these specific ploidy levels may have unique metabolic pathways or genetic predispositions that enhance malic acid production. The

consistency of malic acid content across other ploidy levels, excluding non-integral ones, suggests that environmental influences may play a significant role in shaping these fruit traits, potentially overshadowing genetic factors. This observation aligns with broader research indicating that while genetic makeup provides the framework for biochemical processes, environmental conditions often dictate the extent of their expression.

In contrast, pentaploid haskap fruits demonstrate distinctly lower levels of citric acid compared to other ploidy levels. This deviation suggests unique metabolic or genetic mechanisms at play in pentaploid haskap, which may specifically impact the synthesis pathways of citric acid. Such findings provide a window into the complex interplay between genetics and metabolism in polyploid fruits, suggesting a targeted area for further genetic analysis and metabolic profiling. The differing trends observed across various measurement units—milligrams per 100 grams of fresh weight (mg·100 g^{-1} FW) and milligrams per fruit (mg CPF)—further emphasize the complexity of studying ploidy relationships with organic acid content. These variations underscore the importance of selecting appropriate units of measurement that reflect the true nature of biochemical content, influencing both the interpretation and the conclusions of research studies.

Extending this research, further studies could utilize advanced genomic and metabolomic techniques to map the specific genetic modifications that lead to increased malic or decreased citric acid levels in certain ploidy levels. For example, whole-genome sequencing could identify gene duplications or upregulations responsible for these variations. Additionally, metabolic flux analysis could provide insights into how these genetic changes alter metabolic pathways, leading to varied acid profiles. Moreover, considering the broader implications, understanding how ploidy levels affect acid synthesis could be crucial for agricultural practices. This can assist in breeding programs aimed at cultivating haskap berries with desired organic acid profiles for better flavor and nutritional value. These insights could also guide cultivation practices that align with environmental conditions favoring the optimal production of target acids, enhancing both yield and quality.

In summary, the dynamic relationship between ploidy levels and organic acid content in haskap fruits highlights a fascinating area of genetic and metabolic research, with significant implications for plant science and agricultural biotechnology. This understanding could help develop novel approaches to plant breeding and cultivation that

enhance desired phytochemical qualities in fruits and other plant products.

3.4.3 Exploring the dynamics between ploidy levels and biochemical profiles

The interplay between ploidy levels and the biochemical composition of haskap fruits underscores the intricate and variable nature of genetic influences on plant metabolism. This relationship is highly dependent on the type of biochemical substance being analyzed and the metric of measurement used, highlighting the complex mechanisms that drive these interactions. Notably, biochemicals such as sorbitol, malic acid, quinic acid, and mannose demonstrate varying degrees of correlation with ploidy, revealing the nuanced nature of genetic expression in haskap berries. Within the spectrum of sugars in haskap fruits, fructose, mannose, and glucose are commonly observed in higher concentrations, whereas galactose, sucrose, and sorbitol are less prevalent. Among the organic acids analyzed, citric acid typically predominated, followed by malic and quinic acids. Interestingly, both quinic acid and sorbitol exhibited a robust linear relationship with ploidy levels when measured in milligrams per 100 grams of fresh weight (mg·100 g^{-1} FW). However, this strong correlation significantly weakens when using milligrams per fruit (mg CPF) as a unit of measurement, underscoring the critical impact of measurement choice on the outcomes of such studies. The variability in how different substances correlate with ploidy levels suggests underlying genetic and physiological mechanisms that may influence these biochemical pathways differently. For instance, the strong correlation between sorbitol and higher ploidy levels could indicate a ploidy-induced enhancement in the enzymes responsible for sorbitol synthesis. In contrast, the weaker correlations observed with other sugars and acids may reflect more complex genetic interactions or greater susceptibility to environmental influences.

The findings from this study are crucial for understanding the specific genetic configurations that optimize the synthesis of key biochemicals in haskap berries. This knowledge is invaluable not just for academic research but also for practical applications in agriculture. By identifying how ploidy levels affect biochemical compositions, breeders can more effectively select and cultivate haskap varieties that exhibit desired traits, enhancing both the nutritional value and marketability of the fruit.

Moreover, the implications of this study extend beyond agriculture into the culinary and pharmaceutical industries, where specific biochemical profiles can significantly impact the flavor, health benefits, and therapeutic potential of haskap

berries. For instance, higher concentrations of beneficial acids and sugars can make the berries more attractive for use in functional foods or as natural additives in medicinal products. Further research is essential to delve deeper into the genetic and environmental factors that shape these biochemical profiles.

In summary, the study of ploidy and biochemical content interaction in haskap fruits not only enhances our understanding of plant biochemistry and genetics but also paves the way for targeted breeding strategies and expanded commercial uses of this versatile berry.

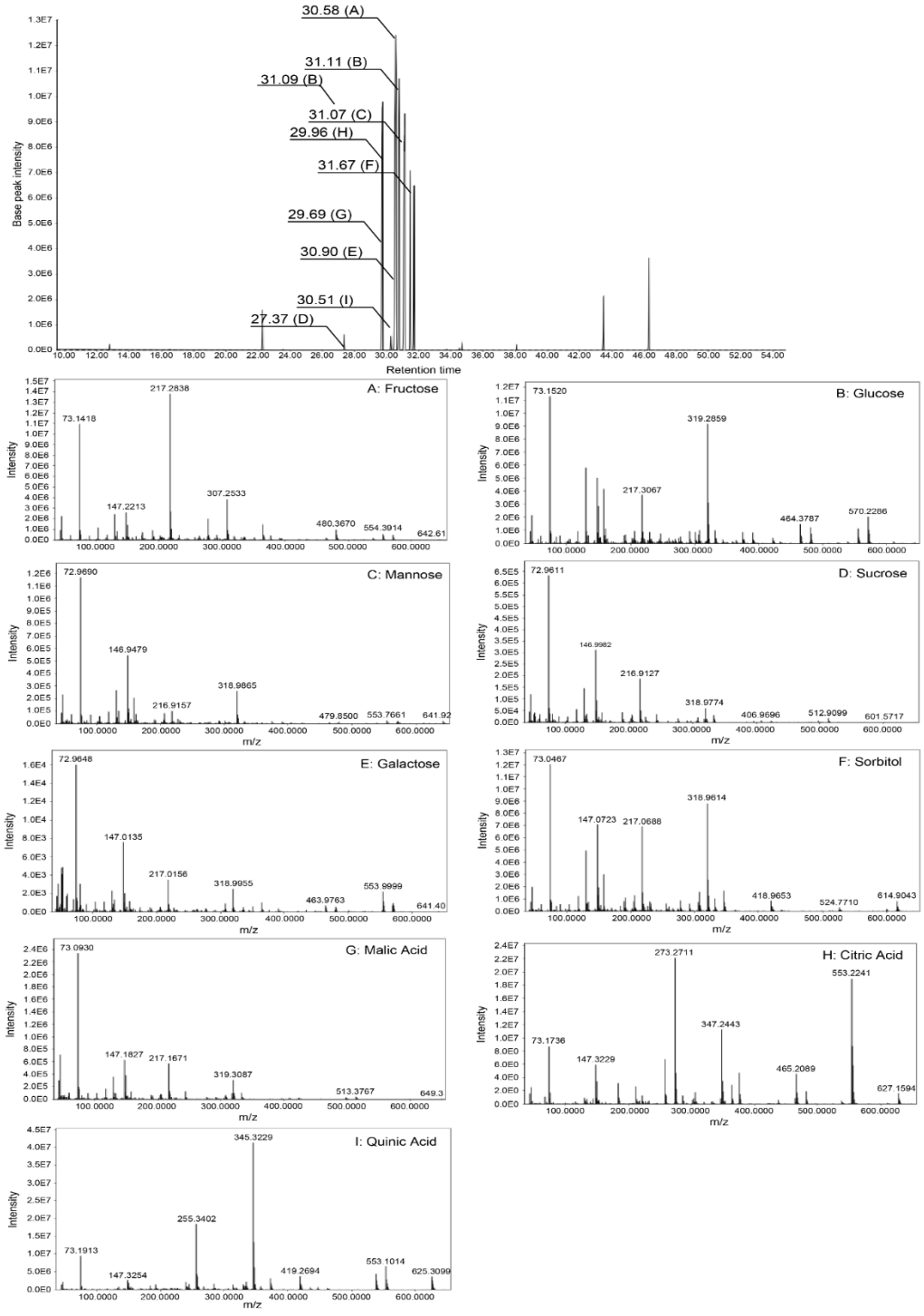


Fig. 6. Gas chromatogram and mass spectra for sample P4-37. Gas chromatogram (A), and mass spectra of fructose (B), glucose (C), mannose (D), sucrose (E), galactose (F), sorbitol (G), malic acid (H), citric acid (I), and quinic acid (J) are shown.

The figures are from " Li, J., & Hoshino, Y. (2024). Elucidating the impact of ploidy level on biochemical content accumulation in haskap (*Lonicera caerulea* L. subsp. *edulis* (Turcz. ex Herder) Hultén) fruits: A comprehensive approach for fruit assessment. " (DOI: 10.1016/j.scienta.2023.112831)

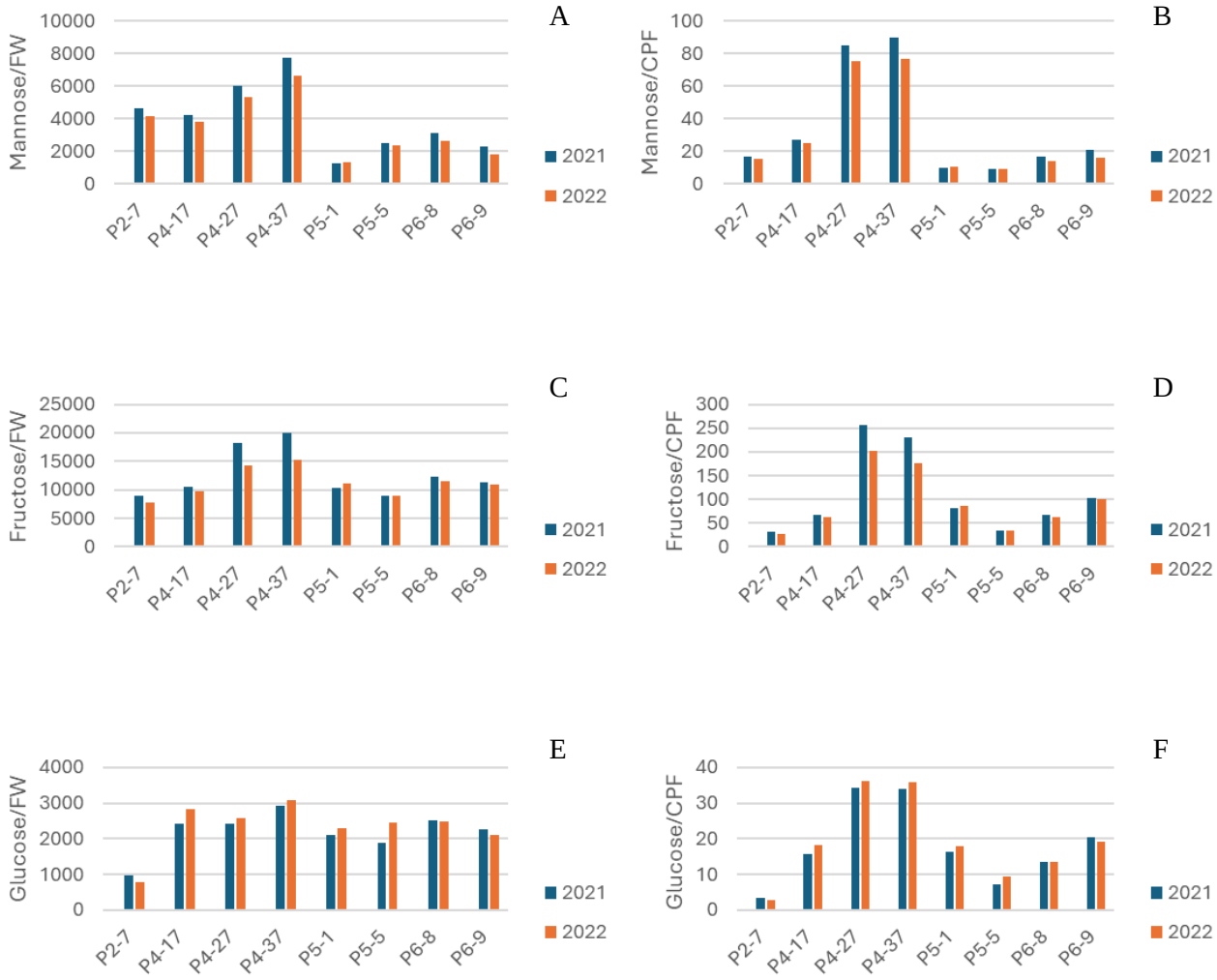


Fig. 7. Content of the mannose (A, B), fructose (C, D), and glucose (E, F) in different ploidy haskap samples collected in 2021 and 2022 in unit of FW (A, C, E) and CPF (B, D, F).

The figures are modified based on " Li, J., & Hoshino, Y. (2024). Elucidating the impact of ploidy level on biochemical content accumulation in haskap (*Lonicera caerulea* L. subsp. *edulis* (Turcz. ex Herder) Hultén) fruits: A comprehensive approach for fruit assessment. " (DOI: 10.1016/j.scienta.2023.112831)

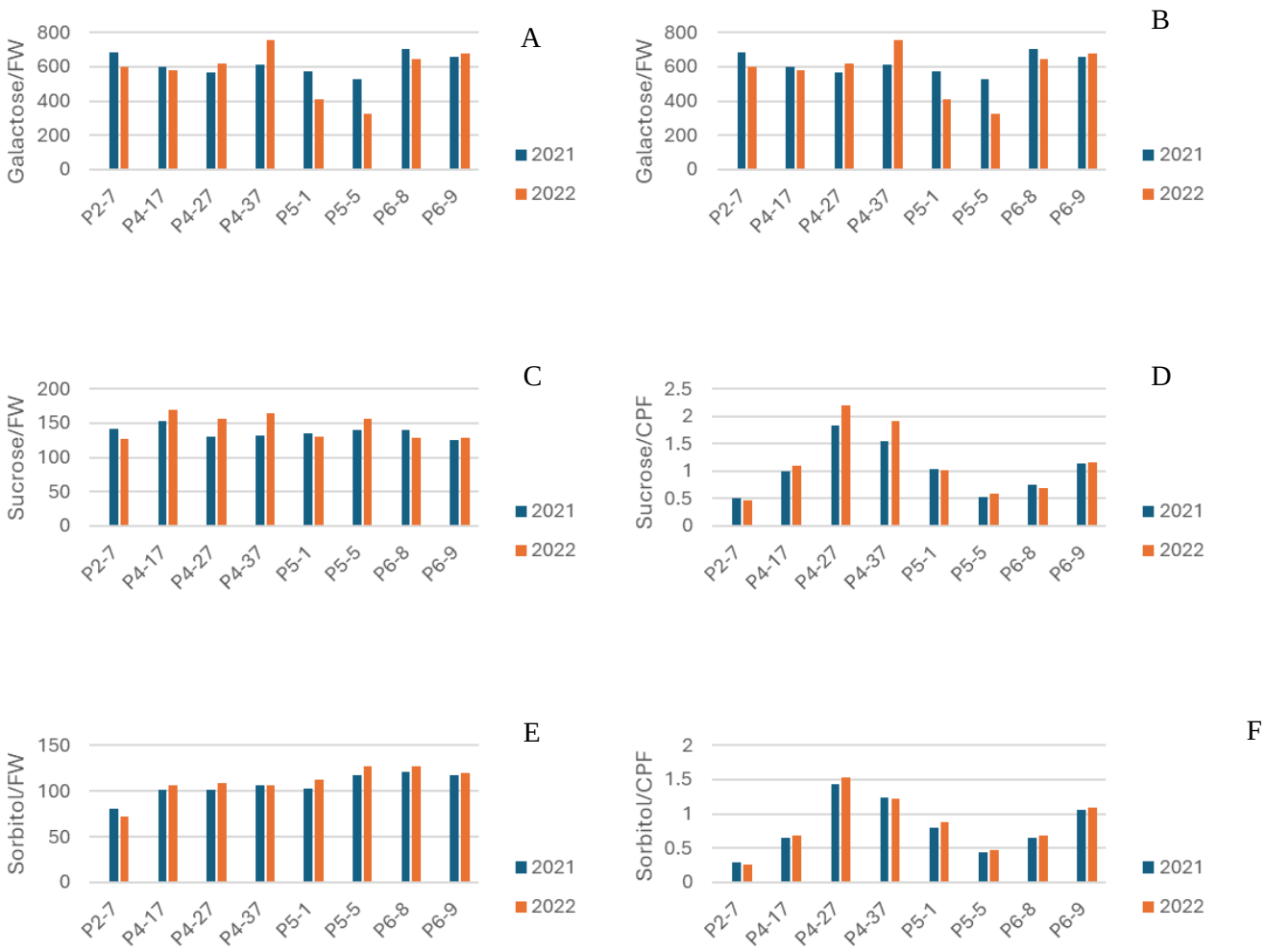


Fig. 8. Content of the galactose (A, B), sucrose (C, D), and sorbitol (E, F) in different ploidy haskap samples collected in 2021 and 2022 in unit of FW (A, C, E) and CPF (B, D, F).

The figures are modified based on " Li, J., & Hoshino, Y. (2024). Elucidating the impact of ploidy level on biochemical content accumulation in haskap (*Lonicera caerulea* L. subsp. *edulis* (Turcz. ex Herder) Hultén) fruits: A comprehensive approach for fruit assessment. " (DOI: 10.1016/j.scienta.2023.112831) © 2024 Jixiao Li, Yoichiro Hoshino, licensed under CC BY 4.0.

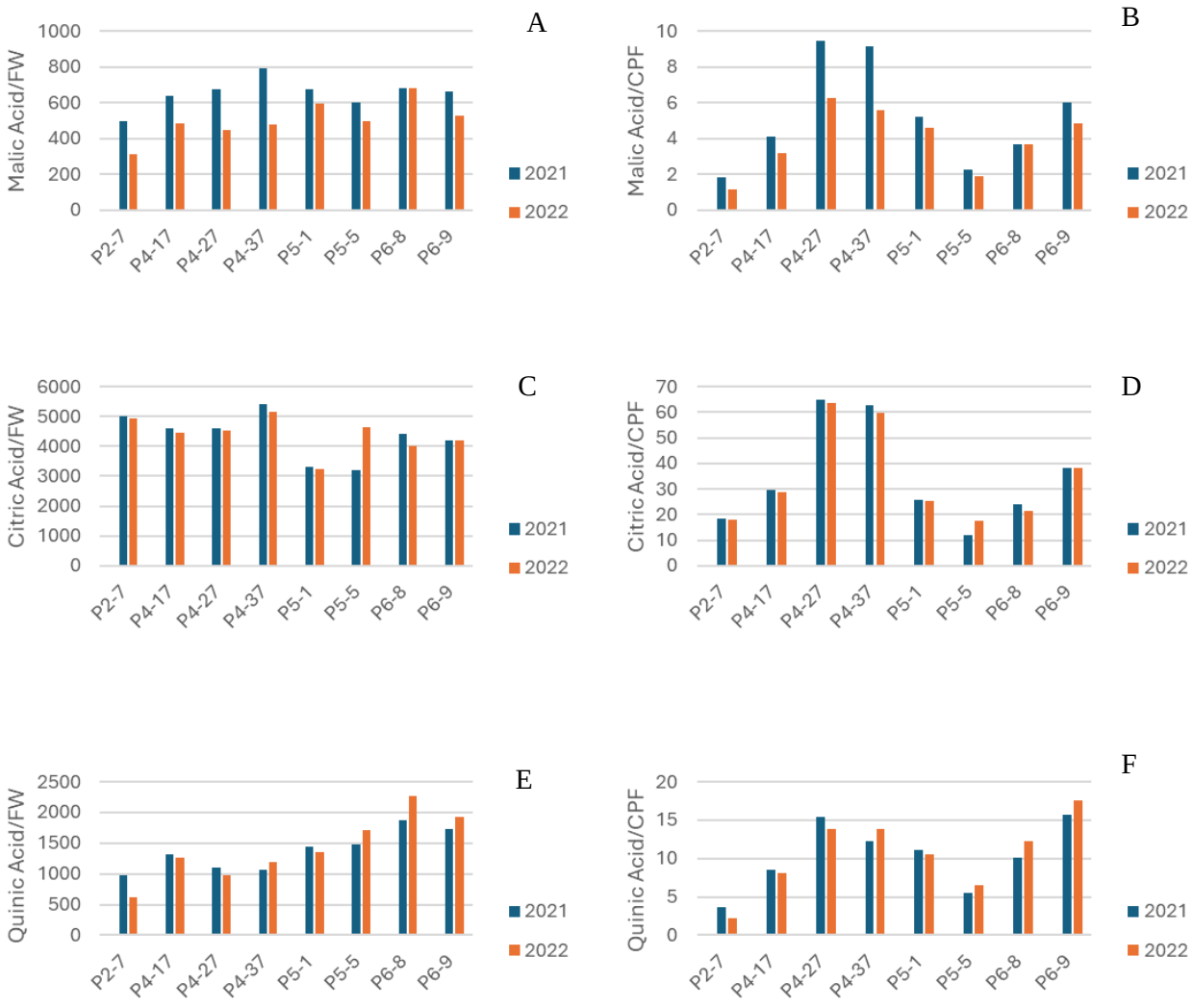


Fig. 9. Content of malic acid (A, B), citric acid (C, D), and quinic acid (E, F) in different ploidy haskap samples collected in 2021 and 2022 in unit of FW (A, C, E) and CPF (B, D, F).

The figures are modified based on " Li, J., & Hoshino, Y. (2024). Elucidating the impact of ploidy level on biochemical content accumulation in haskap (*Lonicera caerulea* L. subsp. *edulis* (Turcz. ex Herder) Hultén) fruits: A comprehensive approach for fruit assessment. " (DOI: 10.1016/j.scienta.2023.112831)

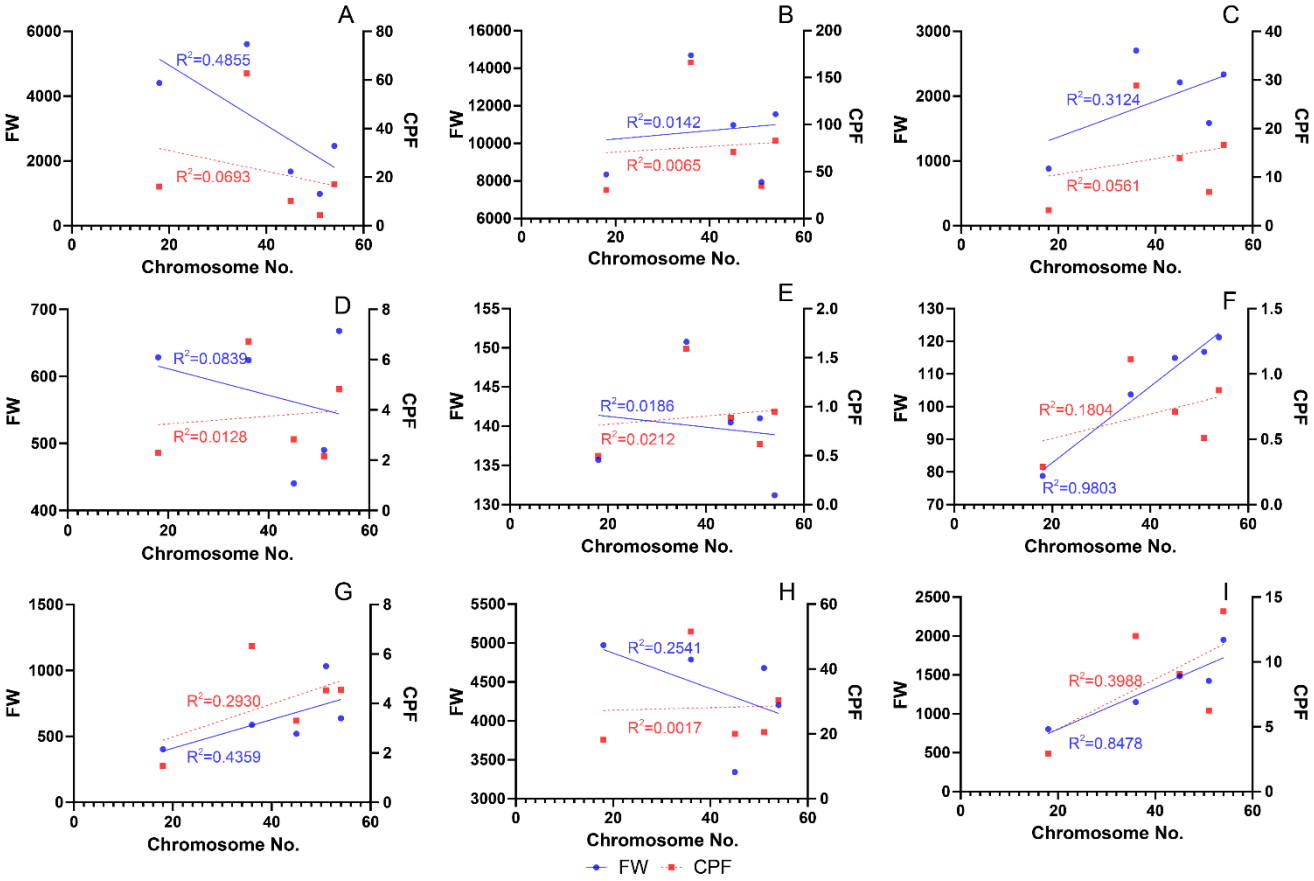


Fig. 10. Analysis of the linear regression between chromosome number and biochemical substance. (A) Mannose, (B) fructose, (C) glucose, (D) galactose, (E) sucrose, (F) sorbitol, (G) malic acid, (H) citric acid, and (I) quinic acid.

Chapter 4

General discussion and future outlook

This research delivers an in-depth analysis of the morphological characteristics and quality attributes of haskap berries, specifically examining how variations in ploidy levels affect these traits. The findings of this study underscore the profound impact that ploidy levels have on multiple dimensions of fruit development and overall quality, providing essential insights into agricultural practices.

Ploidy is a critical genetic factor that influences numerous biological functions in plants, including cell size, metabolic capabilities, and stress response. In haskap berries, variations in ploidy levels significantly affect fruit size, shape, and biochemical composition, such as sugar content, acidity, and antioxidant capacity. These changes, in turn, play a crucial role in determining the overall quality and marketability of the fruit. This study highlights how different ploidy levels can lead to variations in fruit firmness, skin thickness, and juiciness, which are critical quality parameters for consumer preference and processing suitability. For instance, higher ploidy levels are often associated with a larger cell size, which can result in bigger and juicier fruits. This is particularly advantageous for fresh market consumption and can also affect the texture and flavor profile of fruits, making them more appealing to consumers.

4.1 Impact of ploidy on morphological traits

This investigation reaffirms the significant role that ploidy levels play in defining the morphological characteristics of haskap fruits. Specifically, tetraploid variants of these berries tend to show considerable increases in traits such as mass, volume, peel thickness, and seed mass when compared to their diploid counterparts. These morphological improvements are consistent with observations made in other types of fruits; for instance, in yellow-fleshed kiwifruits, both fruit weight and firmness are substantially affected by the ploidy level of the pollen donor, demonstrating the broader applicability of these findings.

Polyploidization, the process of genome doubling that results in increased ploidy, has profound effects not only on the size of individual cells but also on a wide array of morphological, physiological, and biochemical characteristics across plant species. It influences critical aspects such as plant stature, branching patterns, the number and

robustness of roots and shoots, and leaf size and shape. Additionally, it impacts reproductive traits, including the number and size of flowers, seeds, and pollen grains. The interplay of these changes can be observed in the strong positive correlation between fruit mass and volume in haskap berries. There is also a notable negative correlation with peel thickness, illustrating the complex interactions between morphological traits and the overall quality characteristics of the fruit. These relationships highlight how ploidy levels can influence not just a single trait but a comprehensive suite of characteristics that determine the commercial and nutritional value of a fruit. The relevance of ploidy to fruit development is underscored by a wide range of studies across different plant species. For example, research in grapes has shown that increases in ploidy levels can lead to larger berry sizes and changes in biochemical composition, such as sugar content and acidity, which are critical for wine production. Similar studies in berries such as strawberries and blackberries have linked higher ploidy levels to enhanced fruit size and improved resistance to environmental stresses.

Due to these extensive impacts, understanding the effects of ploidy on fruit morphology and quality is crucial for the development of breeding strategies. By selecting for specific ploidy levels, breeders can potentially enhance desirable traits such as fruit size, sweetness, and texture. Moreover, advanced genetic techniques, such as CRISPR/Cas9, offer opportunities to further explore and manipulate ploidy levels with precision, potentially allowing for the custom-tailoring of fruit characteristics to meet specific market demands or grower needs. Future research should continue to explore these relationships, particularly how environmental factors interact with genetic traits influenced by ploidy levels. Such studies are vital to fully harness the potential of ploidy manipulation in agriculture, providing critical insights that can lead to the production of superior fruit cultivars. This study not only enhances our understanding of plant developmental biology but also supports the cultivation of high-quality fruits that can withstand the challenges posed by a changing climate and evolving market preferences.

4.2 Influence of ploidy on sugar biosynthesis

The study of ploidy and its influence on sugar biosynthesis in haskap fruits provides significant insights into the genetic foundations of plant metabolism and fruit quality. Variations in sugar content across different ploidy levels underscore the profound

impact that genetic multiplication can have on the pathways that lead to sugar accumulation. Notably, tetraploid fruits often exhibit enhanced levels of fructose and glucose, which suggests sophisticated genetic mechanisms that regulate the biosynthesis of these essential sugars.

The enhanced sugar content in tetraploid haskap berries can be attributed to the increased gene dosage effect, where multiple copies of genes related to sugar metabolism may lead to enhanced enzymatic activities that drive the synthesis of fructose and glucose. This is consistent with findings from studies on other fruit crops, such as grapes and apples, where increased ploidy levels have been linked to higher sugar concentrations, impacting not only taste but also the market value of the fruits.

Moreover, the variability in the accumulation of sugars such as mannose and sorbitol across different ploidy levels further highlights the complex interplay of genetic factors. Each sugar appears to respond differently to changes in gene copies, indicating that specific genes or gene clusters may exert variable effects on their biosynthetic pathways. This complexity is illustrated by the behavior of mannose and sorbitol, which show significant variations across ploidy levels, suggesting that the genetic control over these sugars involves multiple regulatory mechanisms. Additionally, this research reveals a noticeable reduction in galactose accumulation in odd and intermediate ploidy levels, such as pentaploid and aneuploid samples. This finding could point to disruptions or modifications in the genetic pathways responsible for galactose biosynthesis, which are affected by changes in chromosome numbers. These alterations could inhibit the usual synthesis routes or activate alternative pathways that reduce the production of galactose. In contrast, the minimal variation observed in sucrose content across different ploidy levels, combined with the relatively low abundance of sucrose, suggests that environmental factors rather than genetic modifications may play a more significant role in its biosynthesis. Factors such as temperature, sunlight exposure, and soil nutrients could influence sucrose levels more prominently, overshadowing the genetic effects. The consistent increase in sorbitol content with rising ploidy levels is particularly intriguing, indicating a direct genetic impact of polyploidy on the biosynthesis and accumulation of this sugar alcohol. Sorbitol, often involved in osmoregulation and stress response in plants, could confer additional advantages to higher ploidy levels under varying environmental stresses, suggesting a possible adaptive benefit of increased ploidy.

In summary, the influence of ploidy on sugar biosynthesis in haskap fruits reveals a complex landscape of genetic and environmental interactions. Understanding these dynamics can aid in breeding programs aimed at enhancing fruit sweetness and overall quality. Further research should explore the specific genetic pathways affected by ploidy alterations, potentially using genomic and transcriptomic approaches to map the exact changes in gene expression associated with different ploidy levels. This could lead to more targeted breeding strategies that exploit these genetic variations to produce optimally flavored and nutritionally superior fruits.

4.3 Relationship between ploidy and organic acid content

The study of the relationship between ploidy levels and the content of organic acids in haskap fruits opens a window into the sophisticated interplay of genetics and metabolism in plants. This relationship is crucial as it influences not only the flavor profile of the fruit but also its nutritional and health-promoting properties.

Ploidy significantly affects the physiological and metabolic processes of a plant, including the biosynthesis of organic acids. In haskap berries, an increase in ploidy level corresponds with higher concentrations of quinic acid. This trend is consistent with observations in other plant species, such as the studies conducted on sage (*Salvia officinalis*), which demonstrated that higher ploidy levels enhanced the production of essential oils and other phytochemicals, including organic acids. The increase in the levels of quinic acid with higher ploidy levels could be linked to enhanced gene expression related to its biosynthetic pathways, suggesting a genetic basis for the modulation of this acid. Furthermore, the variation in malic and citric acid contents across different ploidy levels in haskap berries highlights the complexity of metabolic pathways influenced by ploidy. Malic acid, known for its role in the Krebs cycle, exhibits varied levels that suggest a differential genetic regulation that may be tied to the efficiency of photosynthesis and respiration processes, which are influenced by chromosome numbers. Citric acid, another critical component of the Krebs cycle, also exhibits unique patterns across ploidy levels, underscoring the intricate genetic controls affecting its synthesis. The metabolic pathways responsible for the synthesis of these acids are highly complex and involve multiple enzymes and cofactors, which are themselves subject to genetic control. For example, the enzyme phosphoenolpyruvate carboxylase, which plays a

pivotal role in the formation of oxaloacetate from pyruvate—a precursor for malic acid—could be overexpressed in higher ploidy levels, leading to enhanced malic acid production. This enzymatic activity could be enhanced due to the gene dosage effect, where multiple gene copies in polyploid cells lead to enhanced RNA and protein synthesis.

Moreover, the interaction between ploidy and environmental factors also plays a crucial role. For instance, light intensity, water availability, and nutrient status can affect the physiological state of the plant and, by extension, the metabolic pathways that synthesize organic acids. Research on citrus fruits revealed that environmental stresses could alter the concentration of organic acids, interacting with genetic factors to influence the overall profile observed in polyploid plants (Zheng et al., 2015).

In light of these findings, future research could benefit from a deeper exploration of the specific genetic mechanisms at play, possibly through genomic and proteomic studies. Such studies could provide a more detailed understanding of how ploidy influences gene expression levels and enzyme activities involved in organic acid metabolism. Additionally, integrating metabolic engineering and CRISPR technology could offer pathways to manipulate these metabolic processes in haskap berries, aiming to enhance desired organic acid profiles for improved flavor and health benefits.

Overall, the relationship between ploidy and organic acid content in haskap berries is a testament to the complex and dynamic nature of plant genetics and metabolism, offering promising avenues for agricultural and nutritional advancements through targeted genetic breeding and biotechnological interventions.

4.4 Biochemical composition of haskap and comparison with other fruits

The investigation into the biochemical composition of haskap reveals notable trends and patterns consistent with findings from studies on other fruits, particularly concerning the influence of ploidy levels. Research indicates that the majority of chemical compounds in haskap, including sugars and organic acids, increase as the ploidy level rises from diploid to tetraploid. This trend aligns with studies on other fruits such as grapes and watermelons, where higher ploidy levels are associated with enhanced sugar content. For instance, tetraploid grapes and watermelons typically exhibit higher concentrations of glucose and fructose compared to their diploid counterparts,

demonstrating a clear additive effect of increased ploidy on sugar accumulation (Liang et al., 2011; Wan et al., 2010). Similarly, tetraploid haskap variants show significantly higher levels of sugars, suggesting a comparable genetic mechanism.

However, the patterns observed in haskap for ploidy levels beyond tetraploid present a more complex picture. While research on other fruits often focuses on diploid, triploid, and tetraploid levels, this analysis extends to pentaploid and hexaploid in this haskap study. Most biochemical content, including key nutrients, tends to decline in these higher ploidy levels. Notably, only specific compounds such as sorbitol and quinic acid continue to increase beyond the tetraploid stage. This contrasts with the general trend seen in many other fruits where studies are sparse for ploidy levels above tetraploid, thus providing new insights into the polyploidy effects in haskap.

Further findings suggest that the relationship between ploidy and biochemical composition is not always linear. For example, in watermelons, while total sugar content increases with ploidy, the trends for specific sugars like fructose and sucrose can vary (Wan et al., 2010). Similarly, in haskap, although total sugar content generally rises with increasing ploidy up to tetraploid, individual sugar profiles exhibit significant variability (Li & Hoshino, 2024). This indicates that ploidy-induced changes in biochemical composition are influenced by complex genetic and metabolic interactions, rather than a simple linear augmentation.

Another critical aspect highlighted by the research is the phenomenon of the gigas effect, commonly associated with polyploidy. This effect, characterized by increased cell size and enhanced vigor, is typically evident in the transition from diploid to tetraploid. However, in haskap, this effect does not consistently extend to higher ploidy levels, as seen by the decline in most biochemical compounds in pentaploid and hexaploid variants. This finding is significant as it challenges the conventional understanding of the gigas effect and suggests that higher ploidy levels may introduce metabolic burdens that counteract the benefits observed at lower ploidy increases.

The comparison of haskap's biochemical composition with other fruits underscores the diverse responses to polyploidy across species. For instance, the variations in sugar and acid metabolism in haskap reflect similar trends observed in blueberries, where maturation stages significantly influence biochemical profiles, and the role of specific enzymes in sugar and acid metabolism becomes crucial (Li et al., 2020).

Such parallels highlight the importance of considering species-specific responses when analyzing the impact of polyploidy.

In summary, this study provides a detailed examination of the biochemical changes in haskap across different ploidy levels and places these findings within the broader context of polyploidy research in other fruits. The insights gained deepen the understanding of the genetic and metabolic mechanisms underlying these changes and offer practical implications for breeding programs aimed at optimizing fruit quality and nutritional value. Future research should continue to explore these complex interactions, particularly focusing on higher ploidy levels, to fully elucidate the potential and limitations of polyploidy in enhancing fruit crop traits.

4.5 Implications for breeding and cultivation

The detailed examination of the interplay between morphological traits and quality characteristics in haskap fruits, as observed in this study, offers valuable insights that are critical for refining breeding programs and cultivation techniques. The findings emphasize the importance of traits such as fruit size, which are directly linked to agricultural yield and economic value. These traits are particularly crucial as they significantly affect consumer preferences and purchasing decisions, highlighting the importance of targeted breeding to meet market demands. The positive correlation between chromosome number (ploidy) and specific structural characteristics of the fruit, such as peel thickness and seed mass, underscores the potential adaptive mechanisms of haskap plants. These traits may serve crucial roles in the reproductive strategies and natural defense mechanisms of the plant, possibly offering protection against pests and diseases while supporting the overall reproductive success of the plant. This correlation provides breeders and growers with a scientific basis to select for optimal ploidy levels that enhance these desirable traits, thus improving not only the marketability but also the resilience of the plants.

Due to the influence of fruit size and appearance on marketability, understanding how ploidy levels affect these traits allows for more precise genetic selection in breeding programs. By selecting plants with ideal ploidy levels, growers can cultivate fruits that not only meet but exceed consumer expectations for size, appearance, and taste, which can lead to higher market values and increased consumer satisfaction. Furthermore, the

implications of this study extend beyond immediate agricultural productivity and into the realm of sustainable agriculture. Future research could delve into how variations in ploidy impact other bioactive components of haskap fruits, such as antioxidants, vitamins, and phytochemicals. These components are crucial for their health benefits, and understanding their interaction with ploidy could lead to the development of fruit varieties with enhanced nutritional profiles. Such research would not only add value to haskap berries as a health-promoting fruit but also align with global trends toward healthier food options.

Moreover, the potential to enhance the natural defense capabilities of haskap plants through ploidy manipulation offers an intriguing avenue for reducing reliance on chemical pesticides. By naturally increasing the resistance of the plant to environmental stresses and pests through selective breeding, growers can achieve more sustainable cultivation practices, reducing environmental impact and enhancing the appeal of haskap berries as an eco-friendly fruit option. To fully exploit these insights, interdisciplinary approaches involving genetics, agronomy, and environmental science are necessary. For instance, advanced genomic tools such as CRISPR and QTL mapping could be used to identify and manipulate the genes responsible for desirable traits influenced by ploidy levels. Additionally, agronomic studies focusing on the best cultivation conditions for optimized ploidy expressions can help in establishing more effective and sustainable cultivation protocols.

In conclusion, the complex relationships revealed between ploidy levels, morphological traits, and quality characteristics in haskap berries not only provide a roadmap for future breeding and cultivation strategies but also underscore the potential for these approaches to enhance the sustainability, nutritional value, and marketability of this promising fruit crop.

4.6 Expanding the implications of ploidy research in haskap cultivation and beyond

The findings of this study underscore the profound influence of ploidy on the genetic enhancement and agricultural practices associated with haskap berries. By strategically manipulating ploidy levels, researchers and cultivators can significantly optimize crop yield, improve fruit quality, and enhance adaptability to varying

environmental and market conditions. The detailed exploration of the impact of ploidy in this study not only offers actionable insights into the cultivation and breeding of haskap but also sets a precedent for applying similar genetic strategies to other crop species.

Ploidy manipulation provides a powerful tool for addressing some of the critical challenges in modern agriculture, such as climate change, pest resistance, and the need for higher nutritional values. The ability to control and utilize variations in ploidy allows for the development of crop varieties that are better suited to withstand environmental stressors and meet consumer preferences for taste and nutritional content. For instance, polyploid crops often exhibit greater biomass and enhanced secondary metabolite production, which are desirable traits for both biofuel production and pharmaceutical applications. The research conducted on haskap berries offers essential references that are applicable not only within the domain of fruit cultivation but also in broader agricultural contexts. For example, the techniques and findings could inform the breeding strategies of other economically significant crops such as wheat, potatoes, and apples, where ploidy level manipulations have already shown promise in improving crop characteristics. Further investigations into the genetic and physiological mechanisms underlying the observed ploidy effects are necessary to deepen our understanding and refine the applications. Such studies could involve advanced genomic tools to dissect the genetic basis of ploidy-related traits, coupled with physiological studies to elucidate how these genetic changes translate into improved plant performance. For instance, transcriptomic analyses and gene-editing technologies such as CRISPR/Cas9 could be used to identify and manipulate specific genes responsible for advantageous traits in polyploid plants. Moreover, extending the research to include a wider array of environmental conditions and exploring the interaction between ploidy levels and ecological factors could provide deeper insights into the adaptability of polyploid plants. This approach would not only enhance the robustness of the crops but also contribute to more sustainable agricultural practices by reducing dependency on chemical inputs.

Ultimately, the knowledge gained from this study and similar studies has the potential to revolutionize the way we approach plant breeding and cultivation, offering opportunities to enhance crop quality and sustainability. By broadening the applications of ploidy research, we can unlock new possibilities in the culinary and pharmaceutical fields, creating varieties with specific traits that cater to diverse needs and markets. This

study paves the way for a new era of ploidy-based enhancements in agriculture, promising significant advancements in the quality and utility of crops across various industries.

References

Adams, K. L., & Wendel, J. F. (2005). Polyploidy and genome evolution in plants. *Current opinion in plant biology*, *8*(2), 135-141.

Ahuja, M. R. (2005). Polyploidy in gymnosperms: revisited. *Silvae Genetica*, *54*(1-6), 59-69.

Allario, T., Brumós, J., Colmenero-Flores, J., Tadeo, F., Froelicher, Y., Talón, M., Navarro, L., Ollitrault, P., & Morillon, R. (2011). Large changes in anatomy and physiology between diploid Rangpur lime (*Citrus limonia*) and its autotetraploid are not associated with large changes in leaf gene expression. *Journal of experimental botany*, *62*(8), 2507-2519.

Allwood, J. W., Erban, A., de Koning, S., Dunn, W. B., Luedemann, A., Lommen, A., ... & Goodacre, R. (2009). Inter-laboratory reproducibility of fast gas chromatography–electron impact–time of flight mass spectrometry (GC–EI–TOF/MS) based plant metabolomics. Metabolomics, 5, 479-496.

Balao, F., Herrera, J., & Talavera, S. (2011). Phenotypic consequences of polyploidy and genome size at the microevolutionary scale: a multivariate morphological approach. *New Phytologist*, *192*(1), 256-265.

Bieniek, A. (2021). Biological Properties of Honeysuckle (*Lonicera caerulea* L.): A Review: The nutrition, health properties of honeysuckle. *Agrobiodiversity for Improving Nutrition, Health and Life Quality*. https://doi.org/10.15414/ainhlq.2021.0027.

Catalano, C., Abbate, L., Motisi, A., Crucitti, D., Cangelosi, V., Pisciotta, A., ... & Carra, A. (2021). Autotetraploid emergence via somatic embryogenesis in Vitis vinifera induces marked morphological changes in shoots, mature leaves, and stomata. *Cells*, *10*(6), 1336.

Celli, G., Ghanem, A., & Brooks, M. (2014). Haskap Berries (*Lonicera caerulea* L.)—a Critical Review of Antioxidant Capacity and Health-Related Studies for Potential Value-Added Products. *Food and Bioprocess Technology*, 7, 1541-1554. https://doi.org/10.1007/s11947-014-1301-2.

Chandra, A., & Dubey, A. (2009). Assessment of ploidy level on stress tolerance of *Cenchrus* species based on leaf photosynthetic characteristics. *Acta physiologiae plantarum*, *31*, 1003-1013.

Chandra, A., & Dubey, A. (2010). Effect of ploidy levels on the activities of delta(1)-pyrroline-5-carboxylate synthetase, superoxide dismutase and peroxidase in *Cenchrus* species grown under water stress. *Plant physiology and biochemistry : PPB*, 48 1, 27-34 . https://doi.org/10.1016/j.plaphy.2009.10.003.

Chen, J., Coate, J., & Meru, G. (2020). Editorial: Artificial Polyploidy in Plants. *Frontiers in Plant Science*, 11. https://doi.org/10.3389/fpls.2020.621849.

Chen, Z. J. (2007). Genetic and epigenetic mechanisms for gene expression and phenotypic variation in plant polyploids. *Annu. Rev. Plant Biol.*, *58*, 377-406.

Chen, Z. (2007). Genetic and epigenetic mechanisms for gene expression and phenotypic variation in plant polyploids. *Annual review of plant biology*, 58, 377-406 . https://doi.org/10.1146/ANNUREV.ARPLANT.58.032806.103835.

Cheng, Z., Bao, Y., Li, Z., Wang, J., Wang, M., Wang, S., Wang, Y., Wang, Y., & Li, B. (2022). *Lonicera caerulea* (Haskap berries): a review of development traceability, functional value, product development status, future opportunities, and challenges. *Critical Reviews in Food Science and Nutrition*, *63*(27), 8992-9016.

Comai, L. (2005). The advantages and disadvantages of being polyploid. *Nature reviews genetics*, *6*(11), 836-846.

De Silva, A. K. H., & Rupasinghe, H. V. (2020). Polyphenols composition and anti-diabetic properties in vitro of haskap (Lonicera caerulea L.) berries in relation to cultivar and harvesting date. *Journal of Food Composition and Analysis, 88*, 103402.

Corneillie, S., Storme, N., Acker, R., Fangel, J., Bruyne, M., Rycke, R., Geelen, D., Willats, W., Vanholme, B., & Boerjan, W. (2018). Polyploidy Affects Plant Growth and Alters Cell Wall Composition1[OPEN]. *Plant Physiology*, 179, 74 - 87. https://doi.org/10.1104/pp.18.00967.

Dashbaldan, S., Becker, R., Pączkowski, C., & Szakiel, A. (2019). Various patterns of composition and accumulation of steroids and triterpenoids in cuticular waxes from screened Ericaceae and Caprifoliaceae berries during fruit development. *Molecules, 24*(21), 3826.

De Kort, H., Legrand, S., Honnay, O., & Buckley, J. (2022). Transposable elements maintain genome-wide heterozygosity in inbred populations. *Nature Communications, 13*(1), 7022.

Fujita, R., Hayasaka, T., Jin, S., Hui, S., & Hoshino, Y. (2020 a). Comparison of anthocyanin distribution in berries of Haskap (*Lonicera caerulea* subsp. *edulis* (Turcz. ex. Herder) Hultén), Miyama-uguisukagura (*Lonicera gracilipes* Miq.), and their interspecific hybrid using imaging mass spectrometry. *Plant science : an international journal of experimental plant biology*, 300, 110633 . https://doi.org/10.1016/j.plantsci.2020.110633.

Fujita, R., Jin, S., Hayasaka, T., Matoba, K., & Hoshino, Y. (2020 b). Evaluation of Fruit Anthocyanin Composition by LC/MS in Interspecific Hybrids Between Haskap (*Lonicera caerulea* subsp. *edulis* (Turcz. ex. Herder) Hultén) and Miyama-uguisukagura (*Lonicera gracilipes* Miq.). *The Horticulture Journal*. https://doi.org/10.2503/hortj.utd-139.

Gawroński, J., Żebrowska, J., Pabich, M., Jackowska, I., Kowalczyk, K., & Dyduch-

Siemińska, M. (2020). Phytochemical Characterization of Blue Honeysuckle in Relation to the Genotypic Diversity of Lonicera sp. *Applied Sciences*. https://doi.org/10.3390/APP10186545.

Gołba, M., Sokół-Łętowska, A., & Kucharska, A. Z. (2020). Health properties and composition of honeysuckle berry *Lonicera caerulea* L. An update on recent studies. *Molecules*, *25*(3), 749.

Grygorieva, O., Klymenko, S., Kuklina, A., Vinogradova, Y., Vergun, O., Sedlackova, V. H., & Brindza, J. (2021). Evaluation of *Lonicera caerulea* L. genotypes based on morphological characteristics offruits germplasm collection. *Turkish Journal of Agriculture and Forestry*, *45*(6), 850-860.

Hegarty, M. J., & Hiscock, S. J. (2008). Genomic clues to the evolutionary success of polyploid plants. *Current biology*, *18*(10), R435-R444.

Husband, B. C., Baldwin, S. J., & Suda, J. (2013). The incidence of polyploidy in natural plant populations: major patterns and evolutionary processes. In *Plant genome diversity volume 2: physical structure, behaviour and evolution of plant genomes* (pp. 255-276). Vienna: Springer Vienna.

Joseph, S., Edirisinghe, I., & Burton-Freeman, B. (2014). Berries: anti-inflammatory effects in humans. *Journal of agricultural and food chemistry*, 62 18, 3886-903 . https://doi.org/10.1021/jf4044056.

Kalt, W., Cassidy, A., Howard, L. R., Krikorian, R., Stull, A. J., Tremblay, F., & Zamora-Ros, R. (2020). Recent research on the health benefits of blueberries and their anthocyanins. *Advances in Nutrition*, *11*(2), 224-236.

Kan, M., Huang, T., & Zhao, P. (2022). Artificial chromosome technology and its potential application in plants. *Frontiers in Plant Science, 13*, 970943.

Kännaste, A., Copolovici, L., & Niinemets, Ü. (2014). Gas chromatography–mass spectrometry method for determination of biogenic volatile organic compounds emitted by plants. *Plant isoprenoids: methods and protocols*, 161-169.

Khattab, R., Brooks, M., & Ghanem, A. (2016). Phenolic Analyses of Haskap Berries (*Lonicera caerulea* L.): Spectrophotometry Versus High Performance Liquid Chromatography. *International Journal of Food Properties*, 19, 1708 - 1725. https://doi.org/10.1080/10942912.2015.1084316.

Lavania, U. C. (2005). Genomic and ploidy manipulation for enhanced production of phyto-pharmaceuticals. *Plant Genetic Resources*, *3*(2), 170-177.

Lee, H. J., & Sharma, A. (2020). Haskap Berries (*Lonicera caerulea* L.): Phytochemical Constituents and Health Benefits. In *Asian Berries* (pp. 249-278). CRC Press.

Lemoine, R., Camera, S., Atanassova, R., Dédaldéchamp, F., Allario, T., Pourtau, N., Bonnemain, J., Laloi, M., Coutos-Thévenot, P., Maurousset, L., Faucher, M., Girousse, C., Lemonnier, P., Parrilla, J., & Durand, M. (2013). Source-to-sink transport of sugar and regulation by environmental factors. *Frontiers in plant science*, *4*, 272.

Levin, D. (1983). Polyploidy and Novelty in Flowering Plants. *The American Naturalist*, 122, 1 - 25. https://doi.org/10.1086/284115.

Li, J., & Hoshino, Y. (2024). Elucidating the impact of ploidy level on biochemical content accumulation in haskap (*Lonicera caerulea* L. subsp. *edulis* (Turcz. ex Herder) Hultén) fruits: A comprehensive approach for fruit assessment. *Scientia Horticulturae*, *327*, 112831.

Li, J., Suzuki, Y., & Hoshino, Y. (2023). Phenotypic analysis of polyploid and aneuploid haskap (*Lonicera caerulea* L. subsp. *edulis* (Turcz. ex Herder) Hultén) plants and their progeny production. *Euphytica*, *219*(5), 52.1

Li, W. D., Biswas, D. K., Xu, H., Xu, C. Q., Wang, X. Z., Liu, J. K., & Jiang, G. M. (2009). Photosynthetic responses to chromosome doubling in relation to leaf anatomy in *Lonicera japonica* subjected to water stress. *Functional plant biology*, *36*(9), 783-792.

Li, X., Li, C., Sun, J., & Jackson, A. (2020). Dynamic changes of enzymes involved in sugar and organic acid level modification during blueberry fruit maturation. *Food Chemistry*, *309*, 125617. https://doi.org/10.1016/j.foodchem.2019.125617

Liang, Z., Sang, M., Ma, A., Zhao, S., Zhong, G., & Li, S. (2011). Inheritance of sugar and acid contents in the ripe berries of a tetraploid × diploid grape cross population. *Euphytica*, 182, 251-259. https://doi.org/10.1007/s10681-011-0487-x.

Madani, H., Escrich, A., Hosseini, B., Sanchez-Muñoz, R., Khojasteh, A., & Palazón, J. (2021). Effect of Polyploidy Induction on Natural Metabolite Production in Medicinal Plants. *Biomolecules*, 11. https://doi.org/10.3390/biom11060899.

McClintock, B. (1939). The behavior in successive nuclear divisions of a chromosome broken at meiosis. *Proceedings of the National Academy of Sciences*, 25(8), 405-416. https://doi.org/10.1073/PNAS.25.8.405.

Miyashita, T., Araki, H., & Hoshino, Y. (2011). Ploidy distribution and DNA content variations of *Lonicera caerulea* (Caprifoliaceae) in Japan. *Journal of plant research*, *124*, 1-9.

Miyashita, T., & Hoshino, Y. (2015). Interploid and intraploid hybridizations to produce polyploid Haskap (*Lonicera caerulea* var. *emphyllocalyx*) plants. *Euphytica*, *201*, 15-27.

Moing, A. (2000). Sugar alcohols as carbohydrate reserves in some higher plants. *Developments in crop science*, 26, 337-358. https://doi.org/10.1016/S0378-519X(00)80017-3.

Neenu, M. G., Aswathi, A., & Prasath, D. (2024). Synthetic polyploidy in spice crops: A

review. *Crop Science*, *64*(1), 2-23.

Oh, E. U., Jeong, S. Y., Kim, J. Y., & Song, K. J. (2021). Response of fruit set and quality and seed formation to ploidy levels of pollen donor in yellow-fleshed kiwifruits. *Horticulture, Environment, and Biotechnology*, *62*, 9-15.

Olfati, J. A., Samizadeh, H., Peyvast, G., Rabiei, B., & Khodaparast, S. A. (2010). Parental line selection for cucumber hybrid seed production by principal components analysis. *International journal of vegetable science*, *16*(4), 316-325.

Omere, E., Nwaoguala, C., & Emede, T. (2023). Polyploidy and its relevance in crop improvement. *Nigerian Journal of Biotechnology*. https://doi.org/10.4314/njb.v39i2.2.

Osborn, T., Pires, J., Birchler, J., Auger, D., Chen, Z., Lee, H., Comai, L., Madlung, A., Doerge, R., Colot, V., & Martienssen, R. (2003). Understanding mechanisms of novel gene expression in polyploids. *Trends in genetics*, *19*(3), 141-147.

Otto, S., & Whitton, J. (2000). Polyploid incidence and evolution. *Annual review of genetics*, 34, 401-437 . https://doi.org/10.1146/ANNUREV.GENET.34.1.401.

Patel, J., & Kumar, G. (2023). Climate Resilient Agriculture Practices - The Future of Farming. *Plant Science Today*. https://doi.org/10.14719/pst.1835.

Peterson, B. J., & Moran, R. E. (2019). Plant Growth and Development. In *Stress Physiology of Woody Plants* (pp. 15-47). CRC Press.

Prentis, P. J., Wilson, J. R., Dormontt, E. E., Richardson, D. M., & Lowe, A. J. (2008). Adaptive evolution in invasive species. *Trends in plant science*, *13*(6), 288-294.

Qi, Z., Zhao, R., Xu, J., Ge, Y., Li, R., & Li, R. (2021). Accumulation pattern of flavonoids during fruit development of *Lonicera maackii* determined by metabolomics. *Molecules*, *26*(22), 6913.

Rakhmangulov, R., Barabanov, I., Erastenkova, M., Ivanov, A., Kovalenko, T., Mezhina, K., Petrosyan, I., Kharchenko, A., Shaimardanov, D., Shaimardanova, E., Anisimova, I., Tikhonova, N., Ukhatova, Y., & Khlestkina, E. (2023). The new directions in genetics, breeding and biotechnology of ornamental and berry crops in the N.I. Vavilov Institute of Plant Genetic Resources (VIR). *Plant Biotechnology and Breeding*. https://doi.org/10.30901/2658-6266-2022-4-o3.

Ramsey, J., & Schemske, D. W. (2002). Neopolyploidy in flowering plants. *Annual review of ecology and systematics*, *33*(1), 589-639.

Ruiz-Matute, A. I., Hernández-Hernández, O., Rodríguez-Sánchez, S., Sanz, M. L., & Martínez-Castro, I. (2011). Derivatization of carbohydrates for GC and GC–MS analyses. *Journal of chromatography B*, *879*(17-18), 1226-1240.

Rupasinghe, H., Arumuggam, N., Amararathna, M., & Silva, A. (2018). The potential health benefits of haskap (*Lonicera caerulea* L.): Role of cyanidin-3-O-glucoside. *Journal of Functional Foods*, 44, 24-39. https://doi.org/10.1016/J.JFF.2018.02.023.

Rupasinghe, H., Yu, L., Bhullar, K., & Bors, B. (2012). Short Communication: Haskap (*Lonicera caerulea*): A new berry crop with high antioxidant capacity. *Canadian Journal of Plant Science*, 92, 1311-1317. https://doi.org/10.4141/CJPS2012-073.

Sabooni, N., & Gharaghani, A. (2022). Induced polyploidy deeply influences reproductive life cycles, related phytochemical features, and phytohormonal activities in blackberry species. *Frontiers in Plant Science*, *13*, 938284.

Sattler, M. C., Carvalho, C. R., & Clarindo, W. R. (2016). The polyploidy and its key role in plant breeding. *Planta*, *243*, 281-296.

Scalzo, J., Battino, M., Costantini, E., & Mezzetti, B. (2005). Breeding and biotechnology for improving berry nutritional quality. *BioFactors*, 23.

https://doi.org/10.1002/biof.5520230406.

Senica, M., Stampar, F., & Mikulič-Petkovšek, M. (2019). Different extraction processes affect the metabolites in blue honeysuckle (*Lonicera caerulea* L. subsp. *edulis*) food products. *Turkish Journal of Agriculture and Forestry*, *43*(6), 576-585.

Sharma, A., & Lee, H. J. (2021). *Lonicera caerulea*: An updated account of its phytoconstituents and health-promoting activities. *Trends in Food Science & Technology*, *107*, 130-149.

Sharma, V. R., Malik, S., Kumar, M., & Sirohi, A. (2018). Morphological classification of genetic diversity of garlic (*Allium sativum* L.) germplasm for bulb and yield-related traits using principal component analysis. *Int. J. Curr. Microbiol. App. Sci*, *7*(6), 2016-2022.

Silva, A., & Rupasinghe, H. (2020). Polyphenols composition and anti-diabetic properties in vitro of haskap (*Lonicera caerulea* L.) berries in relation to cultivar and harvesting date. *Journal of Food Composition and Analysis*, 88, 103402. https://doi.org/10.1016/j.jfca.2019.103402.

Smith, G. A., Hecker, R. J., & Martin, S. S. (1979). Effects of Ploidy Level on the Components of Sucrose Yield and Quality in Sugarbeet 1. *Crop Science*, *19*(3), 319-323.

Smith, L., Villaret-Cazadamont, J., Claus, S. P., Canlet, C., Guillou, H., Cabaton, N. J., & Ellero-Simatos, S. (2020). Important considerations for sample collection in metabolomics studies with a special focus on applications to liver functions. *Metabolites*, *10*(3), 104.

Soltis, D. E., Visger, C. J., Marchant, D. B., & Soltis, P. S. (2016). Polyploidy: pitfalls and paths to a paradigm. *American journal of botany*, *103*(7), 1146-1166.

Soltis, P., & Soltis, D. (2000). The role of genetic and genomic attributes in the success

of polyploids. *Proceedings of the National Academy of Sciences of the United States of America*, 97 13, 7051-7 . https://doi.org/10.1073/PNAS.97.13.7051.

Stebbins Jr, G. L. (1940). The significance of polyploidy in plant evolution. *The American Naturalist*, *74*(750), 54-66.

Stebbins, G. L. (1971). Chromosomal evolution in higher plants. *Chromosomal evolution in higher plants.*

Sun, Q., Sun, H., Bell, R. L., Li, H., & Xin, L. (2011). Variation of phenotype, ploidy level, and organogenic potential of in vitro regenerated polyploids of Pyrus communis. *Plant Cell, Tissue and Organ Culture (PCTOC)*, *107*, 131-140.

Te Beest, M., Le Roux, J. J., Richardson, D. M., Brysting, A. K., Suda, J., Kubešová, M., & Pyšek, P. (2012). The more the better? The role of polyploidy in facilitating plant invasions. *Annals of botany*, *109*(1), 19-45.

Tossi, V., Tosar, L., Laino, L., Iannicelli, J., Regalado, J., Escandon, A., Baroli, I., Causin, H., & Pitta-Álvarez, S. (2022). Impact of polyploidy on plant tolerance to abiotic and biotic stresses. *Frontiers in Plant Science*, 13. https://doi.org/10.3389/fpls.2022.869423.

Trojak-Goluch, A., Kawka-Lipińska, M., Wielgusz, K., & Praczyk, M. (2021). Polyploidy in industrial crops: Applications and perspectives in plant breeding. *Agronomy*, *11*(12), 2574.

Turner, P., Ximenes, F., Penman, T., Law, B., Waters, C., Grant, T., Mo, M., & Brock, P. (2019). Accounting for biodiversity in life cycle impact assessments of forestry and agricultural systems—the BioImpact metric. *The International Journal of Life Cycle Assessment*, *24*, 1985-2007.

Van de Peer, Y., Mizrachi, E., & Marchal, K. (2017). The evolutionary significance of polyploidy. *Nature Reviews Genetics*, *18*(7), 411-424.

Vorsa, N., & Polashock, J. J. (2005). Alteration of anthocyanin glycosylation in cranberry through interspecific hybridization. *Journal of the American Society for Horticultural Science*, *130*(5), 711-715.

Wan, X., Liu, W., Yan, Z., Zhao, S., Nan, H. E., Xuqiang, L. U., & Liu, P. (2010). Comparison of fructose, glucose, sucrose and total sugar contents in different ploidy watermelon fruits. *Journal of Changjiang Vegetables*.

Wang, H., Li, Y., Wang, S., Kong, D., Sahu, S., Bai, M., Li, H., Li, L., Xu, Y., Liang, H., Liu, H., & Wu, H. (2020). Comparative transcriptomic analyses of chlorogenic acid and luteolosides biosynthesis pathways at different flowering stages of diploid and tetraploid *Lonicera japonica*. *PeerJ*, *8*, e8690.

Wang, X., Kong, L., Zhi, P., & Chang, C. (2020). Update on cuticular wax biosynthesis and its roles in plant disease resistance. *International Journal of Molecular Sciences*, *21*(15), 5514.

Wojdyło, A., Jáuregui, P. N. N., Carbonell-Barrachina, A. A., Oszmiański, J., & Golis, T. (2013). Variability of phytochemical properties and content of bioactive compounds in *Lonicera caerulea* L. var. *kamtschatica* berries. *Journal of Agricultural and Food Chemistry*, *61*(49), 12072-12084.

Xu, Y., Chen, L., Liu, Y., Hao, Y., Xu, Z., Deng, L., & Xie, J. (2019). Screening, prenatal diagnosis, and prenatal decision for sex chromosome aneuploidy. *Expert review of molecular diagnostics*, *19*(6), 537-542.

Yan, K., Cui, J., Zhi, Y., Su, H., Yu, S., & Zhou, S. (2023). Deciphering salt tolerance in tetraploid honeysuckle (*Lonicera japonica* Thunb.) from ion homeostasis, water balance and antioxidant defense. *Plant Physiology and Biochemistry*, *195*, 266-274.

Yao, Y., Zhang, N., Cao, H., Zong, C., & Sun, M. (2018). Induction and identification of

polyploidy plants from superior individuals of wild *Lonicera edulis* Turcz. in Changbai Mountains. *Agricultural Biotechnology*, *7*(4), 21-24.

Yirga, M. (2021). Polyploidy and its implications in plants breeding – A review. *International Journal of Current Research in Biosciences and Plant Biology*, 8, 1-9. https://doi.org/10.20546/IJCRBP.2021.803.001.

Zehfus, L. R., Gillespie, Z. E., Almendáriz-Palacios, C., Low, N. H., & Eskiw, C. H. (2021). Haskap berry phenolic subclasses differentially impact cellular stress sensing in primary and immortalized dermal fibroblasts. *Cells*, *10*(10), 2643.

Zhang, L., Hu, J., Han, X., Li, J., Gao, Y., Richards, C., Zhang, C., Tian, Y., Liu, G., Gul, H., Wang, D., Tian, Y., Yang, C., Meng, M., Yuan, G., Kang, G., Wu, Y., Wang, K., Zhang, H., Wang, D., & Cong, P. (2019). A high-quality apple genome assembly reveals the association of a retrotransposon and red fruit colour. *Nature communications*, *10*(1), 1494.

Zhu, C., Sun, X., Fu, Q., Zhan, Y., Li, S., Liu, Y., Yu, M., Qin, D., Zhang, L., & Huo, J. (2023). Complete chloroplast genome and phylogenetic analysis of *Lonicera caerulea* var. *edulis* (Caprifoliaceae). *Mitochondrial DNA. Part B, Resources*, 8, 314 - 318. https://doi.org/10.1080/23802359.2023.2180309.

Summary

This study provides a comprehensive analysis of the morphological characteristics, basic quality traits, and biochemical composition of haskap fruits with varying ploidy levels. By examining a broad spectrum of fruit attributes, the study elucidates the profound impact that ploidy levels have on fruit traits. Notably, significant variations were observed in fruit mass, volume, peel thickness, seed mass, and sugar content across different ploidy levels. Among these, tetraploid fruits exhibited superior morphological attributes, including larger mass and volume, as well as thicker peels, while aneuploid fruits had the highest sugar content. These findings underscore the importance of considering ploidy levels in the cultivation and breeding of haskap fruits, providing valuable insights and strong guidance for strategies aimed at optimizing both yield and fruit quality.

The significance of these morphological differences cannot be overstated, as they directly impact the marketability and consumer preference for haskap fruits. Larger fruit mass and volume, for instance, are highly desirable traits in the market, as they enhance the visual appeal and perceived value of the fruits. Thicker peels contribute to better protection against physical damage and pests, extending the shelf life and reducing post-harvest losses. Therefore, the cultivation of tetraploid haskap fruits with these advantageous traits can lead to improved economic returns for growers and greater satisfaction for consumers.

This study also delves into the biochemical composition of haskap fruits, identifying a variety of biochemical compounds, including sugars, sugar alcohols, and organic acids, through the application of GC-MS following silylation. This method proved robust and effective, revealing significant variability in the concentrations of these compounds across fruits with different ploidy levels. The predominant sugars identified in the fruits were fructose, mannose, and glucose, each exhibiting substantial variation in concentration depending on the ploidy level. The level of sorbitol, the only detected sugar alcohol, demonstrated a consistent increase with higher ploidy levels, indicating a potential relationship between genetic constitution and sugar accumulation. This study also explored the complex relationship between ploidy levels and organic acids, observing that quinic acid content increased with higher ploidy levels. However, the correlation between ploidy level and the content of other organic acids, such as malic and

citric acids, was not as straightforward, suggesting that additional factors, possibly environmental, may also play a significant role in determining the organic acid profile of the fruits.

These biochemical differences are crucial for several reasons. Sugars and organic acids are key determinants of fruit flavor, which is a critical quality attribute influencing consumer preference and marketability. The variability in sugar content, particularly the high levels observed in aneuploid fruits, suggests the potential for breeding sweeter fruit varieties that could cater to specific market niches. Additionally, the presence of sugar alcohols such as sorbitol, which have lower caloric content compared to sugars, could be leveraged to develop health-oriented products with reduced caloric intake. The intricate relationship between ploidy levels and organic acids, particularly the increase in quinic acid content with higher ploidy, offers insights into how ploidy manipulation can be used to enhance specific flavor profiles and health benefits of haskap fruits.

The intricate relationships between ploidy levels and biochemical composition suggest that both genetic and physiological interactions significantly influence haskap fruit traits. This research highlights the importance of the careful selection of measurement units and consideration of external environmental factors when conducting biochemical studies of fruits. The findings provide valuable insights that can inform and improve breeding and cultivation strategies, ultimately aimed at optimizing the quality of haskap fruits for various applications in the food and pharmaceutical industries. By understanding these complex interactions, breeders can develop haskap varieties with enhanced and targeted biochemical profiles.

Future studies should further explore the impact of environmental conditions on the biochemical composition of haskap fruits to gain a holistic understanding of the factors influencing their quality and nutritional value. Investigating how different environmental variables such as soil type, climate, and agricultural practices affect the biochemical makeup of haskap fruits will provide deeper insights into how to achieve sustainable agricultural production while meeting market demands. For example, studying the effects of varying soil nutrients and irrigation regimes could help identify optimal growing conditions that maximize the desired traits in haskap fruits. Climate factors, such as temperature and humidity, can also significantly influence fruit development and biochemical composition, highlighting the need for region-specific

cultivation practices. Additionally, further research could delve into the effects of ploidy variation on other bioactive components of haskap fruits and how these variations influence the health benefits of the fruit. This would offer more profound insights into achieving sustainable agricultural production and meeting the ever-changing demands of consumers. By precisely controlling and leveraging ploidy variations, there is potential to further optimize crop yield, quality, and adaptability, ensuring that haskap fruits can thrive under diverse environmental conditions and continue to meet the evolving needs of the market.

Exploring the health benefits of bioactive components such as anthocyanins, flavonoids, and polyphenols, which are known for their antioxidant properties, could open new avenues for positioning haskap fruits as functional foods with significant health-promoting potential. Understanding how ploidy levels influence the concentration of these compounds could lead to the development of haskap varieties with enhanced health benefits, catering to the growing consumer demand for nutritionally rich and functional foods.

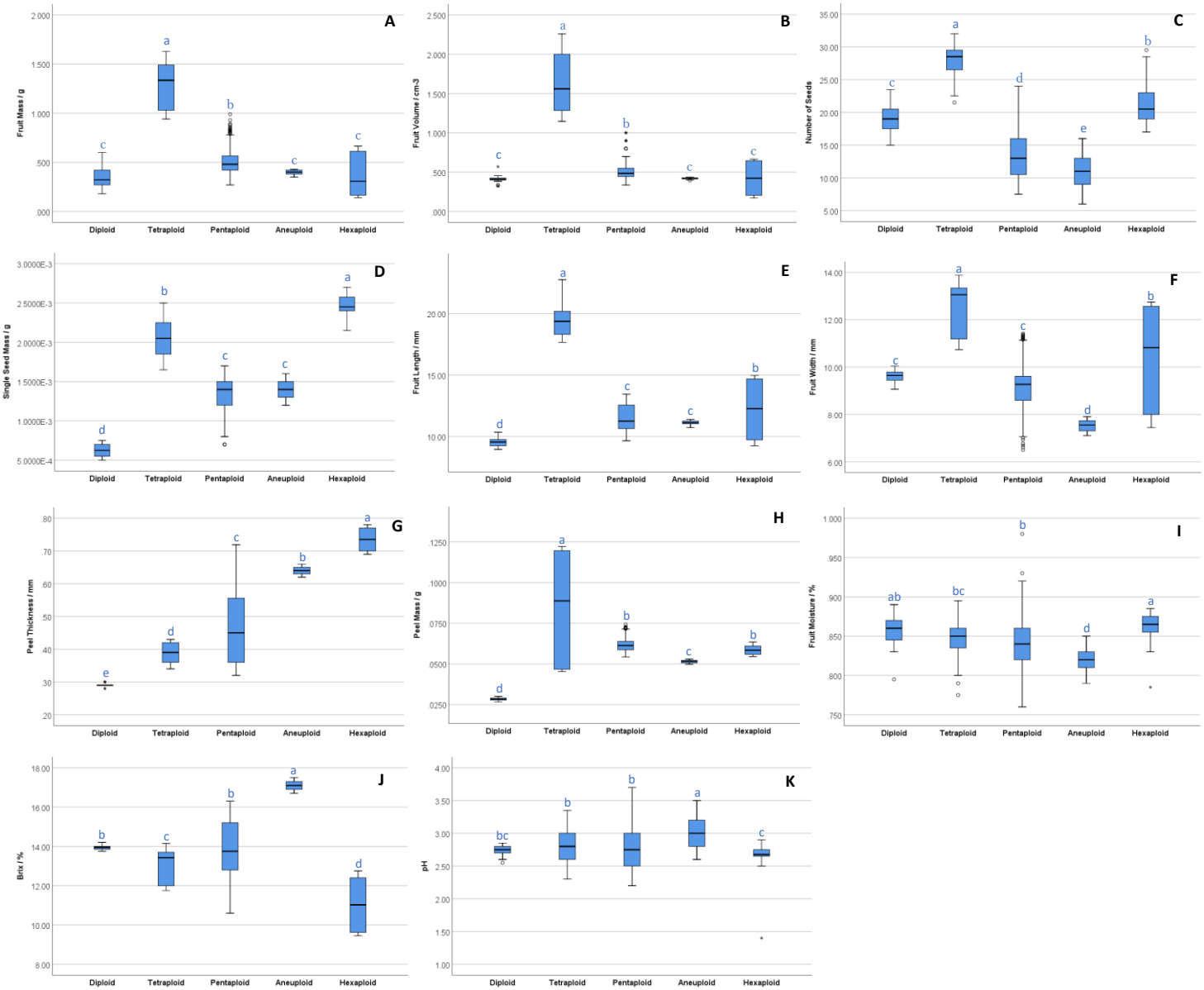
Ultimately, this research significantly contributes to our understanding of the morphological features and quality traits of haskap fruits at different ploidy levels. The comprehensive data provided in this study serve as a valuable resource for further research, cultivation, and breeding efforts aimed at improving haskap fruit production. As the demand for high-quality, nutritionally rich fruits continues to grow, studies such as this one are crucial for guiding future agricultural practices and ensuring that haskap fruits can meet both the nutritional and market demands of consumers worldwide.

The insights gained from this research have practical implications for the entire supply chain, from breeders and growers to retailers and consumers. Breeders can use the findings to develop novel haskap varieties with optimized traits that meet specific market needs. Growers can adopt best practices informed by this study to enhance fruit quality and yield, ultimately leading to higher profitability. Retailers can benefit from offering high-quality, nutritionally superior fruits that attract health-conscious consumers. Finally, consumers can enjoy the benefits of consuming fruits that are not only delicious but also packed with essential nutrients and bioactive compounds that promote health and well-being.

Appendices

Appendix A

Distribution of continuous variables for different ploidy haskap morphological and quality parameters. Including A) fruit mass, B) fruit volume, C) seed count, D) individual seed weight, E) fruit longitudinal diameter, F) fruit transverse diameter, G) peel thickness, H) peel mass, I) fruit moisture, J) fruit sweetness (Brix), K) pH value. Different letters indicate a significant difference determined by analysis of variance (ANOVA; Duncan's test, $p < 0.05$).



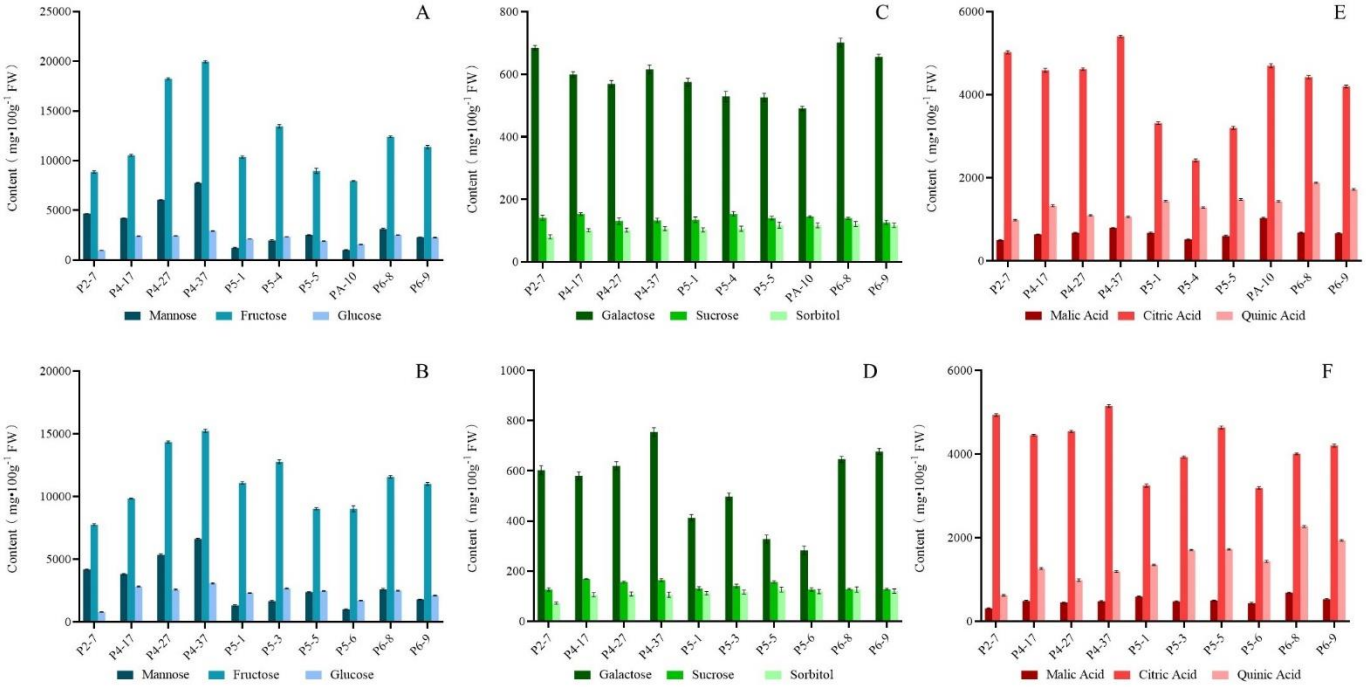
Appendix B

Correlation table of chromosome number with morphological and quality indicator.

	Chromosome No.	**Fruit mass**	**Fruit volume**	**Seed count**	**pH**	**Brix**	**Fruit moisture**	**Fruit length**	**Fruit width**	**Peel thickness**	**Peel mass**	**Single Seed mass**
Chromosome No.	1	-0.321	-0.346	-0.325	0.007	-0.114	-0.07	-0.215	-0.276	0.748	0.083	0.451
Fruit mass	-0.321	1	0.978	0.693	0.244	0.099	-0.089	0.928	0.815	-0.455	0.456	0.324
Fruit volume	-0.346	0.978	1	0.734	0.252	0.072	-0.057	0.934	0.813	-0.456	0.481	0.314
Seed count	-0.325	0.693	0.734	1	0.065	-0.256	0.191	0.764	0.759	-0.324	0.4	0.467
pH	0.007	0.244	0.252	0.065	1	0.543	-0.321	0.173	0.228	-0.191	-0.159	-0.136
Brix	-0.114	0.099	0.072	-0.256	0.543	1	-0.396	-0.04	0.038	-0.273	-0.26	-0.525
Fruit moisture	-0.07	-0.089	-0.057	0.191	-0.321	-0.396	1	-0.085	-0.047	-0.067	0.071	0.058
Fruit length	-0.215	0.928	0.934	0.764	0.173	-0.04	-0.085	1	0.834	-0.239	0.531	0.525
Fruit width	-0.276	0.815	0.813	0.759	0.228	0.038	-0.047	0.834	1	-0.273	0.252	0.422
Peel thickness	0.748	-0.455	-0.456	-0.324	0.228	-0.273	-0.067	-0.239	-0.273	1	0.015	0.489
Peel mass	0.083	0.456	0.481	0.4	0.228	-0.26	0.071	0.531	0.252	0.015	1	0.336
Single seed mass	0.451	0.324	0.314	0.467	0.228	-0.525	0.058	0.525	0.422	0.489	0.336	1

Appendix C

The biochemical content in different ploidy haskap samples collected in 2021 (A, C, E) and 2022 (B, D, F). Results represent the mean ± SD (n = 3), Waller-Duncan's test, $p < 0.05$. Different letters indicate a significant difference determined by analysis of variance (ANOVA; Duncan's test, $p < 0.05$).



The figures are modified from " Li, J., & Hoshino, Y. (2024). Elucidating the impact of ploidy level on biochemical content accumulation in haskap (*Lonicera caerulea* L. subsp. *edulis* (Turcz. ex Herder) Hultén) fruits: A comprehensive approach for fruit assessment. " (DOI: 10.1016/j.scienta.2023.112831)

Appendix D

The content of biochemical content in different ploidy haskap samples from 2021 (A) and 2022 (B) expressed in mg·100 g^{-1} fresh fruit weight (FW). Results represent the mean ± SD (n = 3), Waller-Duncan's test, $p < 0.05$. Different letters indicate a significant difference determined by analysis of variance (ANOVA; Duncan's test, $p < 0.05$).

Subject	Malic Acid		Citric Acid		Quinic Acid	
	2021	2022	2021	2022	2021	2022
P2-7	497.28±11.82^{f}	309.4±12.64^{g}	5020.25±38.88^{b}	4933.45±28.82^{b}	980.75±20.46^{i}	624.1±19.67^{i}
P4-17	637±11.86^{d}	487±13.33^{d}	4588.61±45.97^{d}	4452.52±20.9^{e}	1325.67±25.25^{e}	1260.45±22.27^{f}
P4-27	675.05±10.6^{c}	447.67±13.06^{ef}	4613.63±29.57^{d}	4539.23±24.62^{d}	1094.46±15.85^{g}	981.68±30.64^{h}
P4-37	790.64±14.03^{b}	480.12±15.71^{d}	5403.06±30.09^{a}	5148±35.62^{a}	1058.83±14.96^{h}	1189.58±20.66^{g}
P5-1	674.27±18.85^{c}	592.4±9.63^{b}	3314.67±29.69^{g}	3246.29±38.6^{i}	1437.14±14.05^{d}	1349.14±18.22^{e}
P5-3	/	473.67±18.93^{de}	/	3928.49±23.28^{h}	/	1703.68±14.85^{c}
P5-4	515±15.69^{f}	/	2420.21±33.94^{i}	/	1285.76±15.69^{f}	/
P5-5	599.3±18.25^{e}	497.89±9.52^{d}	3199.72±35.27^{h}	4634.96±32.44^{c}	1475.85±20.57^{c}	1719.33±15.34^{c}
P5-6	/	433.41±18.36^{f}	/	3188.67±28.51^{j}	/	1431.6±23.97^{d}
P6-8	680.03±13.33^{c}	683.16±12.58^{a}	4421.86±42^{e}	4004.04±22.23^{g}	1878.73±13.67^{a}	2268.33±23.67^{a}
P6-9	662.7±13.22^{cd}	529.95±13.76^{c}	4196.78±32.6^{f}	4202.53±37.14^{f}	1721.15±17.5^{b}	1933.35±15.62^{b}
PA-10	1031.07±19.85^{a}	/	4694.79±46.68^{c}	/	1429.17±18.33^{d}	/

Subject	Mannose		Fructose		Glucose	
	2021	2022	2021	2022	2021	2022
P2-7	4649.22±25.57^{c}	4183.87±40.1^{c}	8859.27±115.13^{g}	7746.45±69.8^{h}	968.32±12.4^{i}	794.21±12.86^{i}
P4-17	4202.24±35.25^{d}	3813.53±57.13^{d}	10522.1±90.42^{f}	9836.17±29.09^{f}	2404.88±28.1^{c}	2827.43±31.44
P4-27	6043.23±58.79^{b}	5351.19±72.19^{b}	18231.17±96.46^{b}	14338.97±85.45^{b}	2431.36±18.67^{c}	2572.29±45.42
P4-37	7762.83±33.71^{a}	6620.32±56.76^{a}	19950.79±116.96^{a}	15229.68±116.58^{a}	2918.26±28.35^{a}	3087.92±54.72^{a}
P5-1	1223.89±58.75^{i}	1320.61±52.63^{i}	10362.37±116.79^{f}	11073.21±109.25^{e}	2107.8±14.99^{f}	2298.53±22.75^{f}
P5-3	/	1655.95±59.38^{h}	/	12765.1±150^{c}	/	2673.87±35.6^{c}
P5-4	1974.2±64.88^{h}	/	13440.98±177.34^{c}	/	2331.49±18.98	/
P5-5	2505.35±50.55^{f}	2378.97±35.62^{f}	8960.22±247.32^{g}	9017.89±86.69^{g}	1898.89±36.7^{g}	2466.16±29.46^{e}
P5-6	/	1009.11±42.82^{j}	/	9021.96±242.27^{g}	/	1709.36±15.23
P6-8	3106.11±75.63^{e}	2613.3±60.59^{e}	12387.1±85.09^{d}	11558.59±96.4^{d}	2509.44±17.78	2489.95±33.88^{e}

P6-9	2287.01±20.4^{g}	1790.99±30.19^{g}	11364.68±177.63^{e}	11006.52±106.97^{e}	2252.03±33.86^{e}	2109.07±29.66^{g}
PA-10	1005.38±36.9^{j}	/	7950.09±53.53^{h}	/	1562.33±19.56	/

Subject	Galactose		Sucrose		Sorbitol	
	2021	2022	2021	2022	2021	2022
P2-7	684.04±7.69^{a}	601.84±17.73^{de}	141.02±8.59^{abc}	126.46±6.13^{d}	80.37±6.48^{e}	72.47±4.6^{c}
P4-17	599.64±8.69^{c}	579.84±16.12^{e}	153±4.57^{a}	168.8±1.86^{a}	101.37±4.88^{d}	106.07±7.86^{b}
P4-27	568.96±10.82^{d}	618.73±17.46^{cd}	130.64±10.65^{cd}	156.63±3.07^{b}	101.57±6.51^{d}	108.87±8.54^{b}
P4-37	615.84±13.45^{c}	754.43±16.93^{a}	132.73±6.92^{bcd}	164.45±5.87^{ab}	106.67±6.44^{abcd}	105.7±10.62^{b}
P5-1	575.11±11.81^{d}	412.52±12.48^{g}	134.94±8.64^{bcd}	130.72±7.45^{cd}	102.4±6.62^{cd}	112.13±7.17^{ab}
P5-3	/	497.07±13.83^{f}	/	140.17±7.88^{c}	/	116.93±7.81^{ab}
P5-4	529.06±16.33^{e}	/	153.01±7.38^{a}	/	106.47±8.16^{bcd}	/
P5-5	526.11±12.98^{e}	327.99±16.09^{h}	140.24±5.52^{abc}	156.75±4.81^{b}	117.33±9.6^{ab}	126.53±10.32^{a}
P5-6	/	282.96±17.03^{i}	/	126.84±6.58^{d}	/	118.53±8.32^{ab}
P6-8	701.51±13.84^{a}	645.62±11.3^{bc}	139.39±3.41^{abcd}	128.6±2.37^{d}	121.07±7.72^{a}	126.53±10.66^{a}
P6-9	655.16±8.53^{b}	676.35±12.13^{b}	126.07±6.72^{d}	128.17±2.66^{d}	116.73±7.1^{abc}	119.83±9^{ab}
PA-10	490.59±7.02^{f}	/	144.68±2.89^{ab}	/	116.83±7.99^{abc}	/

Appendix E

The content of biochemical content in different ploidy haskap samples from 2021 (A) and 2022 (B) expressed in mg weight content per single fresh fruit weight (CPF). Results represent the mean ± SD (n = 3), Waller-Duncan's test, $p < 0.05$. Different letters indicate a significant difference determined by analysis of variance (ANOVA; Duncan's test, $p < 0.05$).

Sub.	Malic Acid		Citric Acid		Quinic Acid	
	2021	2022	2021	2022	2021	2022
P2-7	1.82±0.04^{j}	1.13±0.05^{i}	18.33±0.11^{i}	18.02±0.07^{h}	3.58±0.04^{j}	2.28±0.04^{h}
P4-17	4.13±0.08^{g}	3.16±0.09^{e}	29.73±0.23^{d}	28.85±0.07^{d}	8.59±0.1^{g}	8.17±0.08^{f}
P4-27	9.48±0.15^{a}	6.29±0.18^{a}	64.81±0.27^{a}	63.76±0.21^{a}	15.37±0.08^{b}	13.79±0.29^{b}
P4-37	9.16±0.16^{b}	5.56±0.18^{b}	62.59±0.23^{b}	59.64±0.3^{b}	12.27±0.06^{c}	13.78±0.12^{b}
P5-1	5.24±0.15^{d}	4.6±0.07^{c}	25.74±0.15^{e}	25.21±0.22^{e}	11.16±0.03^{e}	10.48±0.06^{d}
P5-3		2.24±0.09^{g}		18.59±0.06^{g}		8.06±0.02^{f}
P5-4	4.76±0.14^{e}		22.36±0.22^{g}		11.88±0.05^{d}	
P5-5	2.26±0.07^{i}	1.88±0.04^{h}	12.06±0.1^{j}	17.47±0.08^{i}	5.56±0.04^{i}	6.48±0.02^{g}
P5-6		2.56±0.11^{f}		18.8±0.11^{g}		8.44±0.08^{e}
P6-8	3.66±0.07^{h}	3.68±0.07^{d}	23.8±0.17^{f}	21.55±0.07^{f}	10.11±0.02^{f}	12.21±0.07^{c}
P6-9	6.03±0.12^{c}	4.82±0.13^{c}	38.21±0.21^{c}	38.26±0.34^{c}	15.67±0.07^{a}	17.6±0.05^{a}
PA-10	4.52±0.09^{f}		20.58±0.16^{h}		6.27±0.04^{h}	

Sub.	Mannose		Fructose		Glucose	
	2021	2022	2021	2022	2021	2022
P2-7	16.98±0.09^{f}	15.28±0.15^{e}	32.35±0.42^{h}	28.29±0.25^{i}	3.54±0.05^{h}	2.9±0.05^{h}
P4-17	27.23±0.23^{c}	24.71±0.37^{c}	68.18±0.59^{f}	63.74±0.19^{e}	15.58±0.18^{e}	18.32±0.2^{c}
P4-27	84.89±0.83^{b}	75.17±1.01^{b}	256.09±1.35^{a}	201.42±1.2^{a}	34.15±0.26^{a}	36.13±0.64^{a}
P4-37	89.93±0.39^{a}	76.7±0.66^{a}	231.13±1.36^{b}	176.44±1.35^{b}	33.81±0.33^{a}	35.77±0.63^{a}
P5-1	9.5±0.46^{g}	10.26±0.41^{g}	80.47±0.91^{e}	85.99±0.85^{d}	16.37±0.12^{d}	17.85±0.18^{c}
P5-3		7.84±0.28^{i}		60.42±0.71^{f}		12.66±0.17^{e}
P5-4	18.24±0.6^{e}		124.17±1.64^{c}		21.54±0.18^{b}	
P5-5	9.44±0.19^{g}	8.97±0.13^{h}	33.77±0.93^{gh}	33.99±0.33^{h}	7.16±0.14^{g}	9.3±0.11^{g}
P5-6		5.95±0.25^{j}		53.2±1.43^{g}		10.08±0.09^{f}
P6-8	16.72±0.41^{f}	14.07±0.33^{f}	66.68±0.46^{f}	62.22±0.52^{ef}	13.51±0.1^{f}	13.4±0.18^{d}
P6-9	20.82±0.19^{d}	16.31±0.27^{d}	103.46±1.62^{d}	100.2±0.97^{c}	20.5±0.31^{c}	19.2±0.27^{b}
PA-10	4.41±0.16^{h}		34.85±0.23^{g}		6.85±0.09^{g}	

Sub.	Galactose		Sucrose		Sorbitol	
	2021	2022	2021	2022	2021	2022
P2-7	2.5±0.03^{g}	2.2±0.06^{f}	0.52±0.03^{f}	0.46±0.02^{h}	0.29±0.02^{g}	0.26±0.02^{g}
P4-17	3.89±0.06^{f}	3.76±0.1^{c}	0.99±0.03^{d}	1.09±0.01^{c}	0.66±0.03^{e}	0.69±0.05^{de}
P4-27	7.99±0.15^{a}	8.69±0.25^{a}	1.84±0.15^{a}	2.2±0.04^{a}	1.43±0.09^{a}	1.53±0.12^{a}
P4-37	7.13±0.16^{b}	8.74±0.2^{a}	1.54±0.08^{b}	1.91±0.07^{b}	1.24±0.07^{b}	1.22±0.12^{b}
P5-1	4.47±0.09^{e}	3.2±0.1^{e}	1.05±0.07^{cd}	1.02±0.06^{d}	0.8±0.05^{d}	0.87±0.06^{c}
P5-3		2.35±0.07^{f}		0.66±0.04^{fg}		0.55±0.04^{ef}
P5-4	4.89±0.15^{d}		1.41±0.07^{b}		0.98±0.08^{c}	
P5-5	1.98±0.05^{h}	1.24±0.06^{h}	0.53±0.02^{f}	0.59±0.02^{g}	0.44±0.04^{f}	0.48±0.04^{f}
P5-6		1.67±0.1^{g}		0.75±0.04e		0.7±0.05^{d}
P6-8	3.78±0.07^{f}	3.48±0.06^{d}	0.75±0.02^{e}	0.69±0.01^{ef}	0.65±0.04^{e}	0.68±0.06^{de}
P6-9	5.96±0.08^{c}	6.16±0.11^{b}	1.15±0.06^{c}	1.17±0.02^{c}	1.06±0.06^{c}	1.09±0.08^{b}
PA-10	2.15±0.03^{h}		0.63±0.01^{ef}		0.51±0.04^{f}	

Appendix F

The linear equation and R^2 value of the relationship between each biochemical substance and the number of chromosomes in different units.

Substance	Unit of FW		Unit of CPF	
	Equation	R^2	Equation	R^2
Malic acid	Y = 10.87X + 193.1	0.4359	Y = 0.06687X + 1.305	0.2930
Citric acid	Y = -22.80X + 5328	0.2541	Y = 0.03959X + 26.52	0.0017
Quinic acid	Y = 27.01X + 260.4	0.8478	Y = 0.1916X + 1.005	0.3988
Mannose	Y = -92.82X + 6814	0.4855	Y = -0.4241X + 39.42	0.0694
Fructose	Y = 22.48X + 9784	0.0142	Y = 0.3034X + 64.68	0.0065
Glucose	Y = 27.69X + 814.6	0.3124	Y = 0.1627X + 7.285	0.0561
Galactose	Y = -1.977X + 650.9	0.0839	Y = 0.01537X + 3.137	0.0128
Sucrose	Y = -0.06875X + 142.6	0.0186	Y = 0.004273X + 0.7331	0.0212
Sorbitol	Y = 1.168X + 59.45	0.9803	Y = 0.009358X + 0.3184	0.1804

Acknowledgments

I express profound gratitude to the Graduate School of Environmental Science at Hokkaido University for its invaluable support throughout my research. This institution has provided a robust academic environment and a platform for extensive environmental research and exploration. The school's commitment to fostering education and research focused on understanding environmental processes and resolving global environmental issues has been instrumental in guiding my academic journey.

Special thanks go to the members of my review committee for their time and effort in evaluating my work. Their insights and feedback have been invaluable. My appreciation extends to the professors, classmates, and colleagues in the Hoshino Lab. Their guidance, support, and cooperation have enabled my research to proceed smoothly and make continuous progress.

My heartfelt gratitude is profoundly directed towards my family, whose unwavering and selfless support has been the cornerstone of my motivation throughout this scholarly endeavor. I extend a special acknowledgment to Ms. Wu Shanshan, whose considerable assistance and encouragement have fortified my resolve amidst the myriad challenges encountered.

The multidisciplinary approach of the graduate school, integrating the Division of Earth System Science, Division of Biosphere Science, Division of Environmental Materials Science, and the Division of Environmental Science Development, has enriched my learning experience. This unique structure has allowed me to gain a comprehensive understanding of environmental science.

I am also grateful for the opportunities provided by the school to engage with diverse and dynamic fields of study, ranging from biogeochemistry to molecular biology and environmental materials science. These experiences have broadened my perspective and enhanced my research skills.

Finally, I acknowledge the supportive community at Hokkaido University, including peers and colleagues, for their camaraderie and collaborative spirit. The intellectual environment fostered by the university has been a cornerstone of my academic development. This thesis would not have been possible without the resources, knowledge, and inspiration provided by the Graduate School of Environmental Science at Hokkaido University.