Highlighting outstanding beetroot varieties for the food industry - Evaluation of agronomic and compositional attributes of organically grown beetroot (Beta vulgaris **L.** **subsp.** *vulgaris) varieties*

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“Dedicated to my wonderful deeply missed mother, whose memories brighten my life”

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List of abbreviations and acronyms

%	Percent
°C	Degree centigrade
ADI	Acceptable Daily Intake
ANOVA	Analysis of variance
BLE	Bundesanstalt für Landwirtschaft und Ernährung
BMEL	Bundesministerium für Ernährung und Landwirtschaft
Bx	Brix
DGE	Deutsche Gesellschaft für Ernährung (German Nutrition Society)
DM	Dry Matter
e.g.	Exempli Gratia
EFSA	European Food Safety Authority
etc.	Et Cetera
g	Gram
ha	Hectare
L	Litre
LDPE	Low Density Polyethylene
mg	Milligram
N	Nitrogen
n.s.	Not Significant
NO	Nitric Oxide
QTL	Quantitative Trait Locus
rpm	Rounds Per Minute
RZ	Rijk Zwaan
subsp.	Subspecies
TDMC	Total Dry Matter Content
TPC	Total Phenolic Content
w/w	Weight for weight
WHO	World Health Organization
µg	Microgram

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1. Introduction

1.1. Health-benefiting plant-based foods

During recent years, the demand for healthy food and the associated interest of consumers in health-promoting compounds in foods has risen sharply, particularly in industrialised countries [1]. A change in consumers' perception of food due to the reveal of the notable impact of food and its compounds on human well-being and health has occurred. The concept of food is not only linked to its fundamental role in satisfying hunger and supplying nutrients but also to promote physical and mental health and the prevention of diseases [2]. Superfoods are a new trend and they are currently an integral part of the media discussion on the topics of food and health. There is no official, legally binding definition of superfoods. Generally, the term superfood is referred to those foods with high levels of bioactive phytochemicals or nutrients, which benefit human health [1].

Due to the nutrient properties of vegetables and fruits, such as vitamins, minerals, and health-promoting phytochemicals such as antioxidants, they are highly recommended in daily diet. Furthermore, vegetables and fruits are considered among the primary sources of dietary fibre [3]. Phytochemicals cannot only play an essential role as an antioxidant, but also they are noted to have antibacterial, antiviral, antifungal, anti-inflammatory, and cholesterol-lowering impacts [4]. It has been reported that adequate consumption of fruits and vegetables (circa 400 g per day) can inhibit the progression of chronic diseases like particular cancers and cardiovascular disease [5]. The World Health Organisation (WHO) reported that, globally, roughly 31% of deaths caused by ischemic heart disease, 14% of gastrointestinal cancer demises, and 11% of stroke might be related to low consumption of vegetables and fruits [6]. Despite the increase in awareness toward a healthier daily diet, it can still be seen that even in developed countries, the daily recommendation for fruit and vegetable consumption is not met. For instance, in the United Kingdom, only 31% of adults consume an adequate amount of vegetables per day [7]. Therefore, the production of value-added products, such as enriching bread as an economic staple food with vegetables, has gained more attention [7].

Furthermore, a global dietary shift with an increase in the consumption of plant-based instead of animal-based foods is seen as a key factor in improving human health. In recent years, many people have therefore increasingly turned to a plant-based diet out of concern for health, sustainability, and animal welfare, and are consciously reducing their consumption of meat and animal-based foods. The 14th DGE (German Society for Nutrition) Nutrition Report [8] comes to a similar conclusion and shows that a plant-based diet is climate-friendly and beneficial to health. According to the report, it is indispensable to reduce consuming red meat by more than 50 % and significantly increase the consumption of legumes, nuts, fruits, and vegetables. Climate protection is a major global challenge. Agriculture must adapt to climate change and make an essential contribution to climate protection. One of the practices, which can be applied, is to further develop varieties that can be grown regionally since the domestic cultivation of crops eliminates long transport routes and consequently can contribute to climate protection.

Therefore, the increase of the demand for plant-based health-promoting foods is without disputation. However, besides meeting the consumers' needs, considering a sustainable and environmentally friendly production method is of importance. In this respect, a combination of various approaches, including dietary change, reduction of food waste, and ameliorated food production through optimized farming practices, must be pursued.

1.2. Beetroot and its compositional characteristics

Lately, classifying beetroot as a superfood [9], simple cultivation, and convenient storability without requiring costly storage equipment enhanced the importance of this vegetable and persuaded farmers and the food industry to increase beetroot production [10]. In 2017, the total world production of beetroot was up to 301 million tons, in which west Europe, with approximately 70% of the total production share, was the largest producer [11,12]. Beetroot (*Beta vulgaris* L. subsp. *vulgaris*), a herbaceous biennial plant, is categorised under the family Chenopodiaceae. Beetroot is a classic winter vegetable that, in its first year, is formed by the enlargement of the shoot axis segment under the cotyledons. The swollen organ of beetroot consists of both root and hypocotyl, which, hereinafter, the term "beet" refers to them in this thesis. The hypocotyl is a transitional region between root and stem. The true root and hypocotyl comprise alternating layers of storage and conductive tissue [13]. Depending on the genotype, its shape can be spherical or cylindrical-long, and the beet colour, depending on the presence of pigments commonly named betalains, can be white, yellow, red, and red with white rings. Beetroot can be replanted in the second year for seed production. Beetroot can resist drought and salinity. Nonetheless, adequate irrigation leads to a uniform growth pattern throughout the season, which consequently maximizes productivity and crop yield. The suitable soil condition for beetroot cultivation is almost neutral to slightly alkaline soils. Hence, beetroot grows well when pH is between 6.0 and 8.0.

Beetroot can be easily integrated into crop rotation, which plays a significant role in reducing the incidence of diseases and pests during its growth period. For instance, beetroot can be sown as a proceeding crop after well-manured crops like potato, in soil with good water-retention properties. Furthermore, rotation in a three- to four-year cycle with legumes is also recommended for beetroot. The growth period of beetroot can vary between 60 to 90 days. The diameter of the beets can reach 5 cm after two months, while the ideal mature size can take three months or longer. The final size of the beets mainly depends on the genotype and spacing rather than the degree of maturity [13]. Mature beets can tolerate and survive mild frosts. In order to produce seed, at the end of the first cultivation season, beetroots are taken out of the soil, and the best beets are sorted in sand or soil in a cool but frost-free place. The beets are replanted in the following spring until the flowers and, subsequently, seeds are produced.

Kale et al. [14] determined the chemical composition of fresh beetroots grown in India. Table 1 indicates the average values of different components determined in three replicates.

Table 1. Average values of compositional characteristics of fresh beetroot (modified after Kale et al. [14])

Composition	Average value (%)
Moisture	87.4 ± 0.3
Proteins	1.35 ± 0.2
Fats	0.3 ± 0.1
Carbohydrates	7.59 ± 0.4
Dietary fibre	1.9 ± 0.2
Ash	1.4 ± 0.2

Beetroot possesses a nutritional composition, including high contents of various bioactive compounds and dietary fibre [15]. Figure 1 illustrates the primary bioactive compounds present in beetroot. Beetroot has been mainly selected as a root vegetable; however, its leaves are edible. The leaves usually have a roughly triangular shape. Depending on the genotype, leaves can be either dark or light green or dark red, and often have a shiny surface [13]. The beetroot foliage is a rich source of carotenoids, specifically beta-carotene and lutein, a dietary source of biosynthesis of vitamin A, flavonoids, vitamin C, and iron [16]. Moreover, beet foliage is a fount of a vitamin B component, namely folate, which has different roles in the body, such as the synthesis of red blood cells, providing organic carbon for various cellular functions, and supporting the operation of the nervous and immune systems [16]. It has been reported that the consumption of 200 g of fresh beetroot can provide more than 40% of the daily suggested amount of folic acid and approximately 15% of the recommended daily amount of iron [17]. Besides the prevention impact against cancer and heart disease, folic acid is essential for pregnant women [17]. The betalains, phenolic compounds, ascorbic acid, and carotenoids [18,19] found in beetroot brought this crop among the ten vegetables with the maximum antioxidant activity [20]. In 2020, from 1450 farms growing beetroot in Germany, the total cultivation area reached 2087.7 ha [21], among which 965.1 ha belonged to organic farms [22]. This reveals that around 46% of beetroots grown in Germany are produced on organic farmland. Consequently, beetroot can be considered a typical organic crop in Germany.

Beetroot can have a broad range of use in the food industry. Besides the fresh use of young leaves and beet, beetroot can be used in pre-cooked semi-finished products, juice [23], bread spread, ketchup [24], energy snack bars [25], vegan/vegetarian patties [26], chips [27], spirit drinks [28], and recently as dietary supplements or in the form of different smoothies or concentrates for athletes [29]. Moreover, beetroot is used to colour not only food products, including pasta, rice, pastries, sausages, soups, sauces and marinades, and dairy products such as flavoured yogurt or ice cream [30], but also in medicines and cosmetics [23].

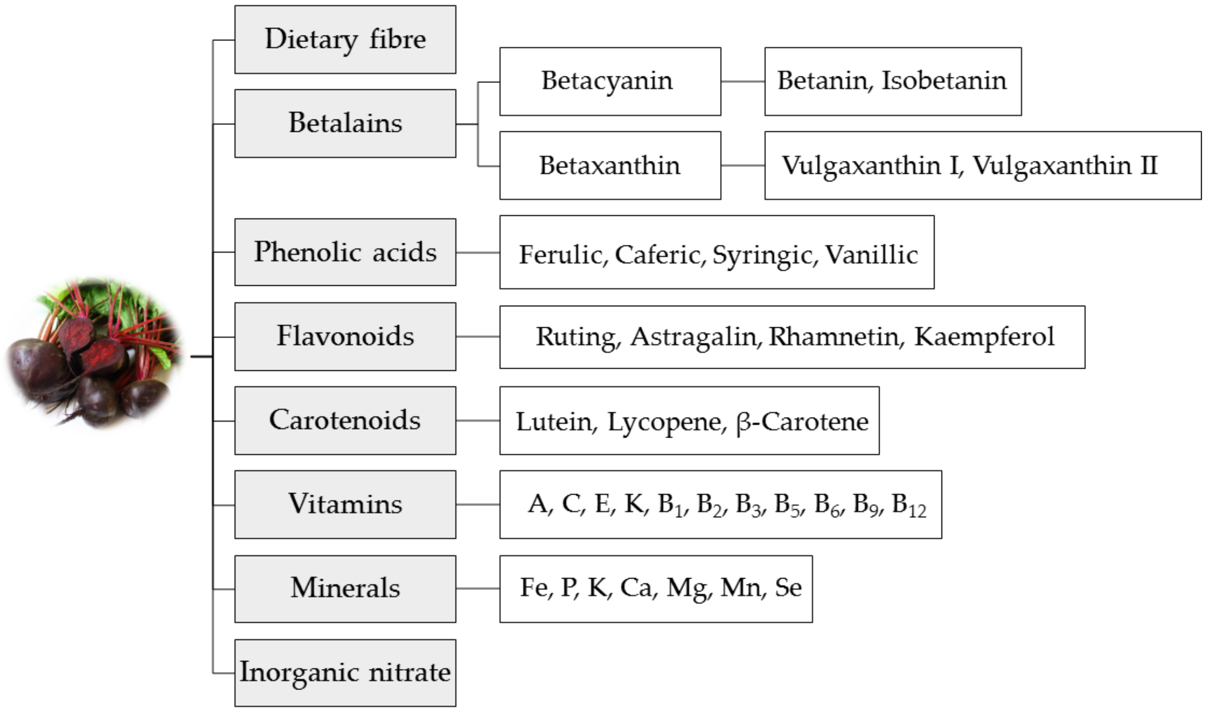

Figure 1: The major biologically active compounds exist in beetroot (modified after Domínguez et al. [31]).

One of the multi-purpose use of beetroot is in the curing process in the meat industry, which involves enhancing the colour and preservation of the final products [32]. Recently, to meet the consumer demand for non-chemical additives and replace them with natural ingredients, plant-based products gained more attention and indicated a high potential to be used as alternatives for commonly used preservatives. Nitrate is a typical compound used in processed meat products to prevent the growth of pathogenic bacteria, inhibit the spoilage caused by retarding lipid oxidation, and cooperate in pink-red colouration in cured meats [33]. In the food industry, colour is one of the most explicit quality attributes, which can provide information about the quality and freshness of foods [34] as well as the implementation of correct technological processes [33]. Due to its special composition, beetroot is among the vegetables containing a high amount of bioactive compounds, allowing the reformulation of conventional food products and transforming them into functional foods. Hereof, developing functional meat products using beetroot has gained particular interest. Beetroot can benefit the meat industry with its natural preservative activity due to its nitrate level, pigments (betalains), and antioxidants (betalains and phenolic compounds) [31]. Besides the use of beetroot in the forms of a fresh vegetable, juice, extract, or powder, the wastes and by-products generated during the beetroot processing can serve as inexpensive sources of functional ingredients for application in the food and meat industry [31]. This results in a green strategy to decrease the waste in vegetable processing systems [35]. Choi et al. [36] studied the influence of fermented beetroot extract and ascorbic acid on colour improvement in meat emulsions. They reported that this combination could be a feasible alternative to synthetic nitrite in meat emulsions.

Furthermore, the application of beetroot peel extract for postponing the lipid oxidation of Mahseer fish steaks during chill storage was investigated [37]. The outcomes indicated lower total bacterial count and higher overall acceptability when comparing the dip treatment in beetroot extract with the control sample. Moreover, during the chilled storage, the treatment prolonged the expiry date of Mahseer fish steak by six days. This reveals the potential of beetroot peel extract for use as a natural preservative in fishery products in order to extend their shelf life [37]. However, the beneficial impacts of using beetroot as a replacement for synthetic nitrite or other preservatives can be reached if a suitable formulation is applied. Sucu and Turp [32] examined the use of beetroot powder as a substitute for nitrite in Turkish fermented sausage (sucuk). They noted that the quality attributes of this reformulated product depend on the amount of used beetroot powder. Additionally, to the use of beetroot in the meat industry, due to the growing interest in vegan, vegetarian, and flexitarian diets, several typical meat-based products have been substituted with plant-based ingredients. Due to its meat-like colour, beetroot is one of the most common ingredients used in the formulation of veggie patties, which not only has a high resemblance to beef meat but also causes a value-added product due to its health-promoting compounds.

As was shown in Figure 1, the functional properties of beetroot are due to a wide range of components. This thesis, will focus on sugar, nitrate, betalains, and the total phenolic contents of beetroot.

1.2.1. Sugar

The sugar composition in beetroot is reported as predominantly sucrose with little glucose and fructose contents [38,39]. Dolores Rodríguez-Sevilla et al. [39] stated that 91.6% of the total soluble sugar in fresh beetroot is composed of sucrose. Wruss et al. [40] determined the sugar composition in seven different beetroot genotypes and reported the studied beetroots on average contained 94.8% sucrose, followed by 3.3% glucose and 1.9% fructose. Low fructose share in the sugar composition of beetroot can be advantageous for use in specific vegetable-based products such as sport-drinks since fructose was reported to reduce exercise capacity in humans [41].

Having information on the sugar content of raw materials used in different food products is of great importance. One of the reasons is to avoid producing products with excessive sugar content, which may exceed the maximum daily consumption of sugar. Furthermore, due to the diseases reported to be associated with sugar consumption, such as obesity or diabetes mellitus type II [42], providing information on the sugar content and composition for patients with a controlled diet is necessary. Another chief reason for investigating the sugar content in different beetroot genotypes is the correlation with other compositional characteristics of beetroot. Previous studies reported a high positive correlation between total sugar contents and total dry matter in beetroot. On the other hand, a high negative correlation between traits and nitrate content was stated [43,44].

The favourable amount of sugar in beetroot can be defined based on its purpose of use and the final product. For instance, for long-term storage of beetroot, beetroots with high sugar content are often desired in order to have beetroot with retained quality [45]. Likewise, in beetroot juice production, beetroot genotypes with higher sugar content are more advantageous, as they may prevent or reduce the amount of added sugar while processing. However, in the case of those food products in which intense thermal practices should be applied, such as baking products or the production of beetroot chips, due to the possibility of the Maillard reaction, the lower sugar level of the chosen beetroot variety could be beneficial.

1.2.2. Nitrate

Vegetables, especially green leafy ones like spinach, radishes, lettuce, and beets, are the primary dietary sources of daily nitrate intake in humans [46], supplying around 72 - 94% of the total intake [43]. There are inconsistent statements concerning the possible health risks of nitrate in the human diet. Hence, the European Union advised the maximum thresholds for nitrate levels in spinach and lettuce, which subsequently, in 1995, it led to the European Commission Regulation for determining Acceptable Daily Intake (ADI) of nitrate ion as 3.65 mg kg^{-1} body weight (equipollent to 219 mg/day for a person with 60 kg weight) [47]. However, the WHO suggested the ADI of nitrate between 0 – 3.7 mg kg^{-1} body weight [43].

Three major impacting factors on nitrate level in plants are environmental, nutritional, and physiological [43], in which light intensity and nitrogen fertilisation have been reported as the chief influencing factors in the nitrate content of vegetables [48]. The nitrate content can differ between plant species and cultivars of identical species [43] and between various parts of a plant [49]. In this regard, the nitrate content level in vegetables was reported to be the highest in the petiole, leaf, stem, and root, respectively. On the other hand, the lowest nitrate content in vegetable organs belongs to seed, fruit, and tuber, respectively [49]. The nitrate level can be affected by the compositional characteristics of a plant. In this respect, with the increase in sugar concentration, the nitrate amount in the vacuoles reduces [50]. Furthermore, a negative correlation between the dry matter content of a plant with its nitrate level was reported [51].

Different studies elucidate the positive influence of dietary nitrate supplementation on workout capacity and muscle efficiency. Murphy et al. [52] stated the consumption of nitrate-rich, whole beetroot by healthy adults could improve running performance. Moreover, Ramick et al. [53] investigated the effect of the consumption of beetroot juice supplements on enhancing exercise capacity in Chronic Kidney Disease (CKD) patients. CKD is associated with fatigue and reduction of exercise capacity, which consequently affects the life quality of the patients. The study revealed that intake of inorganic nitrate through consumption of beetroot juice results in a longer total exercise time and better performance [53].

After ingestion of nitrate, the salivary glands absorb and concentrate the circulating nitrate, which afterward, through the anaerobic bacteria located on the surface of the tongue, is reduced to nitrite [53]. Whilst a small amount of the nitrite enters the stomach, a significant amount launch into the blood circulation, and via haemoproteins and different enzymes, is reduced to nitric oxide (NO) [54]. NO, as a signalling molecule, has a major role in regulating both oxygen utilization and delivery and controls metabolism and vascular tone [55]. The most efficient environment for the reactions leading to NO production is reported to be acidic or hypoxic, which exists at the skeletal muscle level throughout an intense workout [56]. Therefore, the presence of NO is associated with a positive impact on muscle efficiency and muscle fatigue.

1.2.3. Betalain

The common red colour appearing in most fruit and vegetables, for instance, strawberries and red cabbage, comes from anthocyanins, belonging to the flavonoid group of pigments. However, in the order Caryophyllales and the fungal genus *Amanita*, the red colour is due to the pigments called betalain. Accordingly, the pigmentation of beetroot is as well due to the concentration of betalains. Betalains are water-soluble, nitrogenous secondary plant substances [57], which are mainly composed of betanin, isobetanin, vulgaxanthin I, and vulgaxanthin II. Betalains are derived from the amino acid tyrosine [58] and categorized into two chief groups, red-violet betacyanins and yellow-orange betaxanthins [59]. Betacyanins are immonium conjugates of betalamic acid and cyclo-dopa, while betaxanthins are immonium conjugates of betalamic acid and amino acids or amines [60].

The beet colours' differences are caused by variations in levels of different constituents of betalain, especially the relative ratio between the betacyanins and the betaxanthins. Moreover, with cutting the beetroot transversely, distinct light and dark rings are often visible. This results from the differences between the amount of pigment in the storage tissue and the vascular system of the root [13], in which the vascular system, due to the higher pigment content, appears as darker rings. This difference in some genotypes is quite subtle, while in some genotypes like Chioggia, with rosy red and white rings indicating vascular system and storage tissue, respectively, the difference is remarkable [13]. The betalains concentration is mainly distributed towards the outer parts of the beets. Therefore, the betalain content in the peel of beetroot is stated to be higher than the flesh [61,62]. Betalains can be found in the beet part and contribute to the red colour appearing in stalks and leaves.

Besides their use as natural colourants in the food industry, betalains have gained more attention due to their health-promoting characteristics in humans [14]. Beetroot betanin also reduces the activity of cyclooxygenases in the body, enzymes that play a key role in inflammation. By inhibiting cyclooxygenases, beetroot thus has an anti-inflammatory influence [17]. In addition, betanin promotes the release of serotonin, which makes this vegetable act as a mood enhancer [17]. Betalains derived from beetroot extract are one of the few sources of red dye, which have been consented by the European Food Safety Authority (EFSA) to be used as an edible natural colourant (E162) [63]. Comparing betalains with other natural pigments, a wider range of pH stability than anthocyanins [64] and higher stability in water than carotenoids have been reported in previous studies [58].

1.2.4. Total Phenolic content

Phenolic compounds are plants' secondary metabolites, affecting the flavour and colour of fruits and vegetables and determining their quality and health-promoting properties. Phenolic compounds can be classified into three main categories, phenolic acids, flavonoids, and non-flavonoids. The classification depends on the chemical structure and the number of aromatic rings and hydroxyl groups [65]. The plant-based phenolic compounds have shown a broad range of biological roles for both plants and human consumers [60]. The antioxidant capacity of the phenolic compounds varies depending on the plant origin, environmental conditions throughout cultivation, harvest time, post-harvest processes, and storage conditions [66–68]. Phenolic compounds received more attention due to their health-benefiting feature, especially their high antioxidant capacity. The antioxidant activity of phenolic compounds resulted from their free radicals scavenging ability and acting as metal-chelating agents [69]. Besides high antioxidant activity, phenolic compounds are produced in plants as defence mechanisms under different stress conditions, such as infection [70], temperature fluctuation, UV radiation, physical or mechanical damages, etc. [71]. Beetroot is a rich source of phenolic compounds such as coumaric acid and ferulic acid. Kesarwani et al. [72] reported growing conditions, genotype, and the cultivation system as factors affecting phenolic compounds' content. In this regard, several literature studies stated higher levels of phenolic compounds in organically produced crops, including beetroot [72–74].

1.3. Organic cultivation of beetroot

The contents of health-benefiting compounds depend not only on the environmental conditions and production processes but are also notably controlled by the used variety. Using organically-derived inputs such as organic seeds is desirable in an organic farming system [75]. Nowadays, varieties mainly available on the market for mercantile purposes are solely F1 hybrids, which are undesirable or not even allowed in organic production systems. Therefore, developing genotypes such as new open-pollinating ones could be one alternative for organic farming [76]. On the other hand, as the organic seed market is still not economically appealing to plant breeding companies due to the lower yield and inconsistent quality, there is a need for investigation and selection of forgotten cultivars as well as new experimental lines of beetroot, which are promising for the use in organic agriculture.

Due to the intense selection and development of plant species, many cultivars adapted to specific climatic conditions were generated. Although these cultivars would show a good performance in particular situations, at the same time resulted in an extreme decrease of genetic diversity, showing high susceptibility to unpredicted abiotic stresses like heat stresses and drought. Therefore, a rich source of genetic variability for battling against the unexpected conditions resulting from climate change is needed.

Due to their genetic diversity, the open-pollinated cultivars indicate more simplicity and adaptability to a broader range of environmental conditions. Thus, they can be a sustainable solution for dealing with climate change crises. Furthermore, by using these cultivars, the farmers can reproduce the seeds for the next cultivation season instead of relying on external inputs [76], which significantly reduces the production costs. Therefore, open-pollinated cultivars can be of interest to small-scale farms or medium- to low-input agriculture systems such as organic farming. Although these cultivars usually show lower yields compared to the modern hybrids cultivars, in the organic farming system, stability of yield is considered as more important factor [76]. Although organic agriculture is very critical of using hybrids in accordance with its guidelines, farmers and gardeners have so far felt compelled to use F1 hybrids for mercantile reasons. The more powerful the F1 hybrids become and the more traditional cultivars disappear, the narrower the genetic basis of a plant that once existed in many varieties. At some point, no more varieties will be adapted to certain regions, climates, and soil types. There will only be uniform, high-performance varieties that can only exist under high-performance conditions, which leads to gene erosion and threatens biodiversity. The development and official approval of marketable open-pollinated cultivars will help to resolve this dilemma, at least in some regions, and strengthen the position of these cultivars in organic vegetable production. Furthermore, considering the rising demand for healthy products, one of the focuses within the development of open-pollinated varieties should be the content of health-promoting compounds.

Besides food quality, one of the fundamental factors, which should be considered, is the economic feasibility of the production method. Particularly, the selection of management practices should be cost-effective in order to lead to higher returns for farmers [77]. Straus et al. [77] investigated the economic feasibility of different beetroot cultivation systems in Slovenia. The study reported significant differences between the financial results of the investigated cultivation systems. It was noted that the organic cultivation system could be the most economically feasible if double the price compared to the conventionally produced beetroot be considered. Different studies showed consumers' willingness to pay a higher price for organic products [78,79].

Furthermore, previous studies reported lower levels of harmful substances and higher quantities of health-promoting compounds in organic food products [80–83]. For example, Bavec et al. [38] reported that total phenol content, total antioxidant activity, and total sugar content in beetroots (cultivar Rote Kugel) were higher under organic cultivation systems than conventional ones. Straus et al. [77] stated that a lower application of nitrogen fertiliser in the organic farming system enhanced the levels of secondary metabolites and microminerals and, consequently, a higher antioxidant capacity in plant tissue. However, Perner et al. [84] demonstrated that besides the impact of nitrogen deficiency on the increase of plants' antioxidant activities, a sufficient supply of nutrients by adjusting the NH_4^+:NO_3^- ratios led to a similar result. Furthermore, the prohibition of synthetic pesticides in the organic cultivation system led to higher stress levels and, consequently, the activation of plant defence mechanisms. Therefore, higher production of plant secondary metabolites, such as phenolic compounds, in organically grown plants was reported [72]. Moreover, considering the organoleptic quality, a better taste and smell were noted for organic red-coloured beetroot [85].

In order to benefit both farmers and consumers, it is essential to consider how to enhance the yield and agronomic traits of organic crops. Thus, there is a need to breed the existing potential genotypes further or regenerate the forgotten cultivars to adapt them to the specific cultivation conditions of organic farming. Carrillo et al. [74] stated the significant differences between the content of bioactive compounds and their bioactivity of six different organic and conventional beetroot cultivars. Moreover, they claimed that the organic beetroots contained significantly higher total betalains and polyphenol contents and total antioxidant activities compared to conventional beetroots. Results revealed that the impact of the cultivation system on nutritional quality depends on the tested genotype, while some genotypes indicated higher sensitivity to the condition of growing. Hence, from the consumers' perspective, it is difficult to ensure that choosing organic beetroots instead of conventional ones leads to a higher source of health-promoting compounds because the role of the cultivar is undeniable [74].

1.4. Quality preservation of beetroot

Production of high-quality beetroot products depends not only on growing agronomically well-performed beetroot genotypes but also on the stability of their quality during storage to extend their availability and use for an extended time. Like so many vegetables and fruits, the availability of fresh beetroot is seasonal. Hence, different preservation methods, for instance, drying, fermentation, refrigeration, cooking, and vacuum packing, are used to develop food products such as beetroot chips, powder, juice, etc., to extend the shelf life of beetroot. However, in order to benefit beetroot for different purposes, it is important to preserve the whole fresh beet for a longer period after the harvest. This often needs advanced storage technologies, such as storage in a controlled atmosphere, where beetroot usually maintains its quality until the next harvest [86]. Storage in a controlled or modified atmosphere leads to a reduction of storage losses, retention of the vegetables' quality, an extension of the use of vegetables, and, accordingly, farmers' profitably [86,87]. Nevertheless, such technologies are often costly and are not affordable for small- to medium-scale producers [88]. Therefore, affordable and easy-accessible options for extending the shelf life of vegetables are required.

In this regard, Gawęda [89] stated that the improvement of prevalent cold storage methods needs new adjustments, such as selecting suitable packaging. Hereof, storage of two beetroot cultivars at 0.5 °C and 2.0 °C using two kinds of packaging, including plastic boxes and packed in low-density polyethylene bags (LDPE), was investigated. It was reported that the weight loss in the stored beetroot in the LDPE bags was remarkably lower than those stored in the boxes. Therefore, storing beetroot at low temperatures in the LDPE bags leads to retained juiciness. On the other hand, due to the higher transpiration in the stored beetroots in the plastic boxes, higher dry weight and soluble sugars were noted compared to the beetroots stored in the LDPE bags. However, it was revealed that beet shape affects the transpiration level, such that cylindrical-shaped beets transpire easier than round beets [89]. Therefore, the impact of cultivar on the storability of beetroots was disclosed.

Moreover, one of the major health-promoting characteristics of beetroot is its antioxidant activity, which results from the presence of betalains and phenolic compounds [20]. Therefore, to benefit the functional attributes of beetroot, the stability of these compounds is essential [90]. Hereof, the assessment of the betalains stability of powdered beetroot during three months of storage at 4°C indicated that despite the significant decrease in the content of betalains, the stored beetroot powder still contained a high level of antioxidants, which makes their consumption beneficial even after three months [91]. Furthermore, besides low temperature, as one of the influencing factors on better preservation of pigment level, the absence of light during the storage was reported as a delaying factor for colour degradation [92].

1.5. General aims and design of study

The thesis followed the goals of developing well-performed new varieties for beetroot cultivation and production that are adapted to the specific principles of organic agriculture, including a breeding background, which is compatible with organic values. Considering the functional properties of beetroot, with the improvement of the organic cultivation of beetroot using the locally adapted genotypes, the production of an organic domestic superfood can be promoted. The varieties bred within the framework of the project promote the expansion of organic farming and the increase of the market share of organically produced vegetable products while at the same time ensuring the sensory and chemical qualities of the products. Through the targeted selection of beetroot concerning different quality parameters, the healthoriented consumer can be introduced to the consumption of organic products, which meet their expectations of health-benefiting products.

The thesis was derived from a research project titled "Agronomic and sensory evaluation and further breeding of existing beet varieties for new and specific uses (Beta-Divers)". The Federal Ministry of Food and Agriculture of Germany (BMEL) funded the project based on the decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the Innovation Support Program (Grant number: 2818201015). The research project addressed the funding initiative program for the "Promotion of Innovation in Plant Breeding and Plant Protection" and focused on further breeding of existing beetroot varieties and the resulting development of innovative, internationally competitive products for different segments of the value chain (fresh marketing, juice production, semi-finished products, food colourants). Beta-Divers was a joint project, which was conducted through the cooperation of three main grantees, including the University of Hohenheim (Stuttgart, Germany), Kultursaat e. V. (Association for Breeding Research & Crop Maintenance Based on Biodynamic Principles, Echzell, Germany), and Sensient Colors Europe GmbH (Geesthacht, Germany). In addition, the project collaborated with three business partners, namely, Hofgemeinschaft Heggelbach GbR (Herdwangen, Germany), Voelkel GmbH (Hoehbeck, Germany), and Beutelsbacher Fruchtsaftkelterei GmbH (Weinstadt, Germany).

In order to investigate the performance of different open-pollinated beetroot genotypes, a broad range of genotypes was selected for a two-year genotype-screening experiment, which was conducted in conducted at the experimental station Kleinhohenheim (University of Hohenheim) and in two on-farm breeding locations of Kultursaat e.V., located in Bingenheim (Germany) and Oostelbeers (the Netherlands). Section 1.6. presents a detailed explanation of the research locations and the conducted field trials of the genotype-screening experiment. Based on the observations of the genotype-screening experiment, those genotypes with better prospects were selected for further breeding. Breeding of open-pollinated beetroot genotypes using the traditional method of single plant selection was set as one of the objectives of the joint project, which Kultursaat e.V. accomplished. Although the breeding experiment was not part of the three main publications of this thesis, it will be discussed in detail in the general discussion chapter (see Section 6.1.) to give a comprehensive overview of the research project's outcomes.

The main breeding criteria were the adaptation of the varieties to the special cultivation conditions of organic farming, acceptable marketable yield, intensive colouring, and high potential of health-promoting compounds with simultaneously good taste properties. Through precise selection via single plant progenies, the selected traits were developed into high-performance breeding lines and ultimately into new open-pollinated varieties that would be especially suitable for quality-oriented organic farming. Particular emphasis was on selecting genotypes with positive taste characteristics and a high proportion of health-promoting ingredients. Furthermore, besides the contents of health-benefiting compounds in freshly harvested beetroots, their stability during storage was taken into account with the aim of having fresh beetroot with promising quality and preserved nutritional properties throughout the year.

This study intended to reveal the genetic potential of forgotten and not commonly used genotypes and new breeding lines, which perform desirably in terms of agronomic, morphological, and compositional characteristics, as well as their stability and suitability in different locations under organic farming conditions.

1.6. Objectives and outline of the dissertation

Within the general set-up of the cooperation project, the main objectives on which this thesis was founded are as follows:

- to assess the agronomic performance of a broad assortment of new and existing open-pollinated beetroot genotypes cultivated organically in comparison to F1 hybrid genotypes, considering yield potentials, morphological attributes, and their stability in different environmental conditions;
- to determine the content of bioactive compounds of the tested genotypes and to investigate the impact of environmental conditions on the formation of bioactive compounds and whether a notable genetic variability between the examined genotypes exists;
- to evaluate the stability of various bioactive compounds of the studied beetroot genotypes, considering the impact of genotype and the period of cold storage to exploit the functional characteristics of beetroot for a prolonged period after harvest;
- to identify the open-pollinating genotypes of beetroot with prominent and stable agronomic and organoleptic qualities comparable to commercially used hybrid genotypes to introduce them to the market for use in the organic food industry as a substituent for hybrid genotypes;
- to appraise the sensory characteristics of selected open-pollinated genotypes and compare their potentials with the commercial hybrid genotypes, through consumers' perception of the desired beetroot taste characteristics (including sweetness, aroma intensity, bitterness, and earthy flavour), and the degree of acceptability;
- to investigate the possible influence of agricultural practices like nitrogen fertilisation on selected compounds of specific genotypes, to compare it with the impact of inherent compositional characteristics of the examined genotypes;

The last two aforementioned objectives were investigated in the framework of the research project (Beta-Divers) but were not part of the three major publications of the thesis. They will be dealt with in the general discussion section in order to draw a deeper insight into the outcomes of Publication I-III.

In order to achieve the first four described objectives, a genotype-screening experiment was defined, in which six field trials were conducted in 2017 and 2018 at three locations. Two locations belonged to the on-farm breeding stations of Kultursaat e.V., and the third location was the experimental station for organic farming at the University of Hohenheim. The research locations, as well as their latitude, longitude, and altitude, are listed below:

i. De Beersche Hoeve, Oostelbeers, Netherlands (51°28′47 N, 5°15′35 E, and 17 m above sea level)
ii. Horticulture station Heinze, Bingenheim, Hessia, Germany (50°22′28 N, 8°53′45 E, and 129 m above sea level)
iii. Experimental station for organic farming Kleinhohenheim, University of Hohenheim, Stuttgart, Baden-Wuerttemberg, Germany (48°44′14 N, 9°12′01 E, and 430 m above the sea level)

All field trials were carried out under organic farming conditions corresponding to the European Commission's principal regulations for organic agriculture. In the first year of the experiment, on each breeding station 30, and in Kleinhohenheim, 40 genotypes were tested in three field replicates. In the second year of the experiment, 16 genotypes were cultivated at each breeding station, while 36 genotypes were examined in Kleinhohenheim. The genotypes were mainly open-pollinated; however, a few numbers of F1 hybrids as commercial controls and breeding lines were also among the investigated genotypes. The data from all genotypes were analysed, but the indicated results in publications I and II were limited to the 15 genotypes, occurred in all location-by-year combinations. In publication III the data from 36 genotypes, which were investigated in both years at the research station Kleinhohenheim were considered. The investigated genotypes covered not only different beet shapes of beetroot but also different colours. In this regard, flat-spherical, cylindrical, spherical shapes and considering the colour, red, yellow, white, and red-white beets were studied in this work. A detailed description regarding the shape, colour, seed origin of all studied genotypes, and in-depth information about the research stations, precipitation and mean temperature, sowing and harvest dates, fertilisation, and soil management practices can be found in the publications’ chapters.

Publication I presents the evaluation of the agronomic performance of various beetroot genotypes concerning their genetic yield potential, morphological attributes, and their stability in different years and locations. Hereupon, after the harvest of the beetroots and the removal of leaves and stems manually, the beets’ fresh weight was measured in order to calculate the total yield. Then the harvested beetroots were sorted according to our defined criteria for marketability, namely beet diameter of 5–13 cm, no deformation or considerable damages, diseases, etc. Furthermore, the individual beet weight, diameter of beet, and leaves-growth-base width of five randomly selected beetroots meeting standard marketable criteria were measured. Then the beets were evaluated optically (using a 1–9 scale) concerning skin smoothness, corky surface, root tail, scab incidence, and uniformity.

In **Publications II,** the results of the content of total dry matter, total soluble sugar, nitrate, betalain, and total phenolic compounds of the grown beetroot genotypes are reported in order to indicate their genetic potential regarding the content of health-promoting compounds and to assess the effect of environmental conditions on the quantities of specific health-promoting compounds. **Publication III** appraises the stability of compositional characteristics of beetroot genotypes, including the contents of total dry matter, total phenolic compounds, betalain, nitrate, and total soluble sugars during the cold storage, considering the impact of genotype and duration of cold storage.

In **Publications II** and **III**, for the determination of bioactive compounds in freshly harvested beetroots, three beetroots per plot were randomly selected. In addition, for **Publication III**, harvested beetroots of each plot were stored for four months in a cooling chamber at 6 °C. Three beetroots were taken randomly from the cooling chamber one and four months after the storage. After washing the selected beetroots and removing the leaves-growth-base and root tail, a longitudinal cut of each beet, containing flesh and peel, was diced and mixed to have a homogenous sample from each plot. The diced beetroot samples were collected in a plastic flask, directly frozen using liquid nitrogen to inhibit further enzymatic processes, and then kept at −18 °C. Later, the samples were lyophilised, and the dried samples were milled until a fine powder texture was reached. Total dry matter content (TDMC), total phenolic compounds, betalain, nitrate, and total soluble sugars of beetroots of freshly harvested samples, as well as samples taken after one and four months of cold storage, were determined.

2. Publications

Three scientific papers create the overall frame of the present cumulative dissertation. All papers have been published in peer-reviewed journals and are presented in Chapters 3-5. For citation of these three papers, please use the references given below:

Publication I

Yasaminshirazi, K.; Hartung, J.; Groenen, R.; Heinze, T.; Fleck, M.; Zikeli, S.; Graeff-Hoenninger, S. Agronomic Performance of Different Open-Pollinated Beetroot Genotypes Grown Under Organic Farming Conditions. *Agronomy* **2020**, 10, 812.

DOI: 10.3390/agronomy10060812 (Impact factor in 2020: 3.42)

Publication II

Yasaminshirazi, K.; Hartung, J.; Fleck, M.; Graeff-Hoenninger, S. Bioactive Compounds and Total Sugar Contents of Different Open-Pollinated Beetroot Genotypes Grown Organically. *Molecules* **2020**, 25, 4884.

DOI: 10.3390/molecules25214884 (Impact factor in 2020: 4.41)

Publication III

Yasaminshirazi, K.; Hartung, J.; Fleck, M.; Graeff-Hönninger, S. Impact of Cold Storage on Bioactive Compounds and Their Stability of 36 Organically Grown Beetroot Genotypes. *Foods* **2021**, *10*, 1281.

DOI: 10.3390/foods10061281 (Impact factor in 2020: 4.35)

3. Publication I: Agronomic Performance of Different Open-Pollinated Beetroot Genotypes Grown Under Organic Farming Conditions

Yasaminshirazi, K.; Hartung, J.; Groenen, R.; Heinze, T.; Fleck, M.; Zikeli, S.; Graeff-Hoenninger, S. Agronomic Performance of Different Open-Pollinated Beetroot Genotypes Grown Under Organic Farming Conditions. *Agronomy* **2020**, 10, 812.

Following the classification of beetroot as a superfood, the cultivation and production of this vegetable have gained more attention. With roughly 40% share of the organic cultivation of beetroot in Germany, this vegetable can be considered a typical organic product. However, the genotypes, which are commercially used for cultivation, belong to F1 hybrid genotypes, which is critical for the organic cultivation system. Therefore, due to the increase in demand for organic products, improvement of the genotypes, which comply with the principles of organic agriculture and are adapted to its requirements, is needed. Due to their characteristics of self-reproductive ability, preserving plant integrity, and restoring biodiversity, open-pollinating genotypes indicate benefits for the application in organic agriculture. In this regard, in Chapter 3, a wide range of open-pollinated genotypes, which were cultivated under organic conditions in three different pedo-climatic locations, were compared with the commonly used hybrid genotypes. In this study, not only yield and disease resistance but also the morphological traits of these genotypes, due to their influence on consumer choice, processing, and transportation, were taken into account.

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Article

Agronomic Performance of Different Open-Pollinated Beetroot Genotypes Grown Under Organic Farming Conditions

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Abstract: Due to the increase of the organic cultivation of beetroot and its growing importance as a functional food, the potential advantages of open-pollinated genotypes as an alternative for F1 hybrid genotypes has been investigated. In this regard, six field experiments were carried out in 2017 and 2018 in three different locations to investigate the agronomic potential of new and existing open-pollinated genotypes of beetroot and their performance under the specific conditions of organic agriculture. Fifteen beetroot genotypes, including one F1 hybrid as a commercial control and one breeding line, were compared regarding their total and marketable yield, individual beet weight, diameter of beet, and leaves-growth-base width. Furthermore, five randomly selected beetroots meeting common marketable criteria were evaluated optically with regard to skin smoothness, corky surface, root tail, scab incidence, and uniformity. Results of this study indicated a significant impact of genotype on eight of the assessed traits. The cylindrical-shaped genotype, Carillon RZ, demonstrated significantly higher total and marketable yields, with 53.28 ± 3.34 t ha^{-1} and 44.96 ± 3.50 t ha^{-1}, respectively, compared to the yellow-colored genotype, Burpees Golden, which obtained the lowest total yield, 36.06 ± 3.38 t ha^{-1}, and marketable yield, 27.92 ± 3.55 t ha^{-1}. Moreover, the comparison of the open-pollinated genotypes with the F1 hybrid, Monty RZ F1, revealed that except for the traits yield, scab, and uniformity, the open-pollinated genotypes indicated desirable competitive outcomes and thus offer suitable alternatives for organic cropping systems. Overall, the observed genetic variability can be beneficial for breeding and food product development.

Keywords: beetroot; open-pollinated genotype; organic farming; total yield; marketable yield; beet morphology

1. Introduction

With an average of 15% annual increase in cultivation area and a total of approximately 1900 ha (2018), beetroot is a commercially important vegetable in Germany [1]. The high demand for beetroot is also reflected in the quantities purchased per household in Germany, which amounted in 2018 to two kilograms [2]. Approximately 40% of the beetroot produced in Germany grows on organic farmlands, which makes this vegetable a typical organic product [3]. Beetroot is often offered fresh, as well as pre-cooked and vacuum-packed [1].

Beetroot (*Beta vulgaris* L. ssp. *vulgaris)* belongs to the Chenopodiaceae family. It is considered a classic winter vegetable and its pollination is naturally carried out by wind [4]. In the first year, the beet is formed by the thickening of the shoot axis section below the cotyledons. Depending on the genotype, the beet has a round or cylindrical shape. For seed production, beets are replanted in the second year. Recently, with the classification of beetroot as a functional food [5], the importance of this vegetable has further increased. The term functional food generally covers those foods showing health-promoting and disease-preventing benefits due to their nutritive value [6]. Moreover, relatively easy cultivation and good storability without the need for costly storage equipment has increased farmers' as well as the food industry's interest in beetroot production [7]. In addition, information on the morphology of the root crops, especially the shape and size of the storage organ, due to its impact on processing, consumer choice, and transportation, is necessary [8] and consequently it can lead to quality improvement of the crop products [9].

The demand for organic products is constantly increasing [10]. According to different studies which compared the quality of organic and conventional food products, a higher amount of health-benefiting compounds and lower amounts of harmful components were reported in organically produced foods [11–14]. For instance, the study of Bavec et al. [10] depicted that the amount of total sugar content, total phenol content, and total antioxidant activity was higher in organically grown beetroot (cv. Rote Kugel) compared to those which were cultivated under conventional conditions. Moreover, in terms of sensory quality, red beetroot grown under organic conditions had a better taste and smell [15]. In order to meet the requirements and comply with the values of organic agriculture, cultivars need to be adapted to the agronomic conditions of organic farming [16]. One of the main criteria for organic crops is their ability to reproduce. Hence, open-pollinating genotypes are of major interest as the farmers can multiply the seeds themselves instead of depending on external inputs [17,18]. The other important principle of organic plant breeding is that the characteristics of the selected plant can be passed on homogeneously to the next generations [19]. Despite the fact that the F1 hybrids are known to be homogeneous genotypes, the resulting offspring are typically heterogeneous [20]. F1 hybrids are referred to those seeds or plants of the first filial generation which result from a cross-mating of different parental types [21]. Furthermore, the F1 hybrid genotypes of beetroot show a narrower genetic base in comparison with open-pollinated cultivars [22]. Consequently, the open-pollinating cultivars represent more advantages for the use in organic farming compared to F1 hybrids.

The objectives of the present study were to assess the agronomic performance of 14 new and existing open-pollinated beetroot genotypes, including one breeding line, and one F1 hybrid under the specific conditions of organic farming. The genotypes were evaluated for their genetic yield potential and morphological traits and their suitability and stability in different years and locations.

2. Materials and Methods

2.1. Field Experiments and Plant Materials

Field trials were carried out in 2017 and 2018, at three different locations. Two of the locations were the on-farm breeding locations coordinated by Kultursaat e.V.: De Beersche Hoeve (Oostelbeers, Netherlands), and Horticulture station Heinze (Bingenheim, Hessia, Germany). The third location was the research station for organic farming Kleinhohenheim (University of Hohenheim, Stuttgart, Baden-Wuerttemberg, Germany).

The on-farm breeding location De Beersche Hoeve is located at a latitude of 51°28'47 N, longitude of 5°15'35 E, and 17 m above sea level. In 2017, during the growth period (July–October), the mean temperature was 16 °C and the mean precipitation reached 47 mm. In 2018, the mean temperature and precipitation during the growth period (June to September) were 16.5 °C and 27.7 mm, respectively. The soil texture is fine sand with a low clay content.

The Horticulture station Heinze (50°22′28 N, 8°53′45 E) is located at an altitude of 129 m above sea level. In 2017, during the growth period (June to September), the mean temperature was 16.2 °C and the mean rainfall was 53.5 mm. In 2018, the mean temperature and the precipitation during June to September were 20.7 °C and 24.1 mm, respectively. The soil texture is clayey loam.

The research station Kleinhohenheim is located at a latitude of 48°44′14 N, longitude 9°12′01 E, and 430 m above the sea level. In 2017, during the months July to October, the mean precipitation was 77.42 mm and the mean temperature 17.6 °C. In 2018, the mean rainfall reached to 38.2 mm and the mean temperature was 19.0 °C between June and September. The soil texture is considered as loess to loamy clay.

Total monthly precipitation as well as monthly mean temperature of all three research stations during the experimental period in 2017 and 2018 are shown in Figure 1A–C.

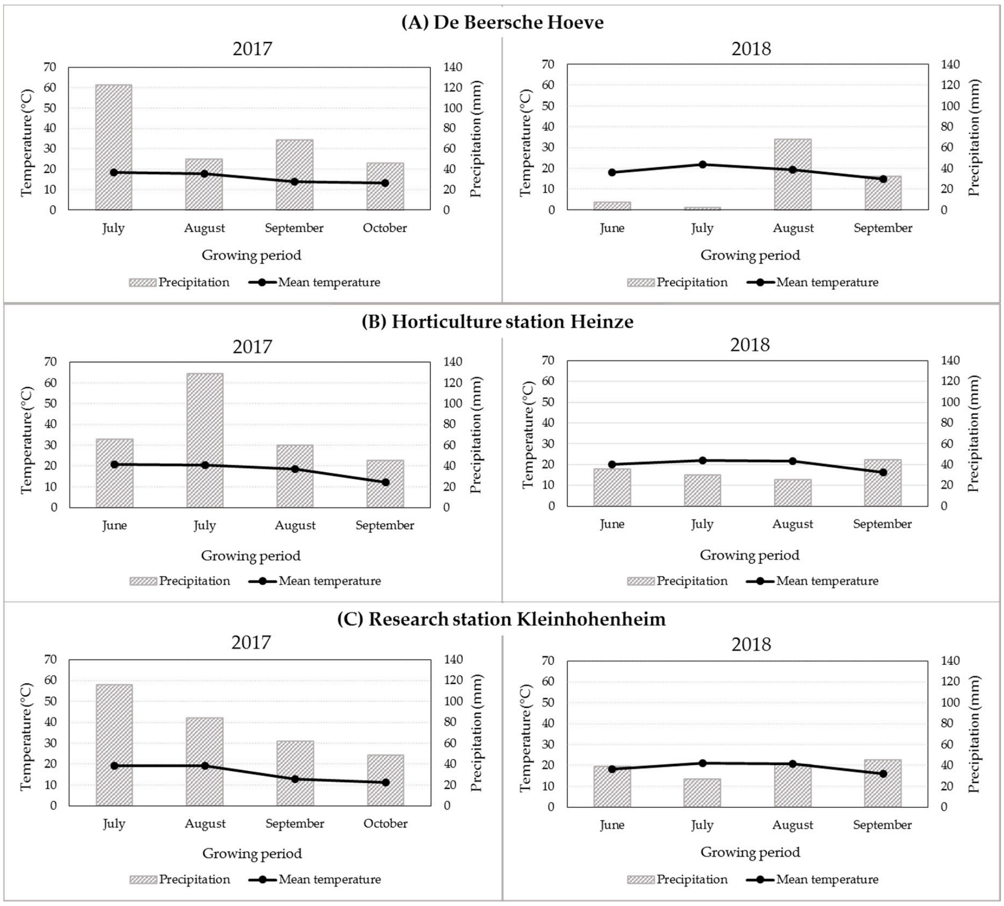

Figure 1. Total monthly precipitation (mm) and monthly mean temperature (°C) of the on-farm breeding station De Beersche Hoeve (the Netherlands) (**A**), the Horticulture station Heinze (Germany (**B**), and research station Kleinhohenheim (Germany) (**C**) during the experimental period in 2017 and 2018.

All field trials were conducted under organic farming conditions in accordance with the European Commission's main regulations for organic farming. Briefly, no synthetic fertilizer was applied to the soil. Pests and disease control were done using biological controls. Frequent mechanical or manual weeding was conducted to suppress the weeds. The genotypes were allocated to the six field trials according to a non-resolvable row column design or a randomized complete block design (Table A1). In 2017, on each on-farm breeding location 30, and in Kleinhohenheim, 40 open pollinating genotypes were cultivated in three field replicates. In 2018, 16 open pollinating genotypes were assessed on each on-farm breeding location, while 36 genotypes were tested in Kleinhohenheim. Over both years

15 genotypes were assessed on all locations forming the orthogonal core of the experiment. Note that data from all genotypes were analyzed, but the presented results were limited to the 15 genotypes which occurred in all location-by-year combinations. The 15 investigated genotypes were 13 red-colored beets: Akela, Betina, Bona, Bordo AS, BoRu1, Carillon RZ, Cervena Kulata, Detroit 3, Jawor, Monty RZ F1, Nobol, Nochowski, Ronjana, one white genotype, Sniezna Kula, and one yellow-colored beet, Burpees Golden. BoRu1 is a breeding line of the breeding organization, Kultursaat e.V., which was used in order to assess its market potential as a new open-pollinated genotype. Table 1 depicts the information regarding shape and color of the beets as well as the seed origin of the 15 selected genotypes. The seeds needed for all six trials were ordered in spring 2017 and stored in a dark and dry place until the sowing day.

Table 1. List of the 15 studied beetroot genotypes (beet shape and color, and seed origin).

Genotype	Beet Color	Shape	Seed Origin
Akela RZ	red	spherical	Rijk Zwaan
Betina	red	spherical	Moravo Seeds (CZ)
Bona	red	spherical	Moravo Seeds (CZ)
Bordo AS	red	spherical	Seklos (LT)
BoRu1	red	spherical	Kultursaat e.V.
Burpees Golden	yellow	spherical	Bingenheimer S. AG
Carillon RZ	red	cylindrical	Rijk Zwaan
Cervena Kulata	red	spherical	Moravo Seeds (CZ)
Detroit 3	red	spherical	Caillard
Jawor	red	spherical	Snówidza (PL)
Monty RZ F1 [1]	red	spherical	Rijk Zwaan
Nobol	red	spherical	Vilmorin (PL)
Nochowski	red	spherical	Spójnia (PL)
Ronjana	red	spherical	Bingenheimer S. AG
Sniezna Kula	white	spherical	Torseed (PL)

[1] Except the genotype Monty RZ F1, all the other genotypes are open-pollinated.

At De Beersche Hoeve, the field experiments were conducted as row-column design in 2017 and as randomized complete block design in 2018. In the first trial year, the field was fertilized with compost equivalent to 60 kg N ha^{-1} and in the second year, compost equivalent to 90 kg N ha^{-1} was incorporated into the soil. The preceding crops were clover grass and Romanesco broccoli in 2017 and 2018, respectively. The field was ploughed two months before each experiment to a depth of 20 cm. Harrowing was done twice before sowing to a depth of approximately 3 cm. The plot size was set as 9 m^2 with three cultivation rows per plot and an inter-row spacing of 50 cm. In the first field trial, sowing was conducted using a precision seed drill on 14 July 2017, and the harvest was done manually on 26 October 2017. In 2018, the beetroots were seeded on 12 June 2018 and harvested on 27 September 2018. In both trials, weed control was done manually and mechanically by hoeing.

At the Horticulture station Heinze, the field experiment was carried out as randomized complete block design in both 2017 and 2018 with three replicates. In 2017, the preceding crop was broccoli, while in 2018, the preceding crop was carrot. In both experimental years, no fertilizer was applied to the field. The field was prepared by ploughing in autumn to a depth of 25 cm and harrowed three months before sowing to a depth of 8 cm. The plot size was 8.75 m^2 with three rows per plot and an inter-row spacing of 42 cm. In 2017, the sowing took place on 08 June 2017 using a hand-sowing machine and the harvest was carried out manually on 30 September 2017. In 2018, the trial started on 09 June 2018 and the beetroots were harvested on 10 September 2018. Weeding was performed by hand and by a wheel hoe.

At the research station Kleinhohenheim, the experimental design was set up as row-column with three replicates in 2017 and as non-resolvable block design, with a block size of ten and a number of treatment of 36, in 2018, with three replicates for 32 genotypes and six replicates for four genotypes. The unequal replications were due to the point that the four genotypes with six replicates belonged to breeding lines, and their performance in the field was unknown. Therefore, in order to have sufficient samples, more replicates were considered for them. In 2017, the preceding crop was clover grass which was considered as green manure, and additionally, the field was fertilized with Vinasse, equivalent to 8.3 kg N ha^{-1}, one month after seeding. In 2018, no additional fertilizer was used due to the N fixation by the pre-crop clover grass. In January 2017, the field was ploughed to a depth of 20 cm and the seed bed was prepared using a rotary harrow to a depth of 6 cm. In the second year of the experiment, ploughing was carried out in January 2018 and harrowing was done about 40 days before sowing. The plot size was 14 m^2 with four rows per plot and an inter-row spacing of 35 cm. In the first experiment, sowing was done on 06 July 2017 and beetroots were harvested manually on 16 October 2017. The sowing and harvest date in the second field trial was on 06 June 2018 and 10 September 2018, respectively. In both years, sowing was conducted using a pneumatic single-seed drill. Inter-row weed control was performed manually and intra-row weeding was carried out mechanically by hoeing.

2.2. Agronomic Traits

2.2.1. Total and Marketable Yield

After the manual harvest of the beetroots, the stems and leaves were cut by hand and immediately the beets' fresh-weight was measured. For the determination of the marketable yield, the harvested beetroots were sorted based on our defined diameter (between 5 and 13 cm) and quality (without any deformation and notable damage).

2.2.2. Diameter of Beet and Width of Leaves-Growth-Base

The average beet size of each genotype as well as the width of the leaves-growth-base was determined by using a Vernier caliper. Five randomly selected marketable beetroots of each plot were measured at the widest part of the beet and at the leaves-growth-base.

2.2.3. Individual Beet Weight

Five randomly selected marketable beets were weighed individually, directly after the harvest, to estimate the average weight of beets of the investigated genotypes and to understand if the yield consisted of a lot of small beets or of bigger and fewer beets per defined area.

2.2.4. Beet Evaluation

With respect to the remarkable influence of the beet morphology and disease resistance of different genotypes on the quality and customer acceptance, the following five traits were evaluated, smoothness of skin, corky surface, root tail, scab (*Streptomyces scabies*), and uniformity (Table 2). In this regard, the marketable beetroots were examined optically using a 1–9 scale. Smoothness and corky surface of beet skin, as well as scab incidence, are among the traits affecting the attractiveness of the beets especially for the fresh market. Information on root tail and uniformity is particularly important for the use of the beet for processing purposes.

Table 2. Evaluation specification of five important outer traits of beetroot using a 1–9 scale.

Scale	Smoothness of Skin	Corky Surface	Root Tail	Scab	Uniformity
1	very rough	no/very little	not detached	no infestation	very low
3	rough	little	little detached	little	
5	average	average	average	average	average
7	smooth	much	detached	many	
9	very smooth	very much	very detached	very much	very high

2.3. Statistical Analysis

According to the design of the experiment, the data was analyzed by the following linear mixed model:

$$y_{ijklmn} = \mu + b_{ljk} + r_{mjk} + c_{njk} + a_j + l_k + \tau_i + (al)_{jk} + (\tau a)_{ij} + (\tau l)_{ik} + (\tau al)_{ijk} + e_{ijklmn}, \quad (1)$$

where a_j and l_k are the fixed effects of the j-th year and the k-th location. b_{ljk}, r_{mjk}, and c_{njk} are the random effect of the l-th block, m-th row, and n-th column within a year-by-location combination. Note that row and column effects were only fitted for row-column designs. Further note that block effects were only fitted if the design has blocks. Dummy variables were used to block out these effects for all other experiments. τ_i is the fixed effect of the i-th genotype. $(al)_{jk}$ is the fixed interaction effect of the j-th year and the k-th location. $(\tau a)_{ij}$,$(\tau l)_{ik}$, and $(\tau al)_{ijk}$ are the random interactions between the corresponding main effects. e_{ijklmn} is the error of y_{ijklmn} with year-by-location specific error variance. The assumptions of normality and homogeneous variances of residuals were checked graphically. If required, a logarithmic transformation of the data was used prior to analysis and results were back-transformed for presentation purpose only. Standard errors were back-transformed using the delta method. Depending on the significance of fixed effects, mean comparisons were done for the highest significant interaction term of all factors using Fisher's Least Significant Difference (LSD) test [23]. Means (or medians in case of back-transformed values) plus minus standard error of the mean (approximated standard error of the median) were presented. A letter display was used to present significant differences between means or medians [23]. Means followed by at least one identical letter were not significantly different from each other.

3. Results and Discussion

The statistical analysis showed that all investigated traits except the root tail and scab incidence were significantly influenced by the genotype ($p < 0.05$). This demonstrated a high variability between the genotypes regarding the agronomic traits. In order to assess the performance of different genotypes under different environmental and climatic conditions, the influence of location and experimental year on different agronomic traits was taken into account, as well. In accordance with the statistical analysis, all traits except scab, and leaves-growth-base width and individual beet weight were significantly influenced by the interaction of location × year ($p < 0.05$). Scab incidence was neither affected by year nor by location. In terms of leaves-growth-base, the results of the analysis of variance (ANOVA) showed a significant effect of year ($p < 0.05$) and individual beet weight was significantly impacted by both year and location. Outcomes of the ANOVA of the investigated agronomic traits are presented in Tables 3 and 4.

Table 3. Mean values and analysis of variance (ANOVA) of results of total yield (t ha^{-1}), marketable yield (t ha^{-1}), beet diameter (mm), leaves-growth-base width (mm), and individual beet weight (g) of 15 different genotypes of beetroot grown in three research stations within the trial year 2017 and 2018. Results represent the mean values ± standard error. Means followed by at least one identical letter were not significantly different from each other.

Genotype	Total Yield (t ha^{-1})	Marketable Yield (t ha^{-1})	Beet Diameter (mm)	Leaves-Growth-Base Width (mm)	Individual Beet Weight (g)
Akela	49.56 ^{abc} ± 3.34	39.37 ^{abc} ± 3.50	78.40 ^{a} ± 0.31	27.78 ^{egh} ± 1.83	252.54 ^{ac} ± 17.42
Betina	48.11 ^{ad} ± 3.34	34.55 ^{cd} ± 3.50	80.59 ^{a} ± 0.31	37.78 ^{b} ± 1.83	266.44 ^{ac} ± 17.42
Bona	50.41 ^{abc} ± 3.47	39.64 ^{abc} ± 3.54	80.29 ^{a} ± 0.31	32.49 ^{cde} ± 1.86	279.01 ^{a} ± 17.42
Bordo AS	50.34 ^{abc} ± 3.97	42.29 ^{ac} ± 4.17	81.50 ^{a} ± 0.31	36.10 ^{bc} ± 1.83	283.33 ^{a} ± 17.42
BoRu1	46.73 ^{ad} ± 3.34	37.44 ^{ad} ± 3.50	78.51 ^{a} ± 0.31	25.22 ^{gh} ± 1.83	252.56 ^{ac} ± 17.42
Burpees Golden	36.06 ^{e} ± 3.38	27.92 ^{d} ± 3.55	71.25 ^{b} ± 0.31	27.52 ^{egh} ± 1.83	214.71 ^{c} ± 17.42
Carillon RZ	53.28 ^{a} ± 3.34	44.96 ^{a} ± 3.50	48.54 ^{c} ± 0.31	23.63 ^{h} ± 1.83	239.87 ^{ac} ± 17.42
Cervena Kulata	44.12 ^{cde} ± 3.34	32.38 ^{cd} ± 3.50	79.26 ^{a} ± 0.31	33.85 ^{bd} ± 1.83	259.48 ^{ac} ± 17.42
Detroit 3	46.35 ^{ad} ± 3.34	33.28 ^{cd} ± 3.50	80.55 ^{a} ± 0.31	27.83 ^{egh} ± 1.83	266.90 ^{ab} ± 17.42
Jawor	46.02 ^{ad} ± 3.34	35.98 ^{ad} ± 3.50	80.04 ^{a} ± 0.31	32.58 ^{bde} ± 1.83	258.66 ^{ac} ± 17.42
Monty RZ F1	51.42 ^{ac} ± 3.34	39.96 ^{abc} ± 3.50	76.99 ^{ab} ± 0.31	32.44 ^{cde} ± 1.83	258.56 ^{ac} ± 17.42
Nobol	40.15 ^{de} ± 3.34	31.16 ^{bd} ± 3.50	76.31 ^{ab} ± 0.31	25.78 ^{fgh} ± 1.83	221.77 ^{bc} ± 17.42
Nochowski	43.44 ^{cde} ± 3.34	32.17 ^{cd} ± 3.50	79.08 ^{a} ± 0.31	43.14 ^{a} ± 1.40	267.27 ^{ab} ± 17.42
Ronjana	42.72 ^{bde} ± 3.39	33.21 ^{cd} ± 3.52	75.93 ^{ab} ± 0.31	30.88 ^{def} ± 1.40	252.89 ^{ac} ± 17.42
Sniezna Kula	49.70 ^{abc} ± 3.34	35.60 ^{ad} ± 3.50	79.43 ^{a} ± 0.31	29.21 ^{dg} ± 1.41	265.87 ^{ac} ± 17.42
Factor			***p*-value of the F-test of the corresponding factor**		
Location	<0.0001	<0.0001	<0.0001	n.s. [1]	<0.0001
Year	n.s.	0.0259	<0.0001	<0.0001	0.0034
Genotype	0.0013	0.0262	<0.0001	<0.0001	0.0002
Location × year	0.0005	<0.0001	<0.0001	n.s.	n.s.

[1] not significant.

Table 4. Mean values and analysis of variance (ANOVA) of results of the evaluation of beet regarding the skin smoothness, corky surface, root tail, scab, and uniformity of 15 different genotypes of beetroot grown in three research stations within the trial year 2017 and 2018, using 1–9 scale. Results represent the mean values ± standard error. Means followed by at least one identical letter were not significantly different from each other.

Genotype	Smoothness of Skin	Corky Surface	Root Tail	Scab	Uniformity
Akela	5.66 ^{abcd} ± 0.40	4.47 ^{bc} ± 0.34	6.67 ± 0.42	3.99 ± 0.34	6.16 ^{ab} ± 0.38
Betina	4.90 ^{be} ± 0.40	4.48 ^{bc} ± 0.34	5.32 ± 0.42	3.94 ± 0.34	6.28 ^{ac} ± 0.38
Bona	5.65 ^{abcd} ± 0.40	4.43 ^{bc} ± 0.34	6.18 ± 0.42	4.02 ± 0.34	5.98 ^{ab} ± 0.38
Bordo AS	5.05 ^{ce} ± 0.40	4.93 ^{bc} ± 0.34	5.65 ± 0.42	3.81 ± 0.34	5.35 ^{bc} ± 0.38
BoRu1	5.08 ^{ce} ± 0.40	4.65 ^{bc} ± 0.34	6.21 ± 0.42	4.09 ± 0.34	6.16 ^{ab} ± 0.38
Burpees Golden	4.59 ^{de} ± 0.40	4.90 ^{bc} ± 0.34	5.23 ± 0.42	3.50 ± 0.35	5.27 ^{bc} ± 0.38
Carillon RZ	6.03 ^{abcd} ± 0.40	4.52 ^{bc} ± 0.34	5.00 ± 0.42	3.20 ± 0.34	6.12 ^{ab} ± 0.38
Cervena Kulata	4.67 ^{de} ± 0.40	5.04 ^{ab} ± 0.34	5.68 ± 0.42	4.12 ± 0.34	6.23 ^{ab} ± 0.38
Detroit 3	3.96 ^{e} ± 0.40	4.34 ^{bc} ± 0.34	6.45 ± 0.42	3.96 ± 0.34	5.19 ^{b} ± 0.38
Jawor	6.06 ^{ac} ± 0.22	4.90 ^{bc} ± 0.34	6.34 ± 0.42	3.80 ± 0.34	6.03 ^{ab} ± 0.38
Monty RZ F1	6.0 ^{abcd} ± 0.22	4.46 ^{bc} ± 0.34	5.78 ± 0.42	3.43 ± 0.34	6.61 ^{a} ± 0.38
Nobol	4.58 ^{de} ± 0.22	5.97 ^{a} ± 0.34	6.24 ± 0.42	3.87 ± 0.34	5.67 ^{ab} ± 0.38
Nochowski	5.39 ^{abcd} ± 0.22	4.08 ^{c} ± 0.34	5.65 ± 0.42	3.72 ± 0.34	6.15 ^{ab} ± 0.38
Ronjana	5.11 ^{ce} ± 0.22	4.17 ^{bc} ± 0.34	6.56 ± 0.42	4.65 ± 0.34	5.84 ^{ab} ± 0.38
Sniezna Kula	6.35 ^{a} ± 0.23	2.85 ^{d} ± 0.34	5.50 ± 0.42	4.65 ± 0.34	6.65 ^{a} ± 0.38
Factor	***p*-value of the F-test of the corresponding factor**				
Location	<0.0001	0.0002	<0.0001	n.s. [1]	n.s.
Year	n.s.	0.0003	n.s.	n.s.	n.s.
Genotype	0.0024	0.0002	n.s.	n.s.	0.0292
Location × year	<0.0001	0.0039	<0.0001	n.s.	<0.0001

[1] not significant.

3.1. Total and Marketable Yield

The total yield ranged between 36.06 ± 3.38 t ha^{-1} and 53.28 ± 3.34 t ha^{-1}. Among all 15 genotypes, the three highest total mean yields were noted for Carillon RZ, Monty RZ F1, and Bona with 53.28 ± 3.34 t ha^{-1}, 51.42 ± 3.34 t ha^{-1}, and 50.41 ± 3.47 t ha^{-1}, respectively, which were statistically non-significantly different from each other (Table 3). On the other hand, the three genotypes, namely, Burpees Golden, Nobol, and Ronjana with 36.06 ± 3.38 t ha^{-1}, 40.15 ± 3.34 t ha^{-1}, and 42.72 ± 3.39 t ha^{-1}, respectively, showed the lowest total yield (Table 3).

The marketable yield of the studied beetroot genotypes varied between 27.92 ± 3.55 t ha^{-1} and 44.96 ± 3.50 t ha^{-1} for the genotypes Burpees Golden and Carillon RZ, respectively, and were statistically different from each other (Table 3). Although the second-highest total yield belonged to the genotype Monty RZ F1, the same order was not seen for the marketable yield. The genotype Bordo AS, which gained the fourth-highest total yield with 50.34 ± 3.97 t ha^{-1}, had the second-highest marketable yield with 42.29 ± 4.17 t ha^{-1}, which indicated its higher share of marketable yield compared to the F1 hybrid genotype, Monty RZ F1.

The results revealed that the highest total and marketable yield belonged to the cylindrical genotype, Carillon RZ. Although the spherical shape has been better accepted in the fresh market [24], an increasing use of cylindrical shape genotypes in beetroot processing, due to the higher percentage of utilizable uniform slices in canning and slicing practices, has been reported [25]. Nevertheless, this uniformity in the top, middle, and bottom of the cylindrical-shape beets can be affected by population density and genotype [25]. There are different factors which affect the yield, such as sowing [26] and harvesting [27,28] date, water availability [29], cultivation system [30], and plant density [26]. Feller and Fink [27] stated that the effect of sowing date on the beetroot yield is significant. Takács-Hájos and Rubóczki [31] studied the differences in beetroot yield, depending on sowing date (April, July, and August), and noted that the highest yield was reached at the earliest sowing date in April. Furthermore, Akhiyarov et al. [32] reported a total yield ranging from 35.30–53.70 t ha^{-1}

for a beetroot cultivar sown at five different dates, between the beginning of April and end of May over three years. In the current research, despite the fact that the sowing dates were either in June or July, the total yield was in a similar range as in the study of Akhiyarov et al. [32]. The study of Kikkert et al. [26] depicted that the marketable yield increased significantly with delayed harvest dates. Among all three harvest dates that were investigated for one beetroot genotype within two years, beetroots with 120 days growing period indicated a marketable yield of 48.9 t ha^{-1}, while those with 100 and 80 growing days revealed a marketable yield of 44.2 and 28.7 t ha^{-1}, respectively. In the present study, the range of the growing period was between 93–107 days, the marketable yield for all investigated genotypes varied between 27.92 ± 3.55 t ha^{-1} and 44.96 ± 3.50 t ha^{-1}.

Considering the cultivation system as one of the yield influencing factors, it was reported that the yield of beetroots grown under organic cultivation systems was 75% of that for the conventional system [30]. Conventionally cultivation of seven different beetroot genotypes in Austria in 2013 showed a yield range of 25.5–32.5 t ha^{-1} [33]. However, in the present work, despite the fact that the beetroots were grown under organic conditions, total yields were considerably higher. Furthermore, according to Stagnari et al. [29], water stress can significantly decrease the yield of beetroot. In 2018, due to the extremely hot and dry summer in Germany, lower average yields in both locations was noted.

3.2. Beet Diameter and Leaves-Growth-Base Width

Except for the cylindrical genotype, Carillon RZ, which significantly had the lowest beet diameter with 48.54 ± 0.31 mm, the range of the beet diameter for all spherical genotypes varied between 71.25 ± 0.31 mm and 81.50 ± 0.31 mm belonging to Burpees Golden and Bordo AS, respectively, which were significantly different from each other (Table 3). Ijoyah et al. [34] compared the beet width of five different spherical-shape beetroot genotypes, and reported a diameter range of 3.64–7.55 cm. Moreover, the beet diameter of the cylindrical-shaped genotype, Regulski Cylinder, which was grown organically in Poland, was 5.43 cm in 2006 and 2008, and 5.0 cm in 2009 [7], which in all cases are slightly larger than the mean beet diameter of the studied cylindrical-shape genotype in the present study. This may be due to the difference in the investigated genotype and the cultivation period, which was longer in the study of Szopińska and Gawęda [7]. The genotypes Betina and Detroit 3 with 80.59 ± 0.31 mm and 80.55 ± 0.31 mm, respectively, had the second and third largest beet diameter. Following the yellow beetroot genotype, Burpees Golden, the cultivars Ronjana with 75.93 ± 0.31 mm and Nobol with 76.31 ± 0.31 mm had the smallest beet diameter (Table 3). The size of the beet can be mainly influenced by nitrogen fertilization [35] and water availability [26]. In the present work, in accordance with the outcomes of ANOVA, beet size was significantly impacted by genotype and the interaction of location × year ($p < 0.0001$). Burpees Golden, which possessed the smallest beet diameter, was noticed as the genotype with the lowest total and marketable yields. However, the same relation was not seen for the genotype Monty RZ F1, which was categorized among the smallest-sized beets, but resulted in the second-highest total and marketable yields. Based on Ugrinovic [35], among the marketable beetroots (beet diameter between 6–15 cm), those with the beet diameter of 6–9 cm can be categorized as the first-class beets for the marketable purposes. Accordingly, all 14 spherical-shape genotypes in this study can be considered as first class beets.

The smallest leaves-growth-base width belonged to the cylindrical genotype, Carillon RZ, with 23.63 ± 1.84 mm, followed by the genotype Boru1 and Nobol with 25.22 ± 1.84 mm and 25.78 ± 1.84 mm, respectively. No significant difference was observed between them (Table 3). In contrast, the genotype Nochowski with 43.14 ± 1.84 mm had a significantly larger leaves-growth-base compared to all studied genotypes (Table 3), followed by Betina and Bordo AS with 37.78 ± 1.84 mm and 36.21 ± 1.84 mm, respectively. This trait is important in the processing of beetroot, as the leaves-growth-base is included in the part which should be cut during processing. Therefore, a smaller leaves-growth-base width is desirable in order to achieve less flesh loss. According to the outcomes of this study, as the genotype demonstrated a significant effect on the leaves-growth-base width

($p < 0.0001$), selection of the suitable genotype, especially for processing purposes, may lead to lower waste.

3.3. Individual Beet Weight

According to the ANOVA, genotype, experimental year, and location influenced the weight of the beetroot. The beets weight varied between 214.71 ± 17.42 g and 283.33 ± 17.42 g (Table 3).

The fresh beet weight of 15 beetroot genotypes, which were sown in July in Hungary, ranged between 88.93–198.67 g, which is notably lower compared to the findings of the present study [31]. Furthermore, investigation of seven beetroot genotypes in Austria demonstrated a beet weight range of 214–366 g, in which the minimum value was similar to the results of this study but the maximum value was higher. Following the same position in beet diameter as well as the total yield, the yellow genotype, Burpees Golden, which owned the smallest beet diameter and the lowest yield, showed significantly lowest value regarding the beet weight with 214.71 ± 17.42 g. On the other hand, the genotype, Bordo AS, with the largest beet diameter, possessed the heaviest beets among all investigated genotypes with a mean beet weight of 283.33 ± 17.42 g. Specifications of the market regarding the size of the root crops have been increasing [36]. Although the agronomic factors affecting the size, such as plant density or harvest time, can be controlled, the size variation within a crop is still great [36]. Therefore, in order to achieve a comprehensive overview of the beet size, beside the beet diameter, the fresh weight of the individual beet was measured in this study. An important factor that determines beet size is the water availability during the growing period [26], as earlier sowing dates showed higher beet weights related to water supply in 15 different genotypes cultivated in Debrecen, Hungary [31]. In the current study, all the field trials were started either in June or July. In addition, the second year of the experiment was considered as an extreme climate condition due to the high temperature and low precipitation level. Therefore, considering the significant effect of the trial year on the beet weight in this study, it can be concluded that the abiotic factors impacted the beet weight.

Due to the importance of the beet size for the fresh market as well as for processing, information about the genetically determined beet size in order to select the suitable genotype for the specific utilization should be taken into account. For instance, the genotypes with beet sizes too large for use in the fresh market could be interesting for juice production or the use in the production of pre-cooked beetroot.

3.4. Beet Evaluation

Morphology of the beet can have a notable influence on the quality, and its attractiveness is a key factor for the fresh market [24]. It is reported [37] that besides high yield and resistance against diseases and pests, shape [38] and uniformity in color without white flecking [39] are the main criteria in the selection process of a beetroot genotype for breeding [40] and processing purposes. One of the factors which plays a key role in physiology and consequently for the quality of different plant species is drought stress [29]. Accordingly, due to the high temperature as well as low precipitation in the second year of this experiment, it can be assumed that the beet morphology has been influenced by this factor.

3.4.1. Smoothness and Corky Surface of Beet Skin

Evaluation of the beets revealed that the skin of the 15 studied genotypes differed significantly from rough to smooth with a range of 3.96 ± 0.40 to 6.35 ± 0.40 (Table 4). The results depicted that the white genotype, Sniezna Kula, with 6.35 ± 0.40, had the smoothest skin among all evaluated genotypes followed by Jawor and Carillon RZ with 6.06 ± 0.40 and 6.03 ± 0.40, respectively. There was no significant difference noted between them (Table 4). On the other hand, Detroit 3, Nobol, and Burpees Golden with 3.96 ± 0.40, 4.58 ± 0.40, and 4.59 ± 0.41, respectively, were evaluated as three genotypes with the lowest smoothness, although they were not significantly different from each other (Table 4). According to the results of the analysis of the variance showing a significant effect of genotype on skin smoothness ($p = 0.0024$), high variability of the beetroot genotypes regarding these characteristics can be concluded. Furthermore, the interaction of location × year significantly affected the smoothness of the beet skin as well ($p < 0.0001$).

Beetroots with lower corky skin have a more attractive appearance for the fresh market. Corky surface is resulted from the light brown stripes which are formed on the skin of the beet. Nobol, with 5.97 ± 0.34, had a significantly corkier surface compared to Cervena Kulata, with 5.04 ± 0.34, which was evaluated as the genotype with the second-corkiest surface. Contrarily, Sniezna Kula demonstrated the least corky surface with 2.85 ± 0.34 followed by the genotype Nochowski with 4.08 ± 0.34. The values were significantly different from each other (Table 4).

Comparing the results of the two traits, corky surface and beet skin smoothness, it can be seen that the genotype Sniezna Kula, which had the smoothest beet skin, was as well the genotype with the lowest corky surface which may be due to the point that the lower cork on the beet makes the skin smoother. Likewise, Nobol with the second-lowest skin smoothness was noted as the genotype with the highest corky surface. The study of Da Silva et al. (2006) indicated that an increase of the mean tuber weight in potato finally led to a smoother skin [41]. In the current study, the same correlation was not noted for beetroot. Furthermore, it has been stated that one of the factors which improves skin smoothness of carrots is to grow them on ridges [42]. It was reported that the incidence of carrot cavity spots is more common under the wet conditions [42] and due to the fact that the soil on ridges are often softer and dries faster [43] by reason of better aeration, carrots grown on the ridges showed less cavity spots and better root shape and skin smoothness. Similar cultivation methods may improve the skin smoothness of beetroot.

3.4.2. Root Tail

The beetroots were evaluated optically based on the degree of detachment of the root tail from the beet. The results showed that the genotype Akela, with 6.67 ± 0.42, was ranked as the genotype with the highest degree of detachment of the root tail from the beet followed by Ronjana and Detroit 3 with 6.56 ± 0.42 and 6.45 ± 0.42, respectively. The cylindrical genotype, Carillon RZ, with 5.0 ± 0.42, was ranked as the genotype with the lowest detachment of the root tail from the beet compared to the other studied genotypes. Although the value was not significantly different from that of the cultivars Burpees Golden and Betina, which were rated around the average level with 5.23 ± 0.42 and 5.32 ± 0.42, respectively (Table 4). There were no significant differences between genotypes analyzed (Table 4). However, the statistical analysis of the results demonstrated a significant influence of the interaction of location × year ($p < 0.0001$) on the root tail. This is in line with the findings of previous studies [44,45] which noted the impact of environmental conditions, especially soil type, on the size and shape of the root vegetables. Moreover, the shape of the beetroot as well as the root tail is considered as a major factor due to the loss which may occur during processing [24]. To be more precise, the higher the degree of detachment of the root tail from the beet, the lower loss will result.

3.4.3. Scab (*Streptomyces Scabies*)

According to Table 4, scab incidence ranged between 3.20 ± 0.34 and 4.65 ± 0.34, which based on the defined 1-9 scale in Table 2, it can be concluded that all evaluated genotypes demonstrated little to average scab formation. Sniezna Kula and Ronjana, with both 4.65 ± 0.34, depicted the highest incidence of scab among all 15 genotypes. The cylindrical genotype, Carillon RZ, with 3.20 ± 0.34 and the F1 hybrid, Monty RZ F1, with 3.43 ± 0.34, were the most resistant genotypes to scab occurrence. There were no significant differences between the genotypes analyzed (Table 4). Scab (*Streptomyces scabies*) is a bacterial disease which causes warty growth on the skin and produces corky spots on the surface of different root crops, including beetroot and potato [46,47]. Despite the fact that the scab arises in all types of beet (*Beta vulgaris* L.), the occurrence of this corky lesion in beetroot due to the use in the fresh market as well as pre-cooked product in transparent packaging (such as glass bottles), is more undesirable compared with sugar beet which is pulped [48]. Crop rotation and ensuring the soil pH close to neutral are two major ways to prevent scab emergence [47]. A study by Lapwood et al. [48] on 12 different beetroot genotypes, including ten spherical and two cylindrical, showed that the two cylindrical genotypes were among the most resistant ones against the scab incidence. In line with these results, our research has as well depicted that regarding scab disease, the open-pollinated cylindrical genotype, Carillon RZ, is as resistant as the F1 hybrid, Monty RZ F1, which is typically known as the genotype with the highest resistance to the biotic and abiotic stressor.

3.4.4. Uniformity

The study showed that, based on the defined ranking, using 1–9 scale (Table 2), all 15 genotypes had an average to high uniformity. The highest uniformity was noted for the genotypes Sniezna Kula, Monty RZ F1, and Betina with 6.65 ± 0.38, 6.61 ± 0.38, and 6.28 ± 0.38, respectively. Detroit 3 with 5.19 ± 0.38, Burpees Golden with 5.27 ± 0.38, and Bordo AS with 5.35 ± 0.38 were scored as the genotypes with the lowest uniformity, through which the results were not significantly different from each other (Table 4). Strang et al. [49] reported a uniformity of the beet shape for Burpees Golden as 6.5 out of 10 (equivalent to 5.8 in our rating scale), which is close to the rate which has been noted in the present study. Due to the influencing factors such as growing space, and balanced and sufficient nutrients and water supply, uniformity of the beetroot in terms of shape and size is difficult to attain [26]. One of the influencing factors on the shape uniformity of the beets is uniform water supply in the soil [31]. Interim water deficiency can change the shape of the beet from the common spherical to oval or heart-shape, which is not desirable for the fresh market or processing purposes [24]. In the present study, due to the dry summer and low precipitation in 2018, it can be assumed that the lower water availability in the second year of the experiment affected the mean beet uniformity. Another major factor leading to non-uniformity in the maturity stage as well as the size of beets at harvest was reported to be the non-uniform seed germination [50] which occurs in the early stage of crop development [51,52]. Likewise, the cluster shape of the beetroot seed which results in up to three seedlings, causes the root size variability [53]. Furthermore, the distribution of the plants within the row and row width have been reported as two other factors affecting the uniformity of the plant [54]. In this regard, the study of Griepentrog et al. (2009) demonstrated the improvement of the spatial uniformity of sugar beet with the increase of row width from 32 to 50 cm [54]. Despite the point that the row distance width in the present study varied in all three locations (35, 42, and 50 cm), the effect of the location on the beet uniformity was not significant (Table 4). However, the interaction of location × year indicated a significant impact on the beet uniformity.

3.5. Overall Outcomes

Comparing the open-pollinated genotypes with the F1 hybrid, Monty RZ F1, indicated that in all investigated traits except for yield, scab, and uniformity, the open-pollinated genotypes revealed desirable competitive results. Additionally, with the available gene pool and selection of genotypes with preferable traits, there would be a possibility for cross-breeding in order to incorporate the outstanding characteristics into a new bred line. Considering the positive aspects of the open-pollinated genotypes for the use in organic farming, including maintaining the plant integrity and passing the plant-specific features to the next generation as well as the self-reproductive ability, the outcomes of the present study revealed the significant influence of the genotype on most of the assessed traits. Therefore, by providing information regarding the agronomic performances of different open-pollinated genotypes, the study recommends the selection of suitable genotypes for various purposes. In terms of beet uniformity as well as the persistence against the common beetroot disease, *Streptomyces scabies*, all studied genotypes indicated a high stability regarding the environmental conditions. Nevertheless, with the significant impact of location on total and marketable yield ($p < 0.0001$), it can be noted that the investigated genotypes may not be recommended for a broad range of environments if yield would be the only trait of interest.

The traits, high total and marketable yield and high degree of uniformity of the breeding line, BoRu1, showed a potential for the cultivation in organic farming. However, other traits like skin smoothness and scab formation still need further improvement by further breeding efforts.

4. Conclusions

With investigating the performance of various beetroot genotypes in different locations, it can be concluded that due to the significant genetic variability in beetroot, selection of suitable genotypes should be aligned with the intended final utilization. The cylindrical genotype, Carillon RZ, with the highest total and marketable yield, smallest leaves-growth-base width and one of the most resistance genotype against the common beet disease, scab, can be suggested as a potential genotype with outstanding characteristics to farmers for cultivation in organic farming. Finally, this study only indicated the agronomic traits of the genotypes and the related impact of environmental conditions, therefore, for the application of the investigated genotypes in different sections, the sensory quality as well as the amount of the health-promoting compounds, should be additionally taken into account.

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Appendix A

Table A1. Detailed information on the six conducted field trial in 2017 and 2018.

Location	Trial Year	Number of Genotypes	Plot Size (m^2)	Field Design	Number of Rows	Number of Columns	Number of Blocks	Number of Replications
De Beersche Hoeve (NL)	2017	30	9	row-column	3	10	3	3
	2018	16	9	randomized complete block	-	-	3	3
Horticulture station Heinze (DE)	2017	30	8.75	randomized complete block	-	-	3	3
	2018	16	8.75	randomized complete block	-	-	3	3
Klein-hohenheim (DE)	2017	40	14	row-column	4	10	3	3
	2018	36	14	non-resolvable block	-	-	12	unequal replication [1]

[1] In case of four genotypes, six replications were applied and the other 32 genotypes were replicated three times.

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4. Publication II: Bioactive Compounds and Total Sugar Contents of Different Open-Pollinated Beetroot Genotypes Grown Organically

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Vegetable consumption is associated with human health promotion due to its various benefits in reducing or preventing the risk of specific diseases such as cardiovascular and cancer. Due to the presence of betalains and phenolic compounds, this crop is among the top ten ranked vegetables containing the highest antioxidant capacity. Furthermore, the high nitrate level in beetroot helps normalize blood pressure and enhances muscle competence. Various studies compared the chemical quality of organic and conventional products and reported a higher amount of health-promoting compounds and a lower level of harmful substances in organically produced foods. However, the assessment of different biologically active compounds considering a wide range of organically grown open-pollinated beetroot genotypes was missing. Therefore, chapter 4 provides outcomes of screened open-pollinated genotypes grown under organic conditions in three different locations within two years regarding their compositional characteristics, revealing the genetic variability and impact of environmental conditions on the content of bioactive compounds. Moreover, according to the specifics of the desired level of bioactive compounds for different products, this chapter identifies the open-pollinating genotypes of beetroot with notable chemical qualities in order to indicate their potential as alternatives for hybrid genotypes.

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Bioactive Compounds and Total Sugar Contents of Different Open-Pollinated Beetroot Genotypes Grown Organically

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Abstract: The growing interest of consumers in healthy organic products has increased the attention to the organic production of beetroot. In this regard, six field experiments were conducted in 2017 and 2018 in three different locations under the specific conditions of organic agriculture, and fifteen beetroot genotypes, including one F1 hybrid as a commercial control and one breeding line, were compared regarding the content of the total dry matter, total soluble sugar, nitrate, betalain, and total phenolic compounds in order to investigate the genetic potential of new and existing open-pollinated genotypes of beetroot regarding the content of their bioactive compounds. The results of this study indicated a significant impact of genotype ($p < 0.05$) on all measured compounds. Furthermore, results revealed a significant influence of the interactions of location × year ($p < 0.05$) on the beetroot composition, and, thus, the role of environmental conditions for the formation of tested compounds. The total dry matter content (TDMC) of beetroots varied between 14.12% and 17.50%. The genotype 'Nochowski', which possessed the highest total soluble sugar content with 14.67 °Bx (Brix), was among the genotypes with the lowest nitrate content. On the contrary, the cylindrical-shaped genotype 'Carillon RZ' (Rijk Zwaan), indicated the lowest sugar content and the highest nitrate concentration. The amount of total phenolic compounds ranged between 352.46 ± 28.24 mg GAE 100 g^{-1} DW (milligrams of gallic acid equivalents per 100 g of dry weight) and 489.06 ± 28.24 mg GAE 100 g^{-1} DW for the red-colored genotypes which is correlated with the high antioxidant capacity of the investigated genotypes. Due to the specifics of the required content of bioactive compounds for various products, the selection of suitable genotypes should be aligned with the intended final utilization.

Keywords: beetroot; open-pollinated genotype; organic farming; betalain; nitrate; total sugar; phenolic compounds; total dry matter; antioxidant

1. Introduction

Consumption of vegetables due to their various benefiting effects, such as the reduced risk of cardiovascular disease, cancer, and diabetes, is associated with human health promotion [1]. Due to the high amount of biologically active compounds, processing, and product consumption of beetroot (*Beta vulgaris* subsp. *vulgaris* L.), a vegetable from the Chenopodiaceae family, has gained attention in recent years [2,3].

Several studies noted beetroot as a vegetable with remarkable health-promoting phytochemicals [2,4]. Beetroot pigmentation can be categorized into two main sources; betalains composing of betanin, isobetanin, vulgaxanthin I, and vulgaxanthin II, and phenolic compounds, including flavonoids, phenolic acids, and other organic and inorganic acids [5]. The presence of betalains and phenolic compounds in beetroot has made this crop to be among the ten vegetables with the highest antioxidant activity [6]. The phytochemicals acting as antioxidants fight against free radicals [7], which generate cancer [8], heart, and kidney illnesses [9]. Furthermore, beetroot contains various minerals such as iron, zinc, phosphorous, potassium, calcium, sodium, magnesium, manganese, and copper [3], and vitamins, such as vitamin A, B6, and C (especially in the foliage). The presence of ascorbic acid and carotenoids in beetroot can further raise the total antioxidant capacity [5,10]. Another bioactive compound, which can be found in high levels in beetroot, is nitrate. Recently, the benefiting effects of nitrate on human health, including normalizing the blood pressure, protection against ischemia-reperfusion damage [3], an increase of muscle efficiency, and fatigue resistance [2], has drawn attention.

Considering the constantly increasing demand for organic products [11], different studies compared the quality of organic and conventional food products and reported lower amounts of harmful components and a higher amount of health-benefiting compounds in organically produced foods [12–15]. Furthermore, regarding the sensory quality, a better taste and smell were reported for red-colored beetroot grown organically [16]. In organic farming, the selection of the genotypes should comply with the principles of organic agriculture. Although organic farming is very critical of the use of F1 hybrid genotypes, farmers and gardeners tend to use F1 hybrids for commercial reasons. In comparison, the open-pollinated genotypes due to their benefiting features, including maintaining the plant integrity and self-reproductive ability [17,18], signify more advantages for the use in organic agriculture compared to F1 hybrids.

The objectives of the present study were to assess the content of bioactive compounds of 15 new and existing beetroot genotypes, consisting of 14 open-pollinated beetroot genotypes, which include one breeding line, and one F1 hybrid genotype, under the specific conditions of organic farming. Genotypes were tested in three pedoclimatic different locations to also test the impact of environmental conditions on the formation of bioactive compounds. Furthermore, this study intended to identify the open-pollinating genotypes of beetroot with outstanding chemical qualities similar to commercially used hybrids in order to introduce them to the organic industry and farmers as a substituent of hybrid genotypes.

2. Results and Discussion

2.1. Total Dry Matter Content

In accordance with the findings of this study, the TDMC of the 15 investigated beetroot genotypes varied between 14.12 ± 4.56% and 17.50 ± 4.56%. The results revealed that the genotypes Nochowski, Betina, and Cervena Kulata with 17.50 ± 4.56%, 17.12 ± 4.56%, and 16.41 ± 4.56%, respectively, contained the highest percentage of TDMC (Table 1). On the other hand, the cylindrical-shaped genotype, Carillon RZ with 14.12 ± 4.56%, Nobol with 14.66 ± 4.60%, and Akela RZ with 14.95 ± 4.56% indicated the lowest TDMC. The TDMC of 15 beetroot genotypes grown in Poland within 2009–2012 ranged between 13.5% to 15.3% [19], which were slightly lower than the range found in the current study. With respect to the outcomes of the analysis of variance (ANOVA), a significant impact of genotype ($p = 0.0029$) and the interactions of year × location ($p < 0.0001$) have been noted in the TDMC (Table 1). The assessment of the effect of cultivation method, genotype, and year on the total dry matter of two beetroot genotypes (cv. Czerwona Kula and cv. Opolski) grown in Poland, showed a content of 15.1% and 19.64% for cv. Czerwona Kula under organic cultivation and 15.1% and 19.46% for cv. Opolski in 2008 and 2009, respectively. In comparison, the TDMC of beetroots grown conventionally was reported as 14.3% and 18.79% for the cv. Czerwona Kula, and 13.6% and 18.66% for the cv. Opolski in 2008 and 2009, respectively [20]. That not only reveals the differences in the TDMC between the trial year and the genotypes but also significantly higher values in the organic cultivation. The lower TDMC of both cultivars in 2008, was due to the abundant rainfall and cooler days in the growing period compared to the year 2009 [20]. In the current study, the extreme climate condition in Germany (low precipitation level and high temperature) in the second year of the field experiment, resulted in the remarkable impact of the experimental year and location on the TDMC. Concerning the cultivation system, Brandt and Mølgaard (2001) stated that due to mineral nitrogen fertilization in conventional production systems, which increases the yield and the water quantity of the plant cells, often raw materials contain more water which leads to diluted content of the nutrients [21]. Moreover, Hallmann and Rembiałkowska (2012) claimed that the higher TDMC found in sweet bell peppers results from the difference in the metabolism of organic and conventional plants [22]. Nevertheless, Sikora et al. (2008) stated no significant influence of cultivation method on the TDMC of two beetroot cultivars Czerwona Kula and Regulski Cylindryczny [23].

Table 1. Mean values and ANOVA of results of the content of nitrate (mg kg^{-1} dry weight (DW)), total soluble sugar (°Bx), total phenolic (mg gallic acid (GAE) 100 g^{-1} DW), and total dry matter (%) of 15 different genotypes of beetroot grown in three research stations within the trial year 2017 and 2018. Results represent the mean values ± standard error. Means followed by at least one identical letter were not significantly different from each other.

Genotype	Nitrate (mg kg^{-1} DW)	Total Soluble Sugar (°Bx)	Total Phenolic Content (mg GAE 100 g^{-1} DW)	Total Dry Matter Content (%)
Akela RZ	8865 ^{a,c} ± 1209	11.94 ^{c,e} ± 0.37	402.55 ^{b,c} ± 28.24	14.95 ^{d,e,f} ± 4.56
Betina	5817 ^{b,c,d} ± 1210	13.88 ^{a,b} ± 0.37	393.94 ^{b,c} ± 28.24	17.12 ^{a,b} ± 4.56
Bona	9364 ^{a,b} ± 1221	12.39 ^{c,d} ± 0.37	395.55 ^{b,c} ± 28.24	15.44 ^{c,e,f} ± 4.56
Bordo	7023 ^{b,c,d} ± 1210	12.80 ^{b,c} ± 0.37	420.29 ^{a,c} ± 28.24	16.21 ^{a,b,c,d} ± 4.56
BoRu1	4597 ^{d} ± 1211	12.47 ^{c} ± 0.37	382.73 ^{c} ± 28.24	15.47 ^{c,e,f} ± 4.56
Burpees Golden	7566 ^{a,d} ± 1211	11.94 ^{c,e} ± 0.37	172.89 ^{d} ± 28.78	15.98 ^{b,e} ± 4.56
Carillon RZ	10924 ^{a} ± 1211	11.19 ^{e} ± 0.37	352.46 ^{c} ± 28.24	14.12 ^{f} ± 4.56
Cervena Kulata	5768 ^{b,c,d} ± 1228	13.94 ^{a,b} ± 0.37	476.84 ^{a,b} ± 28.24	16.41 ^{a,b,c,d} ± 4.56
Detroit 3	7682 ^{a,d} ± 1211	11.99 ^{ce} ± 0.37	402.61 ^{b,c} ± 28.24	15.06 ^{c,e,f} ± 4.56
Jawor	7855 ^{a,d} ± 1211	12.60 ^{c} ± 0.37	415.82 ^{a,c} ± 28.24	15.55 ^{c,e} ± 4.56
Monty RZ F1	5487 ^{c,d} ± 1209	12.33 ^{c,e} ± 0.37	489.06 ^{a} ± 28.24	15.41 ^{c,e,f} ± 4.56
Nobol	9447 ^{a,b} ± 1210	11.28 ^{d,e} ± 0.37	401.23 ^{b,c} ± 28.24	14.66 ^{e,f} ± 4.60
Nochowski	4593 ^{d} ± 1212	14.67 ^{a} ± 0.37	432.90 ^{a,c} ± 28.24	17.50 ^{a} ± 4.56
Ronjana	8595 $^{a.c}$ ± 1210	12.80 ^{b,c} ± 0.37	405.32 ^{a,c} ± 28.24	15.34 ^{c,e,f} ± 4.56
Sniezna Kula	7427 ^{a,d} ± 1211	11.91 ^{c,e} ± 0.37	216.09 ^{d} ± 29.87	16.05 ^{b,e} ± 4.56
Factor	***p*-Value of the F-Test of the Corresponding Factor**			
Location	<0.0001	0.0008	n.s. [1]	0.0013
Year	n.s.	<0.0001	<0.0001	<0.0001
Genotype	0.0191	0.0028	0.0002	0.0029
Location × year	0.0495	<0.0001	0.0114	<0.0001

[1] not significant.

2.2. *Total Soluble Sugar Content*

Due to the importance of carbohydrates as one of the main sources of energy as well as the amount of sugar in the controlled diet of the diabetic patients [24], providing information on the sugar composition and content in vegetables has gained more attention. Furthermore, due to the significant influence of processing practices on different soluble sugars, a need for the information on sugar amount has gained increasing interest for the food processing industry in order to optimize the processing conditions. The sugar contained in beetroot is mainly sucrose with small amounts of glucose and fructose [11].

The total soluble sugar content of the beetroot genotypes in the current work varied between 11.19 ± 0.37 °Bx and 14.67 ± 0.37 °Bx belonged to the genotypes Carillon RZ and Nochowski, respectively, and were statistically different from each other (Table 1). Following Nochowski, the genotypes Cervena Kulata with 13.94 ± 0.37 °Bx and Betina with 13.88 ± 0.37 °Bx possessed the highest total soluble sugar content. The study of Szopińska and Gawęda (2013) stated that the total soluble sugar content of the cylindrical-shaped genotype Regulski Cylinder grown organically in Poland varied between 7.61% and 8.16% within three years [25]. In the current study, higher contents were noted for the cylindrical-shaped genotype, Carillon RZ. Moreover, the amount of the total soluble sugar of 15 beetroot genotypes grown in Poland within 2009–2012 varied between 5.18% to 8.62% [19], which were considerably lower than the range found in the current study.

The amount of sugar content can be impacted by various factors. Statistical analysis of data of the current study determined a significant influence of genotype ($p = 0.0028$) and interactions of year × location ($p < 0.0001$) on the content of total soluble sugar (Table 1). Furthermore, Bavec et al. (2010) investigated the impact of different production systems, namely conventional, integrated, organic, and biodynamic, on the sugar content of red beet. Despite the differences in the sugar content of red beet grown under different farming conditions, no statistically significate influence of farming system was determined [11]. Another influencing factor on the sugar content of beetroot is the applied nitrogen in the early stages of growth and nitrogen availability [3]. In the present study, at the trial station De Beersche Hoeve, in the first year, the field was fertilized with compost equivalent to 60 kg N ha^{-1} and in the second year, compost equivalent to 90 kg N ha^{-1} was incorporated into the soil. At the Horticulture station Heinze, in both experimental years, no fertilizer was applied to the field and at the research station Kleinhohenheim, in the first year the field was fertilized with Vinasse, equivalent to 8.3 kg N ha^{-1}, one month after seeding and in the second year, no additional fertilizer was used due to the N fixation by the pre-crop clover grass.

Providing information on the sugar content of each genotype is of great importance for the food industry for developing beetroot products without exceeding the maximum daily consumption and for achieving the best quality after processing. One of the critical products is prepared beetroot juice which contains approximately 62.0–92.0 g/L sugar [2]. Considering the World Health Organization (WHO) recommended daily sugar consumption of 25–50 g for an adult with normal Body Mass Index (BMI), with consumption of 300–600 mL of processed beetroot juice, the limit of daily sugar consumption can be meet [2]. Moreover, for those beetroot products in which intensive thermal treatment should be applied, considering the possibility of the occurrence of the Maillard reaction, the sugar content of the selected genotype should be taken into account.

2.3. Nitrate Content

The three predominant sources of human nitrate intake reported as water, cured meat, and vegetables [26], in which the green leafy vegetables like spinach, beets, lettuce, and radishes are the major dietary examples [27]. Heretofore, nitrate amount in the vegetables was discussed as a critical topic. That was due to the point that reduction of nitrate to nitrite leads to methemoglobinemia, which its reaction with secondary amines may sequentially form carcinogenic *N*-nitrosamines [28]. Latterly, with the reveal of the remarkable physiological importance of nitric oxide, the significant contribution of the dietary nitrate for the formation of the nitric oxide was disclosed [29].

Recently, the potential beneficial effects of dietary nitrate, including reducing the risk of cardiovascular diseases, gastric cancer, and hypertension [26] draw a lot of attention. Several studies noted the possible positive effects of nitrate on muscle efficiency, oxygen transport in the blood, and muscle fatigue of athletes, especially endurance athletes such as professional cyclists or marathon runners [30], therefore, consumption of beetroot as a good source of nitrate has drawn a lot of interest [31,32]. Depending on the final product and purpose of use, in order to have the suitable nitrate content, the choice of the appropriate cultivar has been highly emphasized [33,34].

The nitrate content of the investigated beetroot genotypes in the present study ranged between 4593 ± 1212 mg kg^{-1} DW and 10,924 ± 1211 mg kg^{-1} DW. Among all 15 genotypes, the three highest nitrate content were noted for Carillon RZ, Nobol, and Bona with 10,924 ± 1211 mg kg^{-1} DW, 9447 ± 1210 mg kg^{-1} DW, and 9364 ± 1221 mg kg^{-1} DW, respectively, which were statistically non-significantly different from each other (Table 1). On the other hand, the three genotypes, namely, Nochowski, BoRu1, and Monty RZ F1 with 4593 ± 1212 mg kg^{-1} DW, 4597 ± 1211 mg kg^{-1} DW, and 5487 ± 1209 mg kg^{-1} DW, respectively, showed the lowest nitrate content (Table 1). The reported nitrate values in Table 1 were expressed on a dry weight basis. Most studies express the nitrate content on a fresh weight (FW) basis, wherefore total dry matter content should be taken into account to compare our results to the literature. The findings of the current study were in agreement with the reported mean nitrate value of beetroots from different European countries, which was 1379 mg kg^{-1} FW [35]. The range of nitrate content of beetroot in Thessaloniki, Greece was between 443–981 mg kg^{-1} FW [36]. In the study of Rubóczki et al. (2015) the nitrate content in ten different beetroot genotypes grown in Hungry determined by a spectrophotometric method ranged between around 700–850 mg kg^{-1} FW [37]. Kosson et al. (2011) noted the mean nitrate contents of 1338.5 mg kg^{-1} FW and 1737.3 mg kg^{-1} FW for two beetroot genotypes grown in Poland, in 2008 and 2009, respectively [20].

The level of nitrate uptake and accumulation in vegetable tissues can be influenced by three main factors: environmental factors, such as water availability and irradiance, genetic factors, and agricultural factors, such as the application of fertilizers, herbicides, and other nutrients [38]. In accordance with the statistical analysis, the nitrate content was significantly affected by genotype ($p = 0.0191$) and interactions of year × location ($p = 0.0495$) (Table 1). This is in agreement with the findings of Kosson et al. (2011) who noted the significant impact of variety and year on the accumulation of nitrate in two different beetroot genotypes [20]. Furthermore, the effect of cultivation method (organic and conventional) was investigated in the study of Kosson et al. (2011) and 33% lower nitrate accumulation in the beetroot grown organically was reported [20]. In the current work, all beetroots were grown under organic conditions. Moreover, Vasconcellos et al. (2016) assessed the differences in the nitrate content of four beetroot products, including juice, powder, chips, and cooked beetroot. It was revealed that beetroot juice with 12,252.90 mg kg^{-1} DW had significantly higher nitrate concentration compared to all other products, which indicated the range of 1649.66–2031.20 mg kg^{-1} DW [5]. Therefore, it can be concluded that the amount of nitrate in beetroot products can be affected by processing. According to the previous studies, the variability in the nitrate content may occur among cultivars of the same species [39,40] genotypes and plant tissues [33,41–43]. In our study, each analyzed sample was a homogenized mixture of three randomly selected beetroot plants of each genotype, which were replicated three times in the field. Consequently, the values demonstrated in Table 1, represent the mean nitrate content of all three replications of each genotype cultivated in three different locations within two years to evaluate the genetic potential in different environmental conditions. Thus, due to the combination of various factors affecting the nitrate content, to have a better evaluation of different beetroot genotypes, a closer look at a larger number of plants per genotype might be appropriate.

2.4. Betalain Content

Color is one of the important attributes indicating the aesthetic and quality and consequently the acceptance of food [44]. Recently, due to the point that consumers prefer natural pigments compared to the synthetic ones, focus on the use of carotenoids, anthocyanins, and chlorophylls as examples of plant-based colorants [45] have been increased. Due to the limited edible sources of betalains, these pigments have been less commonly used for food coloring than other natural pigments [46]. Betalains are water-soluble, nitrogenous pigments [46] which are divided into two main structural groups, red-violet betacyanins and yellow-orange betaxanthins [47]. Previous studies reported the high antioxidant capacity of betalains [48]. Furthermore, the other important roles of betalains in human health are their anti-cancer, antilipidemic, and antimicrobial activities [47].

In the present work, two main subgroups of betalain as representatives of the sample pigment content were quantified. Regarding betacyanin content, the studied red-colored genotypes ranged from 5.35 ± 0.49 mg g^{-1} DW to 7.89 ± 0.72 mg g^{-1} DW for the genotypes Cervena Kulata and Monty RZ F1, respectively (Figure 1). The white- and yellow-colored genotypes possessed the lowest betacyanin content with 0.08 ± 0.01 mg g^{-1} DW and 1.13 ± 0.14 mg g^{-1} DW, respectively, which were statistically different from each other (Figure 1). Following the F1 hybrid, Monty RZ, two genotypes Nochowski with 7.75 ± 0.70 mg g^{-1} DW and Ronjana with 7.39 ± 0.67 mg g^{-1} DW showed the highest betacyanin content. The betaxanthin content of the red-colored genotypes varied between 3.81 ± 0.32 mg g^{-1} DW and 5.51 ± 0.46 mg g^{-1} DW. The three highest betaxanthin content belonged to the genotypes Nochowski, Monty RZ F1, and Ronjana with 5.51 ± 0.46 mg g^{-1} DW, 5.49 ± 0.46 mg g^{-1} DW, and 5.32 ± 0.45 mg g^{-1} DW, respectively (Figure 1). The lowest betaxanthin contents were noted as 0.04 ± 0.01 mg g^{-1} DW and 0.09 ± 0.01 mg g^{-1} DW, belonging to the yellow-colored genotype, Burpees Golden, and the white-colored one, Sniezna Kula, respectively (Figure 1).

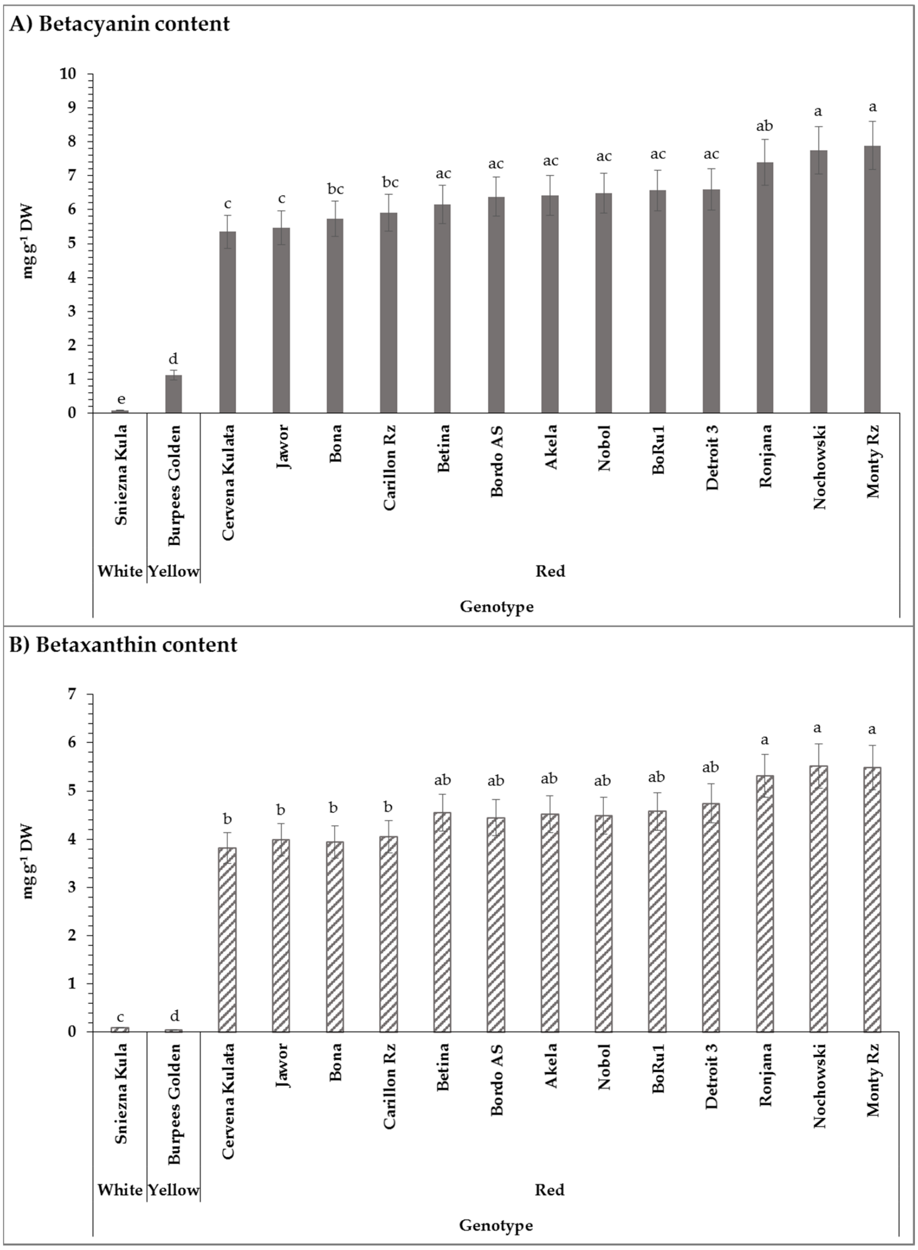

Figure 1. Mean values of (**A**) betacyanin content (mg g^{-1} DW) and (**B**) betaxanthin content (mg g^{-1} DW) of 15 different genotypes of beetroot grown in three research stations within the trial year 2017 and 2018. Means covered with at least one identical letter were not significantly different from each other.

The results of ANOVA showed a significant effect of genotype ($p < 0.0001$) and interactions of year × location ($p = 0.0277$) on the betacyanin content. The effect of genotype ($p < 0.0001$) and interactions of year × location ($p = 0.0069$) was noted to be significant on the content of betaxanthin. Means and p-values are available in Table S1 in the supplementary material. The significant influence of genotype on betacyanin and betaxanthin content has been reported by Sawicki et al. (2016) [49]. Felczynski and Elkner (2008) stated the significant impact of weather conditions and consequently year of the experiment on the composition of the betalains in beetroot. Their study investigated the ratio of betanin to vulgaxanthin, which are respectively the main red and yellow pigments in beetroot, in two red beetroot cultivars, Chrobry and Nochowski, within two years and considerable differences have been noted [39], which is in agreement with the outcomes of the present study.

Kujala et al. (2000) stated that there is a different betalain distribution among the different parts of red beetroot [50]. The study reported higher betacyanin content in outer parts of red beetroot (cv. Little Ball), increasing in the order flesh, crown, and peel. Slatnar et al. (2015) studied the betacyanin and betaxanthin content in the peel and flesh of three different beetroot genotypes, including two red-colored and one yellow-colored genotype, which were grown in Slovenia, using the HPLC method. Regarding the two red-colored genotypes, the betacyanin content of 20.93 mg g^{-1} DW and 11.83 mg g^{-1} DW in the peel and 5.25 mg g^{-1} DW and 4.13 mg g^{-1} DW in the flesh of Pablo and Tanus, respectively, was measured [45]. The beetroot samples used in the current work mainly consisted of flesh with a small portion of the peel. Therefore, the slightly higher betacyanin and betaxanthin contents reported in this study (Figure 2) might be due to the different plant parts. Moreover, the betanin content of four red-colored beetroot genotypes measure by Kujala et al. (2002) ranged between 3.8–7.6 mg g^{-1} DW in the peel and 2.9–5.2 mg g^{-1} DW in the flesh. That for the vulgaxanthin content was reported as 1.4–4.3 mg g^{-1} DW and 1.5–4.0 mg g^{-1} DW in the peel and flesh, respectively [10]. The results of this study indicated a nearly similar range for the betacyanin content but higher betaxanthin values. Inconsistent to the previous findings, the study of Gasztonyi et al. (2001) presented a significantly lower range of betanin content of five red-colored beetroot genotypes with 0.41–0.50 g kg^{-1} (equivalent to mg g^{-1} in the current study) as well as lower range of vulgaxanthin I with 0.32–0.42 g kg^{-1} [51].

With respect to the betacyanin content of the yellow beetroot genotype, Boldor, Slatnar et al. (2015) stated 0.61 mg g^{-1} DW in the peel and 0.13 mg g^{-1} DW in the flesh. Likewise, for the betaxanthin content, the values of 4.71 mg g^{-1} DW and 0.22 mg g^{-1} DW in the peel and flesh, respectively, were reported [45]. The findings of our work regarding the yellow-colored genotype, Burpees Golden, indicated higher betacyanin content and remarkably lower betaxanthin value. Furthermore, the outcomes of Lee et al. (2014) demonstrated the betacyanin content of 0.114 mg g^{-1} FW and betaxanthin content of 0.187 mg g^{-1} FW for the mixed sample of peel and flesh of the yellow-colored beetroot, Burpees Golden, grown in Texas (the USA) [52].

Providing information on the betalain content of each beetroot genotype, due to the effect on the antioxidant activity as well as the color intensity, is important for the food industry in order to choose the suitable genotype for the final product such as for the use of beetroot in functional processed food or the use as the food colorant.

2.5. Total Phenolic Content

Phenolic compounds are plants' secondary metabolites, which affect both sensorial attributes, such as flavor, taste and color, and functional properties, like antioxidant activity, of plant products [53,54]. Furthermore, phenolic compounds also contribute to plants' growth, pigmentation, reproduction [55], and defense mechanisms under different stress conditions. Due to the free radicals scavenging activity, phenolic compounds demonstrate a high antioxidant effect. Antioxidant activity is associated with the prevention of cardiovascular diseases and cancer [56,57].

The total phenolic content ranged from 352.46 ± 28.24 mg GAE 100 g^{-1} DW to 489.06 ± 28.24 mg GAE 100 g^{-1} DW for the red-colored genotypes. The F1 hybrid genotype, Monty RZ, showed the highest total phenolic content followed by Cervena Kulata and Nochowski (Table 1). On the other hand, the genotypes Carillon RZ, BoRu1, and Betina with 352.46 ± 28.24 mg GAE 100 g^{-1} DW, 382.73 ± 28.24 mg GAE 100 g^{-1} DW, and 393.94 ± 28.24 mg GAE 100 g^{-1} DW, respectively, indicated the lowest total phenolic content among the studied red-colored genotypes. The yellow-colored genotype, Burpees Golden, and the white-colored genotype, Sniezna Kula, with 172.89 ± 28.78 mg GAE 100 g^{-1} DW and 216.09 ± 29.87 mg GAE 100 g^{-1} DW, respectively, demonstrated significantly lower total phenolic content compared to the red genotypes (Table 1). In the study of Rubóczki et al. (2015), the content of total polyphenols in ten different beetroot genotypes grown in Hungary was determined using the Folin–Ciocalteu method and varied between 45.47 mg GAE/100 g and 83.37 mg GAE/100 g [37]. Values were considerably lower than the values measured in our study. Furthermore, Ninfali and Angelino (2013) reported total phenols of 1.77 mg GAE g^{-1} DW (equivalent to 177 mg GAE 100 g^{-1} DW) [58], which was lower than the values found in the current study. Kovarovič et al. (2017) investigated the total phenolic compounds of four beetroot varieties in the Czech Republic and noted the range of 368.75 ± 5.14 mg kg^{-1} (equivalent to 36.87 mg 100 g^{-1} DW) to 887.75 ± 7.73 mg kg^{-1} (equivalent to 88.77 mg 100 g^{-1} DW) belonging to the variety Chioggia (white-colored with red strips) and Cylindra, respectively [59]. Kavalcova' et al. (2015) stated a total polyphenols' range of 820.10 mg kg^{-1} to 1280.56 mg kg^{-1} (equivalent to 82.01–128.05 mg 100 g^{-1} DW) [60]. The investigated genotypes in our study exhibited promising results regarding the amount of total phenolic compounds.

The outcomes of ANOVA indicated a significant impact of genotype (p = 0.0002) and the interactions of year × location (p = 0.0114) (Table 1). The total phenol content in fresh fruits and vegetables can be affected by different biotic and abiotic factors. Genotype, environmental conditions such as climate and soil, and plant maturity and ontogeny play the most important role in the quantity of phenolic compounds [55]. It was reported that the level of chemical fertilizers, which are used in conventional agricultural practices, can lead to the disturbance in the natural production of phenolic compounds [61]. In this regard, Carrillo et al. (2019) investigated the impact of the production system on the content of total phenols in six different beetroot genotypes. It was reported that the total polyphenol content of the organic beetroots was significantly higher than the conventional ones, although the difference depended on the cultivar [62]. Nevertheless, the study of Straus et al. (2012) demonstrated a higher total phenolic content in beetroots grown under organic condition compared to the conventional ones, however, the difference was not significant [63].

Similar to the betalain content in beetroot, several studies noted a higher total phenolic content in the peel than in the flesh [50,64]. Based on the point that the analyzed samples in the current work mainly consisted of flesh with a small proportion of peel, the differences between the values reported in other studies and the outcomes of this study might be due to the variation in the plant part. Additionally, differences in the measurement methods [65] (e.g., HPLC, capillary zone electrophoresis, and Folin–Ciocalteu), as well as the extraction of the phenolic compounds techniques [66–68] in various studies, may lead to a variation in the reported values.

3. Methods and Materials

3.1. Plant Materials and Sample Preparation

The analyzed beetroot samples were obtained from six field experiments, which were carried out in two years, 2017 and 2018, at three different locations. Locations consisted of two on-farm breeding locations of Kultursaat e.V.: De Beersche Hoeve (Oostelbeers, Netherlands), Horticulture station Heinze (Bingenheim, Hessia, Germany), and at the research station for organic farming Kleinhohenheim (University of Hohenheim, Stuttgart, Baden-Wuerttemberg, Germany). All beetroots were grown organically. In 2017, on each on-farm breeding location 30, and in Kleinhohenheim, 40 genotypes were cultivated in three field replicates. In 2018, 16 genotypes were investigated on each on-farm breeding location while 36 genotypes were assessed in Kleinhohenheim. Data from all genotypes were analyzed together. However, the presented results were limited to the 15 genotypes, which occurred in all location-by-year combinations. The 15 investigated genotypes can be listed as 13 red-colored genotypes, Akela RZ, Betina, Bona, Bordo, BoRu1, Carillon RZ, Cervena Kulata, Detroit 3, Jawor, Monty RZ F1, Nobol, Nochowski, Ronjana, one white genotype, Sniezna Kula, and a yellow genotype, Burpees Golden. Figure 2 illustrates four beetroot genotypes representing different beet shapes and colors, which were investigated in this study. The detailed information on the seeds' origin, experimental design, and the locations can be found in Yasaminshirazi et al. (2020) [69].

Samples were prepared from three randomly selected beetroots per plot, which fulfilled the criteria for marketability (beet diameter between 5 and 13 cm and without any deformation and notable damages). Beetroots were washed directly after the harvest. After cutting out the root tail and the leaves-growth-base, a sectional cut of the beet including the peel were chopped into small pieces and thoroughly mixed to have a homogenous sample. The chopped pieces were collected in a closed plastic flask and immediately were frozen using liquid nitrogen. Afterward, the samples were lyophilized using the Dieter Piatkowski–Forschungsgeraete freeze-dryer (Munich, Germany). The dried samples were ground by a laboratory knife mill (Retsch GM 200, Haan, Germany) until they reached a fine powder texture. The powder was collected and remained in a closed small plastic flask in a cool dark box until the day the chemical analyses were carried out.

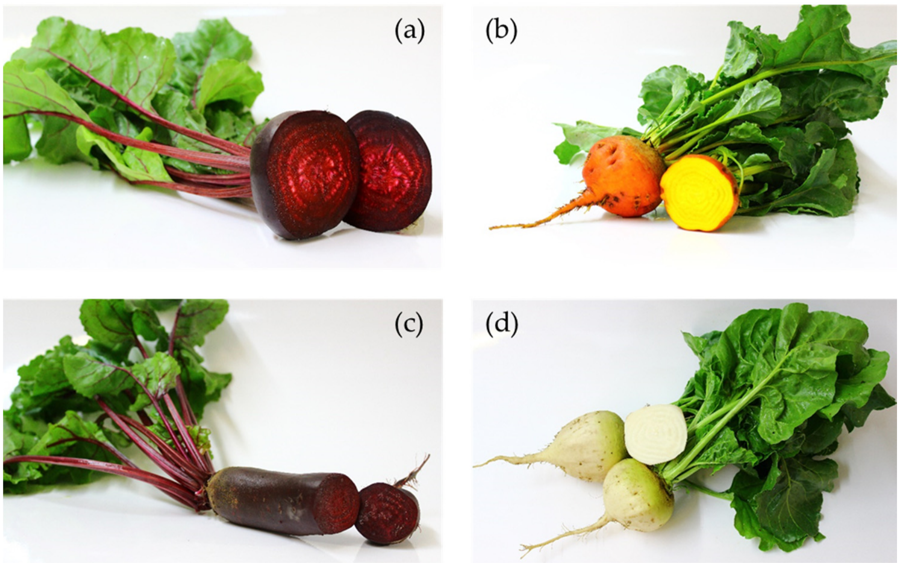

Figure 2. Presentation of four beetroot genotypes according to the beet shape and color (**a**) genotype Ronjana (red and round beet), (**b**) genotype Burpees Golden (yellow and round beet), (**c**) genotype Carillon RZ (red and cylindrical-shaped beet), and (**d**) genotype Sniezna Kula (white and round beet). Pictures are taken by Khadijeh Yasaminshirazi.

3.2. Chemicals and Reagents

The Folin–Ciocalteu reagent and gallic acid were purchased from Merck (Darmstadt, Germany). Na_2CO_3 was provided by AppliChem GmbH (Darmstadt, Germany). Methanol and ethanol were purchased from Carl Roth GmbH (Karlsruhe, Germany) and Th. Geyer (Renningen, Germany), respectively. Regarding the nitrate measurement, sulfanilamide was purchased from AppliChem GmbH (Darmstadt, Germany). Ammonium chloride and hydrochloric acid were provided by Th. Geyer (Renningen, Germany). Sodium nitrite and ammonia solution 25% from Merck (Darmstadt, Germany) and N-(1-naphthyl)-ethylene diamine dihydrochloride from Carl Roth GmbH (Karlsruhe, Germany) were used.

3.3. Total Dry Matter Content

The weight of chopped beetroot samples (*i*) was measured before and after freeze-drying and the total dry matter content (TDMC) was calculated according to Equation (1):

$$\text{TDMC}_\text{i}\ [\%] = \left(\frac{weight\ after\ drying_i}{weight\ before\ drying_i}\right) \times 100 \quad (1)$$

3.4. Total Soluble Sugar Content

The determination of total soluble sugar content was carried out using a digital handheld refractometer (Kruess, Germany). One to two drops of freshly pressed beetroot were used to determine the degree of Brix, which is equivalent to the percentage of total soluble sugar content. Each sample was measured in duplicate and a mean value was calculated directly.

3.5. Quantitation of Nitrate Content

Approximately 0.5 g of each dried beetroot sample was weighed and added in a 100 mL volumetric flask and was filled up with distilled water until the final volume adjusted to 100 mL and was shaken shortly, manually. The volumetric flask was placed in an ultrasonic water bath at 80 °C for 10 min. After the flask was cooled down, the supernatant was filtered using filter paper. The nitrate content quantitation was conducted by flow injection analysis method (FIA) [27] using FIASTAR 5000 (FOSS Analytical AB, Sweden).

3.6. Betalain Content

3.6.1. Extraction of Betalains

Approximately 0.04 g of each dried sample was weighed (Precisa 125 A, Dietikon, Switzerland) and added to 30 mL of 50% (*v/v*) ethanol in a 50 mL falcon tube. The mixture was shaken for two hours with 100 rounds per minute (rpm). Afterward, the obtained extracts were centrifuged (Centrifuge 5810 R, Eppendorf AG, Hamburg, Germany) with 4000 rpm and at 20 °C for 10 min.

3.6.2. Betalains Content Determination

Quantification of two main subgroups of betalains, betacyanin, and betaxanthin, was determined spectrophotometrically using a UV/Visible Spectrophotometer (Ultrospec 3100 Pro, Amersham Bioscience, Buckinghamshire, UK). The absorption of betacyanins at 538 nm and betaxanthins at 480 nm were measured and their concentrations were calculated based on Koubaier et al. (2014) and Sawicki et al. (2016) [49,70].

3.7. Total Phenolic Content

3.7.1. Extraction of Total Phenolic Compounds

Approximately 0.5 g of each dried beetroot sample was weighed and collected in a 15 mL falcon tube where 10 mL of methanol was added. The mixture was shaken for 30 min and afterward centrifuged (Centrifuge 5810 R, Eppendorf AG, Hamburg, Germany) at 4000 rpm at 20 °C for 20 min in order to separate the solid phase from the supernatant. These extracts were utilized for the measurement of the total phenolic content.

3.7.2. Total Phenolic Content Quantification

The total phenolic content measurement was carried out following the methodology of Folin–Ciocalteu [71]. Summarily, 0.6 mL of the extracted sample was added to 60 mL of distilled water in a 100 mL volumetric flask. Then, 5 mL of Folin–Ciocalteu reagent was added to the flask. After two to six minutes, 25 mL of sodium carbonate solution 15% was added to the mixture, and the final volume was adjusted to 100 mL with distilled water. After two hours of incubation at room temperature, absorbance at 760 nm was determined by a UV/Visible Spectrophotometer (Ultrospec 3100 Pro, Amersham Bioscience, Buckinghamshire, UK). To draw a calibration curve, six standard solutions consisting of 0.03 g L^{-1}, 0.12 g L^{-1}, 0.24 g L^{-1}, 0.48 g L^{-1}, 0.9 g L^{-1}, and 1.5 g L^{-1} gallic acid in distilled water were used. Lastly, the total phenolic content was stated as mg GAE 100 g^{-1} DW (milligrams of gallic acid equivalents per 100 g of dry weight).

3.8. Statistical Analysis

According to the design of the experiments, the data were analyzed by the linear mixed model used in Yasaminshirazi et al. (2020) [69] on agronomic traits from the same six experiments:

$$y_{ijklmn} = \mu + b_{ljk} + r_{mjk} + c_{njk} + a_j + l_k + \tau_i + (al)_{jk} + (\tau a)_{ij} + (\tau l)_{ik} + (\tau al)_{ijk} + e_{ijklmn}, \quad (2)$$

where a_j and l_k are the fixed effects of the *j*-th year and the *k*-th location. b_{ljk}, r_{mjk}, and c_{njk} are the random effect of the *l*-th block, *m*-th row and *n*-th column within a year-by-location combination. Note that block effects as well as row and column effects were only fitted if blocks existed and a row-column design was used, respectively. Dummy variables were used to block out these effects for all other experiments but were not mentioned in (2) to simplify the description. τ_i is the fixed effect of the *i*-th genotype. $(al)_{jk}$ is the fixed interaction effect of the *j*-th year and the *k*-th location. $(\tau a)_{ij}$, $(\tau l)_{ik}$, and $(\tau al)_{ijk}$ are the random interaction effects between the corresponding main effects. e_{ijklmn} is the error of y_{ijklmn} with year-by-location specific error variance. For the trait total phenolic content, a group-by-location-specific error variance was assumed, where a group refers to beetroots of the same color (red, yellow, white). For betacyanin and betaxanthin, a group-by-year-specific error variance was found to fit well. The Akaike Information Criterion (AIC) was used to select the best error variance structure [72]. The assumptions of normality of residuals and homogeneity of residual variances (in addition to the heterogeneity specified in the model) were checked graphically. For the compounds betacyanin and betaxanthin, a logarithmic transformation of the data was used before analysis. Estimated means were back-transformed afterward for presentation purposes only. These means represent medians on the original scale. Standard errors were back-transformed using the delta method. Depending on the significance of fixed effects, mean comparisons were done for the highest significant interaction term of all factors using Fisher´s Least Significant Difference (LSD) test [73]. Means (or medians in case of back-transformed values) and their (approximate) standard error were presented. A letter display was created using the statistical software SAS version 9.4 (SAS Institute, Cary, North Carolina, United States) macro %mult [73] to present results from multiple comparisons.

4. Conclusions

Significant differences were found in the content of selected compounds between the assessed open-pollinated beetroot genotypes. This outcome is essential for the selection of genotypes and is of great importance for farmers for having new possibilities for cultivation in organic farming, for the industry for the production and development of beetroot products with desirable characteristics, and for meeting consumers' expectations of health-promoting food products. Furthermore, with the significant impact of the interactions of year × location on all tested compounds, it can be concluded that the abiotic factors influence the biochemical composition of beetroot. Out of the 15 tested genotypes the open-pollinated genotype Nochowski with comparable total phenolic compounds and betacyanin content to F1 hybrid genotype, Monty F1 RZ, indicated a high antioxidant capacity, which makes it a potential genotype for value-added food products. Moreover, this genotype may serve as an option for juice production due to its high sugar and low nitrate content. According to the importance of taste on customers' acceptability, further studies in association with the sensory quality of the genotypes as well as other flavor-relevant components, such as geosmin, are required.

Supplementary Materials: The following are available online, Table S1: Mean values and ANOVA of results of betacyanin (mg g^{-1} DW) and betaxanthin (mg g^{-1} DW) content of 15 different genotypes of beetroot grown in three research stations within the trial year 2017 and 2018.

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5. Publication III: Impact of Cold Storage on Bioactive Compounds and Their Stability of 36 Organically Grown Beetroot Genotypes

Yasaminshirazi, K.; Hartung, J.; Fleck, M.; Graeff-Hönninger, S. Impact of Cold Storage on Bioactive Compounds and Their Stability of 36 Organically Grown Beetroot Genotypes. *Foods* **2021**, *10*, 1281.

Introducing new beetroot genotypes to farmers and the food industry requires a comprehensive investigation, in which different aspects, including agronomic and compositional characteristics and the impact of genetic and environmental conditions on them, have been taken into consideration. A thorough evaluation of the overall content and stability of different bioactive compounds concerning a broad assortment of organically grown beetroots was overlooked. In Chapters 3 and 4, a significant genetic variability regarding agronomic performances and compositional characteristics of different open-pollinated beetroot genotypes was noted. These outcomes indicate the characteristics of the tested genotypes directly after harvest. However, in order to prolong the use of beetroot throughout the year, it is essential to investigate the storability of these genotypes after the harvest and the extent of change in their quality. Cold storage is one of the common postharvest practices used to prolong the shelf life and marketing of fruits and vegetables after the harvest season by minimizing their physiological and chemical changes. Chapter 5 compared the content of total dry matter, betalain, total phenolic compounds, nitrate, and total soluble sugars of different beetroot genotypes stored for up to four months in a cooling chamber at 6 °C with the contents directly after the harvest to investigate the impact of genetic, storage period and year on the stability of the compounds of interest.

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Impact of Cold Storage on Bioactive Compounds and Their Stability of 36 Organically Grown Beetroot Genotypes

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Abstract: In order to exploit the functional properties of fresh beetroot all year round, maintaining the health-benefiting compounds is the key factor. Thirty-six beetroot genotypes were evaluated regarding their content of total dry matter, total phenolic compounds, betalain, nitrate, and total soluble sugars directly after harvest and after cold storage periods of one and four months. Samples were collected from two field experiments, which were conducted under organic conditions in Southwestern Germany in 2017 and 2018. The outcome of this study revealed a significant influence of genotype ($p < 0.05$) on all measured compounds. Furthermore, significant impacts were shown for storage period on total dry matter content, nitrate, and total phenolic compounds. The medians of nitrate content based on the genotypes studied within the experiment ranged between 4179 ± 1267–20,489 ± 2988 mg kg^{-1} DW (dry weight), and that for the total phenolic compounds varied between 201.45 ± 13.13 mg GAE 100 g^{-1} DW and 612.39 ± 40.58 mg GAE 100 g^{-1} DW (milligrams of gallic acid equivalents per 100 g of dry weight). According to the significant influence of the interactions of storage period and genotype on total soluble sugars and betalain, the decrease or increase in the content of the assessed compounds during the cold storage noted to be genotype-specific. Therefore, to benefit beetroots with retained quality for an extended time after harvest, selection of the suitable genotype based on the intended final use is recommended.

Keywords: beetroot; organic farming; storage; bioactive compounds; betalain; nitrate; sugar; phenolic compounds; total dry matter

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1. Introduction

Containing a high amount of health-promoting compounds [1], suitable cultivation and storage [2] brought attention to the use of beetroot (*Beta vulgaris* subsp. *vulgaris* L.) and its products. Classification of beetroot as a super food has increased the importance of this vegetable [3]. Beetroot is included in the ten vegetables with the highest antioxidant capacity due to its promising amount of betalain and phenolic compounds [4] as well as the presence of carotenoid and ascorbic acid [5,6].

Betalains are nitrogen-containing, water-soluble plant secondary metabolites, which are derivatives of betalamic acid [7] and can be found in different parts of plants, but are restricted to the *Caryophyllales* order [8]. Red beetroot, as one of the chief and the most commercially sources [9] of betalain, has been approved by European Union to be used as a natural colorant (E162) [8] in dairy, confectionery, beverages, and meat products [10]. Based on the nature of the substituent of betalamic acid residue, betalains can be divided into two major groups: betacyanin which is responsible for red-violet color, and betaxanthin representing the yellow-orange color [11]. Investigating different beetroot genotypes indicated that the main betacyanins in beetroot are betanin, isobetanin, betanidin, and isobetanidin, and the chief betaxanthin components are vulgaxanthin I and vulgaxanthin

II [12]. In spite of the fact that betalains show low stability at higher temperatures [13], due to its high stability in the wide range of pH values (between three and seven [9]), betacyanins are considered to be a better choice providing a red-violet color range for coloring foods with a low acid content, compared to anthocyanins. Anthocyanins are the most common pigments for this color range [14], despite showing instability at pH values above three [15]. Indicating anti-lipidemic, anti-cancer, and antimicrobial activities made betalain a beneficial component for human health [16]. Moreover, flavonoids, phenolic acids, and various organic and inorganic acids, which are the major phenolic compounds of beetroot [5], further increase the anti-radical activity of this crop, which leads to preventing cancer and cardiovascular disease [17,18]. Total phenolics are part of fruits and vegetables' bioactive compounds, which not only benefit plants with assisting the growth and protective mechanisms under abiotic stress conditions [19], but also promote human health with influencing the functional qualities of plant products [20].

In addition, the potential profiting effects of nitrate in beetroot on human health have drawn a lot of attention. Green leafy vegetables including beetroot are considered as major dietary sources of nitrate [21], which is the chief contributor in nitric oxide production [19,22]. Nitric oxide demonstrated an essential role in the gastric [23] and cardiovascular [24] regulations. Latterly, preventing ischemia-reperfusion damages, regulating the blood pressure [25], enhancing muscle efficiency and endurance [1], were reported as the potential positive effects of dietary nitrate. Therefore, beetroot has been recently used as a dietary supplement for patients with hypertension or cardiovascular diseases [23] or as powder formulation in different products, such as yogurt, drinks, and snacks, for consumption by athletes before physical exercises [5]. Moreover, the contribution of nitric oxide in the improvement of seed germination, growth performance, and mineral adjustment in plants under different abiotic stress conditions has been reported [26–28]. Furthermore, the sugar composition of beetroot was reported as a dominant proportion of sucrose (91.6%) [29], with a small and relatively similar proportion of glucose and fructose [5]. Information on the amount and composition of carbohydrates in vegetables is essential when considering the importance of sugar content in different controlled diets (such as for diabetic patients, athletes, and vegetarians) as well as in the food industry for optimization of processing practices.

Due to the rise in consumers' awareness of the advantages of organic products [30], the demand for such products is steadily growing [29]. Comparing the quality of organic and conventional cultivation, previous studies reported higher contents of total phenolic compounds, betalain, and antioxidant capacities in organically grown beetroots. However, the extent of difference between the cultivation methods highly depends on the genotype [31]. Considering the functional characteristics of beetroot, the stability of its heath beneficial compounds plays an important role [32]. In order to have fresh beetroot throughout the year and be able to use them for the processed products with promising quality, preserving the nutritional properties of beetroot during storage is crucial. Previous studies reported the impact of genotypes, fertilization, and the storage environment on the storability of beetroot [2]. Nevertheless, an evaluation of various biologically active compounds considering a broad range of beetroot genotypes, which were cultivated organically was missing. Cold or refrigerated storage is one of the prevalent postharvest practices, which is applied for extending the shelf-life of vegetables. Due to their perishable nature, vegetables and fruits often need to be stored at low temperatures to minimize their physiological and chemical changes [33], and to extend their marketing after the harvest season [34]. Different studies claimed that advanced storage technologies, such as a controlled or modified atmosphere, are ideal options to preserve bioactive compounds in vegetables [2,35]. However, due to the high prices of such technologies, small-scale producers cannot afford them and cheap and locally-available technologies are demanded [36]. Therefore, using the genetic potential of forgotten varieties or breeding new and promising genotypes with high storability and evaluating their performances under organic farming conditions could be one of the most reasonable solutions.

The present study aimed to determine the impact of genotype and cold storage period on the stability of different bioactive compounds of 36 beetroot genotypes, grown under organic farming conditions in Southwestern Germany. The outcomes of this study can be beneficial for household consumers, who tend to favor fresh beetroot for extended time rather than the processed ones, for farmers to profit their grown beetroots with maintained quality and less storage loss, and for food industries accessing beetroot with retained health-promoting compounds.

2. Materials and Methods

2.1. Chemicals and Reagents

For quantification of nitrate, sulfanilamide (AppliChem GmbH, Darmstadt, Germany), ammonium chloride and hydrochloric (Th. Geyer, Renningen, Germany), sodium nitrite and ammonia solution 25% (Merck, Darmstadt, Germany), and N-(1-naphthyl)-ethylene diamine dihydrochloride (Carl Roth GmbH, Karlsruhe, Germany) were used. Regarding total phenolic content measurement, Folin–Ciocalteu reagent and gallic acid were provided by Merck (Darmstadt, Germany). Na_2CO_3 and methanol were purchased from AppliChem GmbH (Darmstadt, Germany) and Carl Roth GmbH (Karlsruhe, Germany), respectively. Ethanol needed for betalain analysis was purchased from Th. Geyer (Renningen, Germany).

2.2. Plant Materials and Sample Preparation

The beetroots analyzed in the present study were grown under organic conditions at the research station for organic farming Kleinhohenheim, University of Hohenheim, Stuttgart, Baden-Wuerttemberg, Germany (48°44′14 N, 9°12′01 E, 430 m above the sea level). Two field experiments were conducted in which, in 2017, 40 genotypes, and in 2018, 36 genotypes were cultivated. In 2017, the field experiment was conducted as row-column design with three replicates. In 2018, the experiment was carried out as non-resolvable block design, in which a block size of ten and a treatment number of 36, with six replicates for four genotypes and three replicates for 32 genotypes were applied. Although the data from all genotypes were statistically analyzed together, the results shown in the present study were limited to the 36 genotypes, which occurred in both years. Table 1 presents the detailed information on beet color, beet shape, and seed origin of the studied beetroot genotypes.

During the growth period in 2017, the mean precipitation and temperature were 77.42 mm and 17.6 °C, respectively. In 2018, the mean precipitation reached 38.2 mm and the mean temperature was 19.0 °C during the growth period. Detailed information on monthly precipitation and mean temperature, fertilization, sowing and harvest dates, and soil management practices can be found in Yasaminshirazi et al. [37].

Three randomly selected beetroots per plot were collected each year for analysis of the bioactive compounds in freshly harvested beetroots. Additionally, beetroots from each plot were stored in vegetable net sacks in a cooling chamber at 6 °C, directly after harvest. All stored beetroots met the marketability criteria (including beet diameter of 5–13 cm, no deformation or remarkable damages, diseases, etc.). Beetroots for analysis of bioactive compounds were taken from the cooling chamber one and four months after the storage. After washing and cutting the leaves-growth-base and root tail, a sectional cut of each beet (flesh including peel) was diced and mixed in order to have a homogenous sample from each plot. After collecting the diced beetroots in a plastic flask, to prevent any further enzymatic processes, samples were immediately frozen by liquid nitrogen, kept at −18 °C, and then followed by lyophilization using the Dieter Piatkowski–Forschungsgeraete freeze-dryer (Munich, Germany). The dried samples were milled using GRINDOMIX GM 200 (Retsch GmbH, Haan, Germany) up until a fine powder texture was reached. Until analysis, the powdered samples were stored in closed plastic bottles in a dark and dry box at ambient temperature. Total dry matter content (TDMC), total phenolic compounds, betalain, nitrate, and total soluble sugars of beetroots of freshly harvested samples were determined and compared with those of samples taken after one and four months of cold storage.

Table 1. List of the 36 investigated beetroot genotypes indicating the beet color, shape, and seed origin.

Genotype	Beet Color	Shape	Seed Origin
Ägyptische Plattrunde (Ä. P.)	red	flat-spherical	Sativa (DE)
Akela Rijk Zwaan (RZ)	red	spherical	Rijk Zwaan (NL)
Alvro Mono	red	spherical	Vitalis (US)
Betina	red	spherical	Moravo Seeds (CZ)
Bolivar	red	spherical	Hild (DE)
Bona	red	spherical	Moravo Seeds (CZ)
Bordo	red	spherical	Seklos (LT)
Boro F1	red	spherical	Bejo (DE)
BoRu1	red	spherical	Kultursaat e.V. (DE)
Borus	red	spherical	Spójnia (PL)
Burpees Golden (Burpees G.)	yellow	spherical	Bingenheimer S. AG (DE)
Carillon RZ	red	cylindrical	Rijk Zwaan (NL)
Cervena Kulata (Cervena K.)	red	spherical	Moravo Seeds (CZ)
Ceryl	red	spherical	Spójnia (PL)
Chrobry	red	spherical	Spójnia (PL)
Czerwona Kula 2 (Czerwona K. 2)	red	spherical	Spójnia (PL)
Detroit 2 Dark Red (Detroit 2 D. R.)	red	spherical	Samen Schenker (DE)
Detroit 3	red	spherical	Caillard (FR)
Detroit Globe (Detroit G.)	red	spherical	King Seed (UK)
Formanova	red	cylindrical	Sativa (DE)
Forono	red	cylindrical	Bingenheimer S. AG (DE)
Gesche SG	red	spherical	Christiansens Biolandhof (DE)
Jannis	red	spherical	Bingenheimer S. AG (DE)
Jawor	red	spherical	Snówidza (PL)
Libero RZ	red	spherical	Rijk Zwaan (NL)
Monty RZ F1	red	spherical	Rijk Zwaan (NL)
Nobol	red	spherical	Vilmorin (PL)
Nochowski	red	spherical	Spójnia (PL)
Pablo F1	red	spherical	Bejo (DE)
Regulski Okragly (Regulski O.)	red	spherical	Pnos (PL)
Robuschka	red	spherical	Bingenheimer S. AG (DE)
Ronjana	red	spherical	Bingenheimer S. AG (DE)
Hilmar	red	spherical	Hild (DE)
Sniezna Kula	white	spherical	Torseed (PL)
Tondo de Chioggia (Tondo d. Ch.)	red-white	spherical	Bingenheimer S. AG (DE)
UB-E3	red	spherical	U.Behrendt (DE)

2.3. Total Dry Matter Content

Before and after freeze-drying, the diced beetroot samples (i) were weighed. Equation (1) was used to calculate the TDMC:

$$\mathrm{TDMC}_i\ [\%] = \left(\frac{\text{weight after drying}_i}{\text{weight before drying}_i}\right) \times 100, \tag{1}$$

2.4. Total Phenolic Content (TPC)

The TPC quantification was conducted according to the methodology of Folin–Ciocalteu [38].

Briefly, the extraction was prepared by mixing 10 mL of methanol with approximately 0.5 g dried beetroot sample in a falcon tube. After shaking the mixture for 30 min, the tubes were placed in a centrifuge (Centrifuge 5810 R, Eppendorf AG, Hamburg, Germany) at 4000 rounds per minute (rpm) for 20 min (20 °C) for separation of supernatant from the solid phase. Later, 0.6 mL of the prepared extract was mixed with 60 mL of distilled water and 5 mL of Folin–Ciocalteu's reagent in a 100 mL volumetric flask. After two to six minutes, with adding 25 mL of sodium carbonate (15%) and adjusting the final volume with distilled water to 100 mL, the mixture was left for two hours at room temperature. The absorbance at 760 nm was measured spectrophotometrically (Ultrospec 3100 Pro, Amersham Bioscience, Buckinghamshire, UK) and TPC was reported as mg GAE 100 g^{-1} DW. In order to draw a standard curve, six different concentrations of gallic acid solution (0.03–1.5 g L^{-1} gallic acid in distilled water) were used.

2.5. Betalain Content

Determination of two chief subgroups of betalains, namely betacyanin and betaxanthin, was conducted spectrophotometrically (Ultrospec 3100 Pro, Amersham Bioscience, Buckinghamshire, UK) in accordance with the method used by Koubaier et al. [39] and Sawicki et al. [40].

A mixture of roughly 0.04 g of the dried beetroot samples and 30 mL of 50% (v/v) ethanol was shaken for two hours with the speed of 100 rpm and followed by centrifuging the samples at 20 °C for 10 min with the speed of 400 rpm (Centrifuge 5810 R, Eppendorf AG, Hamburg, Germany). The absorption of betaxanthin and betacyanin was measured at 480 nm and 538 nm, respectively.

2.6. Nitrate Content Determination

The nitrate content was determined according to the flow injection analysis method (FIA) [21] using FIASTAR 5000 (FOSS Analytical AB, Hilleroed, Denmark). The detailed extract preparation can be found in Yasaminshirazi et al. [41].

2.7. Total Soluble Sugar Content

The degree of Brix corresponding to the percentage of total soluble sugar content was measured utilizing a digital handheld refractometer (Kruess, Hamburg, Germany). After measuring each sample in duplicate, their mean value was calculated directly.

2.8. Statistical Analysis

Data from both years and experiments were jointly analyzed using a mixed model approach. The model can be described by:

$$y_{ijklmn} = \mu + \tau_i + \varphi_j + a_k + (\tau\varphi)_{ij} + (\tau a)_{ik} + (\varphi a)_{jk} + (\tau\varphi a)_{ijk} + b_{kl} + r_{klm} + c_{kln} + e_{ijklmn}, \quad (2)$$

where y_{ijklmn} is the observation of genotype i after storage period j in the m row, nth column of block l in year k, μ is the intercept, τ_i is the fixed effect of genotype i, φ_j is the fixed effect of the jth storage period, a_k is the fixed effect of the kth year, $(\tau\varphi)_{ij}$, $(\tau a)_{ik}$, and $(\tau\varphi a)_{ijk}$ are the random interaction effects of the corresponding main effects, $(\varphi a)_{jk}$ is the fixed effect of storage period j and year k, and b_{kl}, r_{klm}, and c_{kln} are the random effects of block, row and column within block. e_{ijklmn} is the error of y_{ijklmn}. Note that the effect $(\varphi a)_{jk}$ is the confounded with the effect of sampling day and the interaction effect of year k and storage period j, as thus was taken as fixed in the model. Due to this confounding, the interpretation of common or marginal means should be made with caution, too. Further note that data of the same sample (beetroots form the same plot) were taken for different storage periods within one experiment. Thus, repeated data were taken on each sample. The model accounted for this repeated measures structure by allowing a first order autoregressive variance-covariance structure for random effects including the year and the residual error. Year specific variances and covariances were fitted to all random effects and the error except for year-by-genotype effects. As row and columns existed only in the first year, effects for both were fitted only for first year data. Pre-requirements of normally distributed residuals and homogeneous variance (despite the year specific variances) were checked graphically. In case of nitrate content, data were square-root transformed prior to analysis. For total phenolic compounds, betacyanin, and betaxanthin, data were logarithmically transformed prior to analysis. In both cases, means were back-transformed for presentation purpose only. Back-transformed values were denoted as medians. Standard errors were back-transformed using the delta method. In case of finding significant differences, Tukey test was used for multiple comparisons. A letter display was used to present their results [42].

Additionally, genotype-by-storage period means were calculated for all six traits. These simple means were standardized to have a mean of zero and a variance of one. A Principal Component Analysis (PCA) was applied on these standardized means. The two first components were presented via biplot using the default setting of the %biplot macro for SAS (factype = SYM). Thus, scaling of score and loading plot was done with $\boldsymbol{US}^{\frac{1}{2}}$ and $\boldsymbol{VS}^{\frac{1}{2}}$, respectively, where $\boldsymbol{USV}'$ is the single value decomposition of the two-dimensional approximation of the data matrix.

3. Results and Discussion

3.1. Total Dry Matter Content

The outcome of analysis of variance (ANOVA) demonstrated a significant effect of genotype on the content of total dry matter (p < 0.0001) (Table 2). This is in line with the findings of Kosson et al. [43] who claimed a significant influence of genotype on TDMC. Furthermore, it was revealed that the TDMC changed significantly during the cold storage (p = 0.0005). Likewise, significant influence of the interactions of storage period and year on the TDMC was noted (Table 2). Hagen et al. [44] stated no significant influence of cold storage for six weeks on the percentage of total dry matter in curly kale. Nonetheless, a strong influence of the storage on transpiration and consequently on the weight loss of the stored beetroot has been reported [45]. In this regard, Gawęda [45] assessed the difference of two storage methods on two beetroot cultivars and reported not only a significant difference between the two cultivars, but also a two-times-decline in dry weight of beetroots stored in polyethylene film bags than those in traditional plastic boxes, after 6 months of cold storage.

Based on the significant impacts of genotype and the interactions of storage period and year, Table 3 presents the means of TDMC of the investigated genotypes within the trial (A) and based on three storage periods in each year (B). The TDMC of the tested genotypes ranged between 11.49 ± 0.55% and 18.15 ± 0.55%. The highest contents of total dry matter were noted in the genotypes Nochowski (18.15 ± 0.55%), Chrobry (18.14 ± 0.55%), and Betina (16.94 ± 0.56%) and the lowest in the genotypes Alvro Mono (11.49 ± 0.55%), Libero RZ (12.31 ± 0.56%), and Tondo d. Ch. (13.37 ± 0.58%) (Table 3).

Considering the means of different storage periods in the first year, the TDMC directly after the harvest was 15.19 ± 0.14%. After one month of cold storage, the TDMC increased to 15.63 ± 0.14%. After a storage period of four months, the TDMC significantly decreased compared to the samples taken one month after the harvest and reached 14.95 ± 0.14%, which was not significantly different from the mean of freshly harvested beetroots. Therefore, the overall change in the TDMC was minor. In the second year, the TDMC directly after the harvest was 14.51 ± 0.21%. After one month of cold storage, the content rose significantly to 15.34 ± 0.21%. Afterwards, up to a storage period of four months, the change was not significant (Table 3). A corresponding outcome was noted by Jakopic et al. [46], who reported a higher TDMC in rutabaga turnips after a cold storage of four months. In contrast, a slight decrease in the TDMC of ten organically grown onion genotypes after five months of cold storage was noted [47]. Regarding the influence of the year, the amount of total dry matter did not differ significantly between the values of the samples from the freshly harvested beetroots in each year. Likewise, the TDMC contents after cold storage periods of one and four months did not differ significantly between both years. According to the significant increase in the TDMC within the first one month of cold storage in both years, it can be concluded that the highest amount of water loss could occur at the first four weeks of cold storage.

Table 2. ANOVA of results of total dry matter, total phenolic compounds, betaxanthin, betacyanin, nitrate, and total soluble sugars of 36 beetroot genotypes grown in research station Kleinhohenheim within the years 2017 and 2018 for three storage periods (storage period expressed as cold storage durations of zero (directly after harvest), one, and four months).

Effect	Total Dry Matter Content	Total Phenolic Compounds	Betaxanthin	Betacyanin	Nitrate	Total Soluble Sugars
Genotype	<0.0001	<0.0001	<0.0001	<0.0001	0.0005	<0.0001
Storage period	0.0005	<0.0001	<0.0001	<0.0001	<0.0001	n.s. [1]
Genotype × Storage period	n.s.	n.s.	<0.0001	<0.0001	n.s.	0.0121
Year	n.s.	<0.0001	0.0001	<0.0001	n.s.	0.0008
Storage period × Year	0.0009	0.0001	n.s.	n.s.	n.s.	<0.0001

[1] not significant.

3.2. Total Phenolic Content

In accordance with the results of ANOVA, genotypes can significantly impact the amount of total phenolic compounds ($p < 0.0001$). This is in agreement with Lattanzio et al. [48], who stated the key effect of genotype on the content of phenolic contents in fresh fruit and vegetables. Furthermore, a significant influence of the storage period ($p < 0.0001$), year ($p < 0.0001$), and the interactions between storage period and year were noted (Table 2).

According to the significant impacts of genotype and the interactions of storage period and year, Table 3 exhibits the medians of TPC of the examined genotypes within the trial (A) and based on three storage periods in each year (B). The TPC in investigated red-colored beetroot genotypes varied from 322.93 ± 21.04 mg GAE 100 g^{-1} DW to 612.39 ± 40.58 mg GAE 100 g^{-1} DW measured in genotypes Robuschka and Alvro Mono, respectively. Following Alvro Mono, the cylindrical-shaped genotype Forono with 561.74 ± 37.37 mg GAE 100 g^{-1} DW and Monty RZ F1 with 519.91 ± 33.89 mg GAE 100 g^{-1} DW indicated the highest TPC. Taking all the genotypes into account, the lowest TPC possessed by the yellow-colored genotype Burpees G. (201.45 ± 13.13 mg GAE 100 g^{-1} DW), the red-white-colored Tondo d. Ch. (241.16 ± 20.35 mg GAE 100 g^{-1} DW), and white-colored Sniezna Kula (242.55 ± 16.07 mg GAE 100 g^{-1} DW). Based on the median values of different storage periods in the first year, the TPC directly after the harvest was 294.55 ± 8.57 mg GAE 100 g^{-1} DW. After one month of cold storage, a significant increase up to 341.18 ± 9.93 mg GAE 100 g^{-1} DW was noted.

Table 3. Mean and median values of total dry matter content (%) and total phenolic content (mg GAE 100 g^{-1} DW) of 36 beetroot genotypes grown at the research station Kleinhohenheim within the years 2017 and 2018. Results represent the mean (median) values ± (approximate) standard error. In section (A), means (medians) followed by at least one identical lower-case letter in one column did not differ significantly between genotypes at experiment-wise Type 1 error α = 0.05. In section (B), means (medians) followed by at least one identical lower-case letter in one column did not differ significantly within different storage periods at experiment-wise Type 1 error α = 0.05 and means (medians) followed by at least one identical upper-case letter in one column did not differ significantly within year at experiment-wise Type 1 error α = 0.05.

		(A) Means (Medians) Based on Genotype	
	Genotype	**TDMC (%)**	**Total Phenolic Content (mg GAE 100 g^{-1} DW)**
	Ä. P.	14.77 ^{bcde} ± 0.55	417.20 ^{ac} ± 27.18
	Akela RZ	14.81 ^{bcde} ± 0.55	413.30 ^{ac} ± 26.94
	Alvro Mono	11.49 ^{e} ± 0.55	612.39 ^{a} ± 40.58
	Betina	16.94 ^{ac} ± 0.56	413.71 ^{ac} ± 27.41
	Bolivar	14.85 ^{ad} ± 0.55	364.91 ^{cdef} ± 23.79
	Bona	14.78 ^{bcde} ± 0.55	405.26 ^{ac} ± 26.41
	Bordo	16.79 ^{ac} ± 0.55	449.00 ^{ac} ± 29.26
	Boro F1	13.84 ^{bcde} ± 0.55	414.61 ^{ac} ± 27.03
	BoRu1	14.85 ^{ad} ± 0.55	417.89 ^{ac} ± 27.25
	Borus	16.39 ^{ab} ± 0.56	376.35 ^{bcde} ± 25.94
	Burpees G.	14.87 ^{ad} ± 0.55	201.45 ^{i} ± 13.13
	Carillon RZ	13.81 ^{bcde} ± 0.55	432.35 ^{ac} ± 28.19
	Cervena K.	16.15 ^{ab} ± 0.56	465.96 ^{ac} ± 31.57
	Ceryl	16.25 ^{ab} ± 0.55	426.45 ^{ac} ± 27.80
	Chrobry	18.14 ^{a} ± 0.55	449.16 ^{ac} ± 29.26
	Czerwona K. 2	15.92 ^{ab} ± 0.55	355.25 ^{cdefg} ± 24.04
	Detroit 2 D. R.	15.64 ^{ad} ± 0.56	418.26 ^{ac} ± 28.83
	Detroit 3	14.30 ^{bcde} ± 0.55	403.59 ^{ac} ± 26.73
	Detroit G.	14.89 ^{ad} ± 0.56	350.31 ^{cdefg} ± 24.15
	Formanova	14.46 ^{bcde} ± 0.55	397.19 ^{bcd} ± 26.31
	Forono	15.21 ^{ad} ± 0.55	561.74 ^{ab} ± 37.37
	Gesche SG	15.25 ^{ad} ± 0.55	484.20 ^{ac} ± 31.56
	Jannis	14.68 ^{bcde} ± 0.55	414.71 ^{ac} ± 27.02
	Jawor	15.17 ^{ad} ± 0.55	430.75 ^{ac} ± 28.07
	Libero RZ	12.31 ^{de} ± 0.56	408.49 ^{ac} ± 29.38
	Monty RZ F1	15.65 ^{ad} ± 0.55	519.91 ^{ad} ± 33.89
	Nobol	14.20 ^{bcde} ± 0.55	456.04 ^{ac} ± 29.74
	Nochowski	18.15 ^{a} ± 0.55	486.33 ^{ac} ± 31.69
	Pablo F1	14.58 ^{bcde} ± 0.55	440.53 ^{ac} ± 29.33
	Regulski O.	15.89 ^{ab} ± 0.55	381.10 ^{bcde} ± 24.84
	Robuschka	15.57 ^{ad} ± 0.55	322.93 ^{cefgh} ± 21.04
	Ronjana	15.39 ^{ad} ± 0.55	508.49 ^{ad} ± 33.16
	Hilmar	15.01 ^{ad} ± 0.55	420.64 ^{ac} ± 27.41
	Sniezna Kula	15.65 ^{ad} ± 0.55	242.55 ^{fi} ± 16.07
	Tondo d. Ch.	13.37 ^{bde} ± 0.58	241.16 ^{ei} ± 20.35
	UB-E3	16.06 ^{ab} ± 0.55	393.07 ^{bcd} ± 26.04
		(B) Means (Medians) Based on Storage Period	
	Storage Period		
Year 1	Directly after harvest	15.19 ^{abA} ± 0.14	294.55 ^{cB} ± 8.57
	1 month after cold storage	15.63 ^{aA} ± 0.14	341.18 ^{bA} ± 9.93
	4 months after cold storage	14.95 ^{bA} ± 0.14	437.83 ^{aA} ± 12.78
Year 2	Directly after harvest	14.51 ^{bA} ± 0.21	395.91 ^{aA} ± 12.59
	1 month after cold storage	15.34 ^{aA} ± 0.21	379.09 ^{aA} ± 12.17
	4 months after cold storage	15.46 ^{aA} ± 0.22	425.18 ^{aA} ± 14.47

Likewise, after a storage period of four months the TPC further increased and reached 437.83 ± 12.78 mg GAE 100 g^{-1} DW (Table 3). In the second year, the TPC directly after the harvest was 395.91 ± 12.59 mg GAE 100 g^{-1} DW. After one month of cold storage, it slightly decreased, however, the change was not significant. Afterwards, up to a storage period of four months, an increasing trend in the TPC was noticed (Table 3). The median values of TPC after a cold storage period of four months in both years were higher than those at harvest time, which indicated the potential of the investigated genotypes in providing a promising content of phenolic compounds for an extended time after the harvest. This is in agreement with the study of Jakopic et al. [46], who reported an increase of TPC of rutabaga root after four months of cold storage. Regarding the influence of the year, the amount of total phenolic compounds differed significantly between the values of the samples from the freshly harvested beetroots in each year. Nevertheless, the TPC after cold storage periods of one and four months did not differ significantly between both years.

Evaluation of the stability of TPC of red beetroot (var. Little Ball) during the storage in 5 °C for 196 days indicated a slight decrease in the first 63 days of storage and afterwards the change was minor [49]. In line with the results of the present study, in which both decreasing and increasing trends in the TPC during the storage period was noted, both decreases (in broccoli [50] and pomegranate [51]) and increases (in pigmented potato tuber [52]) in TPC have been reported in the previous studies. This may result from the differences between the impact's extent of cold storage on individual constituents of phenolic compounds [49].

Corleto et al. [32] investigated the stability of TPC in beetroot juice, which were stored for 32 days at four different temperatures and significant differences were noted during the storage at refrigeration temperature (4 °C). Nevertheless, it was revealed that under refrigeration and freezing conditions, antioxidant activity and TPC remain more stable in comparison to storage at room temperature. Assessing the impact of temperature and period of storage on beetroot snack bars indicated that TPC decreased constantly during six months storage at all studied temperatures (6 °C, 22–32 °C, and 37 °C). However, the TPC loss at 6 °C was less than at higher temperatures [53]. High temperature is reported as the main factor causing the reduction of TPC in vegetables due to change in the phenolic profiles [54].

The cylindrical-shaped genotype, Forono, was noted to be among the genotypes with the highest TPC (Table 3), betacyanin, and betaxanthin (Table 4) after the cold storage, which can be correlated to the high antioxidant activity of this genotype. Furthermore, another cylindrical-shaped beetroot, Carillon RZ, was noted as a genotype with an average TPC and betalain content. Additionally, this genotype indicated the highest total and marketable yield as well as high resistance against the common beet disease, scab, among 15 investigated beetroot genotypes in our previous study [37] which further reveals its latent promising characteristics. In contradiction to Forono and Carillon RZ, the other studied cylindrical-shaped beetroot, Formanova, was among the red-colored genotypes with the lowest betalain content and an average TPC. This may explain the impact of genotype rather than the shape on the content of the discussed compounds.

Table 4. Median values of betaxanthin (mg g^{-1} DW) and betacyanin (mg g^{-1} DW) of 36 beetroot genotypes grown at the research station Kleinhohenheim within the years 2017 and 2018, directly after harvest, and after the cold storage periods of one and four months. Results represent the median values ± approximate standard error. Medians followed by at least one identical lower-case letter in one column did not differ significantly between genotypes at experiment-wise Type 1 error α = 0.05. Medians followed by at least one identical upper-case letter in one row did not differ significantly between storage periods at experiment-wise Type 1 error α = 0.05.

Genotype	Betaxanthin (mg g^{-1} DW)			Betacyanin (mg g^{-1} DW)		
	Directly after Harvest	1 Month after Cold Storage	4 Months after Cold Storage	Directly after Harvest	1 Month after Cold Storage	4 Months after Cold Storage
Ä. P.	$4.41^{aA} \pm 0.84$	$4.62^{abA} \pm 0.88$	$4.40^{abA} \pm 0.84$	$6.12^{abcA} \pm 1.10$	$6.17^{aA} \pm 1.11$	$5.42^{aA} \pm 0.97$
Akela RZ	$4.44^{aA} \pm 0.85$	$5.70^{aA} \pm 1.09$	$5.04^{abA} \pm 0.96$	$6.65^{abA} \pm 1.19$	$8.02^{aA} \pm 1.44$	$6.77^{aA} \pm 1.22$
Alvro Mono	$2.69^{aA} \pm 0.51$	$3.08^{adA} \pm 0.59$	$3.05^{bcdA} \pm 0.60$	$3.61^{abcdA} \pm 0.65$	$4.13^{aA} \pm 0.74$	$4.28^{aA} \pm 0.79$
Betina	$4.31^{aA} \pm 0.82$	$4.73^{abA} \pm 0.90$	$5.13^{abA} \pm 1.00$	$5.47^{abcdA} \pm 0.98$	$7.20^{aA} \pm 1.29$	$6.83^{aA} \pm 1.26$
Bolivar	$4.65^{aA} \pm 0.89$	$4.55^{abA} \pm 0.87$	$5.28^{abA} \pm 1.01$	$6.30^{abcA} \pm 1.13$	$5.93^{aA} \pm 1.07$	$6.83^{aA} \pm 1.23$
Bona	$4.07^{aA} \pm 0.78$	$4.76^{abA} \pm 0.91$	$3.71^{beA} \pm 0.71$	$5.79^{abcA} \pm 1.04$	$6.39^{aA} \pm 1.15$	$4.94^{aA} \pm 0.89$
Bordo	$5.03^{aA} \pm 0.96$	$4.48^{abA} \pm 0.86$	$5.23^{abA} \pm 1.00$	$6.99^{abA} \pm 1.25$	$7.05^{aA} \pm 1.27$	$7.28^{aA} \pm 1.31$
Boro F1	$4.75^{aA} \pm 0.91$	$4.88^{aA} \pm 0.93$	$4.58^{abA} \pm 0.87$	$6.50^{abA} \pm 1.17$	$6.17^{aA} \pm 1.11$	$5.74^{aA} \pm 1.03$
BoRu1	$5.38^{aA} \pm 1.03$	$5.88^{aA} \pm 1.12$	$4.99^{abA} \pm 0.95$	$7.23^{aA} \pm 1.30$	$7.56^{aA} \pm 1.36$	$6.34^{aA} \pm 1.14$
Borus	$4.10^{aA} \pm 0.78$	$4.63^{abA} \pm 0.88$	$4.28^{bcA} \pm 0.88$	$5.75^{abcA} \pm 1.03$	$6.40^{aA} \pm 1.15$	$5.63^{aA} \pm 1.10$
Burpees G.	$0.20^{bB} \pm 0.04$	$1.15^{dA} \pm 0.22$	$1.03^{deA} \pm 0.20$	$0.43^{egA} \pm 0.08$	$0.09^{bB} \pm 0.02$	$0.11^{bB} \pm 0.02$
Carillon RZ	$4.83^{aA} \pm 0.92$	$5.17^{aA} \pm 0.99$	$4.72^{abA} \pm 0.90$	$6.65^{abA} \pm 1.20$	$7.47^{aA} \pm 1.34$	$6.51^{aA} \pm 1.17$
Cervena K.	$4.11^{aA} \pm 0.79$	$4.49^{abA} \pm 0.86$	$4.54^{abA} \pm 0.90$	$5.75^{abcA} \pm 1.03$	$6.30^{aA} \pm 1.13$	$5.93^{aA} \pm 1.11$
Ceryl	$5.57^{aA} \pm 1.06$	$6.40^{aA} \pm 1.22$	$5.48^{abA} \pm 1.05$	$7.91^{aA} \pm 1.42$	$8.55^{aA} \pm 1.54$	$7.22^{aA} \pm 1.30$
Chrobry	$5.93^{aA} \pm 1.13$	$4.58^{abA} \pm 0.88$	$4.71^{abA} \pm 0.90$	$7.84^{aA} \pm 1.41$	$7.69^{aA} \pm 1.38$	$7.73^{aA} \pm 1.39$
Czerwona K. 2	$4.51^{aA} \pm 0.88$	$4.41^{abcA} \pm 0.84$	$3.91^{bcA} \pm 0.76$	$6.67^{abA} \pm 1.22$	$6.17^{aA} \pm 1.11$	$5.48^{aA} \pm 1.01$
Detroit 2 D. R.	$4.67^{aA} \pm 0.89$	$4.40^{abcA} \pm 0.84$	$4.63^{abA} \pm 0.95$	$6.65^{abA} \pm 1.19$	$6.22^{aA} \pm 1.12$	$6.20^{aA} \pm 1.21$
Detroit 3	$5.46^{aA} \pm 1.04$	$4.58^{abA} \pm 0.87$	$5.41^{abA} \pm 1.05$	$7.73^{aA} \pm 1.39$	$6.15^{aA} \pm 1.10$	$6.72^{aA} \pm 1.24$
Detroit G.	$4.67^{aA} \pm 0.89$	$4.08^{adA} \pm 0.78$	$4.45^{bcA} \pm 0.90$	$7.02^{abA} \pm 1.26$	$5.59^{aA} \pm 1.00$	$5.63^{aA} \pm 1.10$
Formanova	$3.63^{aA} \pm 0.69$	$3.54^{adA} \pm 0.69$	$3.28^{bcdA} \pm 0.63$	$5.01^{abcdA} \pm 0.90$	$5.08^{aA} \pm 0.94$	$5.00^{aA} \pm 0.90$
Forono	$5.76^{aA} \pm 1.10$	$6.39^{aA} \pm 1.25$	$6.70^{bA} \pm 1.28$	$8.39^{aA} \pm 1.51$	$7.99^{aA} \pm 1.46$	$8.97^{aA} \pm 1.61$
Gesche SG	$6.60^{aA} \pm 1.26$	$5.74^{aA} \pm 1.10$	$5.38^{abA} \pm 1.03$	$7.79^{aA} \pm 1.40$	$7.73^{aA} \pm 1.42$	$7.11^{aA} \pm 1.28$
Jannis	$4.17^{aA} \pm 0.80$	$4.81^{abA} \pm 0.92$	$4.90^{abA} \pm 0.94$	$5.85^{abcA} \pm 1.05$	$6.49^{aA} \pm 1.16$	$6.17^{aA} \pm 1.11$
Jawor	$4.24^{aA} \pm 0.81$	$4.23^{adA} \pm 0.81$	$3.85^{bcA} \pm 0.74$	$5.86^{abcA} \pm 1.05$	$6.18^{aA} \pm 1.11$	$5.84^{aA} \pm 1.05$
Libero RZ	$6.25^{aA} \pm 1.19$	$6.73^{aA} \pm 1.38$	$5.24^{abA} \pm 1.07$	$8.63^{aA} \pm 1.55$	$8.70^{aA} \pm 1.70$	$6.51^{aA} \pm 1.27$
Monty RZ F1	$5.87^{aA} \pm 1.12$	$5.40^{aA} \pm 1.03$	$5.29^{abA} \pm 1.01$	$8.70^{aA} \pm 1.56$	$8.20^{aA} \pm 1.47$	$7.32^{aA} \pm 1.32$
Nobol	$5.72^{aA} \pm 1.09$	$5.38^{aA} \pm 1.03$	$5.83^{abA} \pm 1.11$	$8.18^{aA} \pm 1.47$	$7.64^{aA} \pm 1.37$	$8.16^{aA} \pm 1.47$
Nochowski	$6.08^{aA} \pm 1.16$	$5.05^{aA} \pm 0.97$	$5.95^{abA} \pm 1.14$	$7.77^{aA} \pm 1.39$	$7.57^{aA} \pm 1.36$	$8.49^{aA} \pm 1.53$
Pablo F1	$4.35^{aA} \pm 0.83$	$5.00^{aA} \pm 0.96$	$4.72^{abA} \pm 0.92$	$6.13^{abcA} \pm 1.10$	$6.36^{aA} \pm 1.14$	$5.71^{aA} \pm 1.04$
Regulski O.	$3.91^{aA} \pm 0.75$	$4.96^{aA} \pm 0.95$	$4.48^{abA} \pm 0.86$	$5.93^{abcA} \pm 1.06$	$6.88^{aA} \pm 1.24$	$6.23^{aA} \pm 1.12$
Robuschka	$3.85^{aA} \pm 0.74$	$4.60^{abA} \pm 0.88$	$5.34^{abA} \pm 1.02$	$5.50^{abcA} \pm 0.99$	$6.22^{aA} \pm 1.12$	$6.70^{aA} \pm 1.20$
Ronjana	$6.16^{aA} \pm 1.18$	$4.68^{abA} \pm 0.89$	$6.08^{abA} \pm 1.16$	$8.07^{aA} \pm 1.45$	$7.42^{aA} \pm 1.33$	$9.05^{aA} \pm 1.63$
Hilmar	$4.42^{aA} \pm 0.84$	$4.73^{abA} \pm 0.90$	$5.05^{abA} \pm 0.97$	$5.89^{abcA} \pm 1.06$	$6.54^{aA} \pm 1.17$	$6.63^{aA} \pm 1.19$
Sniezna Kula	$0.13^{bA} \pm 0.03$	$0.18^{eA} \pm 0.05$	$0.16^{gA} \pm 0.05$	$0.12^{gA} \pm 0.03$	$0.17^{bA} \pm 0.04$	$0.15^{bA} \pm 0.04$
Tondo d. Ch.	$0.22^{bA} \pm 0.04$	$0.26^{eA} \pm 0.05$	$0.19^{fgA} \pm 0.05$	$0.30^{fgA} \pm 0.05$	$0.25^{bA} \pm 0.05$	$0.20^{bA} \pm 0.05$
UB-E3	$3.40^{aA} \pm 0.65$	$4.21^{adA} \pm 0.80$	$3.50^{bcdA} \pm 0.68$	$5.31^{abcdA} \pm 0.95$	$5.75^{aA} \pm 1.03$	$4.29^{aA} \pm 0.79$

3.3. Betalain Content

The findings of this study revealed a significant effect of the interaction between genotype and storage period on both betacyanin and betaxanthin content ($p < 0.0001$). Moreover, it was noted that the year impacted the betaxanthin and betacyanin contents significantly (Table 2). Kujala et al. [6] claimed that beetroot genotype, cultivation, and storage conditions can affect the content of betanin and isobetanin (the main betacyanins found in beetroot). Cejudo-Bastante et al. [55] reported the influence of storage duration and temperature on the betalain content of fruits. Generally, temperature has been reported as a key factor for the stability of the betalain [56].

Two major subgroups of betalain, betaxanthin and betacyanin, were measured in this study. Respecting the significant impact of the interactions between genotype and storage period on both betaxanthin and betacyanin contents, Table 4 demonstrates the contents based on different storage periods for each genotype separately. Considering all studied genotypes, the highest betaxanthin contents in beetroots directly after harvest were measured in the genotypes Gesche SG (6.60 ± 1.26 mg g^{-1} DW), Libero RZ (6.25 ± 1.19 mg g^{-1} DW), and Ronjana (6.16 ± 1.18 mg g^{-1} DW) and the lowest values were noted in the white-colored genotype Sniezna Kula, yellow-colored Burpees G., and red-white Tondo d. Ch., with 0.13 ± 0.03 mg g^{-1} DW, 0.20 ± 0.04 mg g^{-1} DW, and 0.22 ± 0.4 mg g^{-1} DW, respectively (Table 4). Comparing the red-colored genotypes, the betaxanthin content after one month of cold storage varied between 3.08 ± 0.59 mg g^{-1} DW and 6.73 ± 1.38 mg g^{-1} DW belonging to the genotypes Alvro Mono and Libero RZ, respectively. Following Alvro Mono, the genotypes Formanova (3.54 ± 0.69 mg g^{-1} DW), Detroit G. (4.08 ± 0.78 mg g^{-1} DW), and UB-E3 (4.21 ± 0.80 mg g^{-1} DW) possessed the lowest betaxanthin values (Table 4). However, the white-colored genotype Sniezna Kula with 0.18 ± 0.05 mg g^{-1} DW, the red-white genotype Tondo d. Ch. with 0.26 ± 0.05 mg g^{-1} DW, and yellow-colored genotype Burpees G. with 1.15 ± 0.22 mg g^{-1} DW indicated the lowest betaxanthin values when taking all studied genotypes into account (Table 4). After the storage period of four months, the betaxanthin content of the red-colored genotypes ranged between 3.05 ± 0.60 mg g^{-1} DW and 6.70 ± 1.28 mg g^{-1} DW belonging to the genotypes Alvro Mono and the cylindrical-shaped Forono, respectively. However, considering all studied genotypes, the lowest betaxanthin contents were observed in the white-colored genotype Sniezna Kula with 0.16 ± 0.05 mg g^{-1} DW, the red-white genotype Tondo d. Ch. with 0.19 ± 0.05 mg g^{-1} DW, and yellow-colored genotype Burpees G. with 1.03 ± 0.20 mg g^{-1} DW (Table 4). Regarding the influence of cold storage, in the yellow-colored genotype Burpees G., a significant increase in the betaxanthin content after one month of cold storage was observed and afterwards, up to a storage period of four months, no significant change occurred (Table 4).

The betacyanin content of the red-colored genotypes directly after the harvest ranged from 3.61 ± 0.65 mg g^{-1} DW to 8.70 ± 1.56 mg g^{-1} DW. The three highest betacyanin contents were noted in genotypes Monty RZ F1, Libero RZ, and Forono, respectively (Table 4). Taking all the examined genotypes into account, the white-colored genotype Sniezna Kula with 0.12 ± 0.03 mg g^{-1} DW, the red-white genotype Tondo d. Ch. with 0.30 ± 0.05 mg g^{-1} DW, and yellow-colored genotype Burpees G. with 0.43 ± 0.08 mg g^{-1} DW possessed the lowest betacyanin contents. After one month of cold storage, the betacyanin content of the red-colored genotypes varied between 4.13 ± 0.74 mg g^{-1} DW and 8.70 ± 1.70 mg g^{-1} DW belonging to Alvro Mono and Libero RZ, respectively, and that after a cold storage of four months, ranged between 4.28 ± 0.79 mg g^{-1} DW and 9.05 ± 1.63 mg g^{-1} DW found in the genotypes Alvro Mono and Ronjana, respectively. After cold storage periods of one and four months, the lowest betacyanin contents were measured in Burpees G., Sniezna Kula, and Tondo d. Ch., respectively (Table 4). Among all investigated genotypes, the betacyanin content in yellow-colored genotype, Burpees G., was significantly influenced by the duration of the cold storage. In this regard, a significant decrease in the betacyanin content after one month of cold storage was noted and afterwards up to a storage period of four months, no significant change was observed (Table 4).

Kujala et al. [49] studied the effect of cold storage at 5 °C on betanin and isobetanin content of the red beetroots (var. Little Ball) grown in Finland and significant differences in the amounts of betanin and isobetanin during the cold storage (0–196 days) were noted. Moreover, it was reported that the content of betanin in red beetroot peel decreased in the first 140 days of cold storage and then slightly increased. In terms of isobetanin, until 98 days, an increasing trend and afterward, up to 140 days of storage, a light decrease were noticed [49]. Maity et al. [53] investigated the effect of storage temperature and duration on betacyanin and betaxanthin contents of compressed beetroot snack bars and the maximum retention was noted in those stored at 6 °C and the content did not change significantly after four months of storage.

Moreover, storage of beetroot powder in three different temperatures (namely 10, 25, and 40 °C) indicated the minimum loss in the content of betacyanin and betaxanthin at the lowest temperature up to five weeks [56]. Yong et al. [57] investigated the effect of seven days of cold storage on betacyanin content of red pitahaya at 4 °C and reported a significant increase after six days of storage and then a slight decrease on day seven. In contrast, Obenland et al. [58] noted no significant impact of two weeks storage of red pitahaya at 5 and 10 °C on the betacyanin content.

3.4. Nitrate Content

The average nitrate content differed significantly between the genotypes ($p = 0.0005$) (Table 2). A corresponding result was found by Kosson et al. [43], who reported a significant influence of variety on the nitrate content in two beetroot genotypes. Moreover, a significant impact of the storage period on the nitrate content was noted ($p < 0.0001$). On the other hand, the interactions between storage period and genotype, year, and interactions between storage period and year on the nitrate content were not significant (Table 2).

Corleto et al. [32] reported a significant impact of different refrigeration temperatures and periods on the nitrate level of freshly pressed beetroot juice. Moreover, their study revealed that in the first eight days of storage at 4 °C, the nitrate value did not change significantly, while between day 8 and 32 of the storage, the nitrate level decreased drastically. In contrast, Chung et al. [59] studied the impact of cold storage on four different leafy vegetables, including spinach, crown daisy, organic Chinese spinach, and organic non-heading Chinese cabbage, and no significant changes in the nitrate content could be proven during the storage period.

According to the significant effect of genotype and storage period on the content of nitrate, Figure 1 demonstrates the medians of nitrate content of the studied genotypes as well as medians based on three storage periods studied within the experiment. Moreover, the precise median and asymptotic standard error values of nitrate content are available in Table S1 in the supplementary material. The nitrate values exhibited in Figure 1 were reported on a dry weight basis, while nitrate content in literature is often also presented on a fresh weight (FW) basis. Consequently, to compare the findings of this work with other studies, the TDMC should be considered. The amount of nitrate of the studied genotypes ranged between 4179 ± 1267 mg kg^{-1} DW and 20,489 ± 2988 mg kg^{-1} DW found in Chrobry and Libero RZ, respectively (Figure 1). Following Libero RZ, the genotypes Bona (16,794 ± 2539 mg kg^{-1} DW) and Alvro Mono (16,438 ± 2539 mg kg^{-1} DW) indicated the highest nitrate contents within the trial. The second and third lowest nitrate content were measured in genotypes Nochowski with 4602 ± 1329 mg kg^{-1} DW, and Hilmar with 5190 ± 1411 mg kg^{-1} DW, respectively.

With regards to the medians based on the storage period, the nitrate content of 9095 ± 414 mg kg^{-1} DW was calculated for all genotypes in both years for samples collected directly after the harvest. After one month of cold storage, the median nitrate content increased significantly and reached a median of 10,977 ± 459 mg kg^{-1} DW. No further significant increase was observed after a cold storage period of four months (Figure 1). Consequently, based on the outcome of this study, the main change in the nitrate content of the investigated genotypes arose within the first four weeks of storage.

According to the point that the nitrate content may not only vary between the genotypes but also among the cultivars of the same species and plant tissues [60–62], assessing a greater number of plants per genotype for having a better evaluation can be recommended.

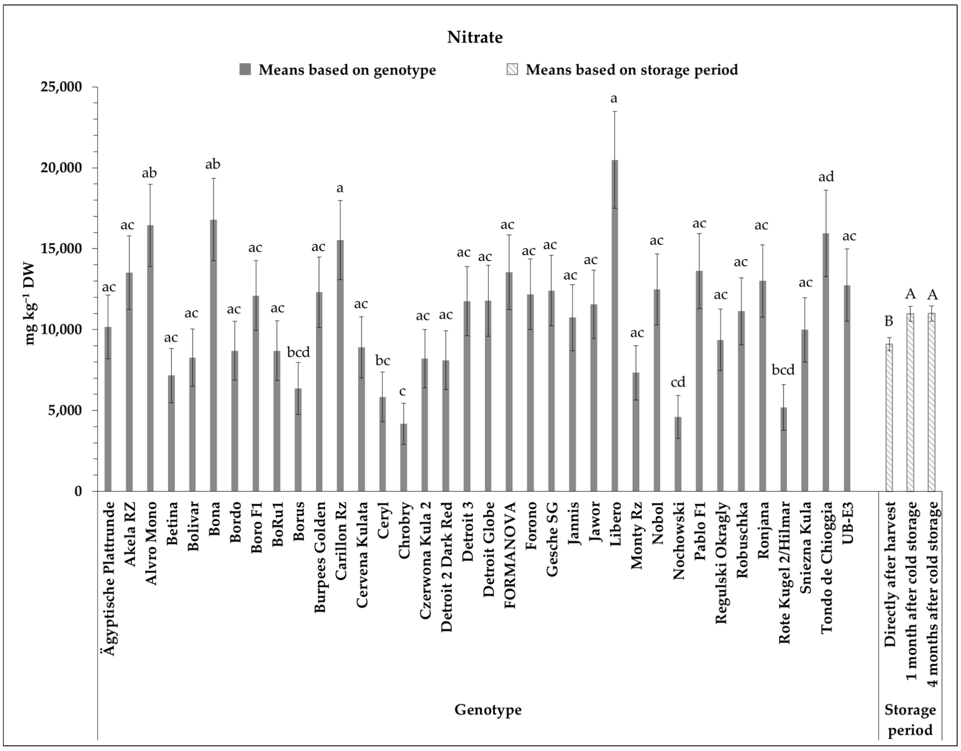

Figure 1. Median values of nitrate content (mg kg^{-1} DW) of 36 beetroot genotypes grown at the research station Kleinhohenheim within the years 2017 and 2018, based on genotype and storage period. Results represent the median values ± asymptotic standard error. Medians covered by at least one identical lower-case letter did not differ significantly between genotypes at experiment-wise Type 1 error α = 0.05. Medians covered by at least one identical upper-case letter did not differ significantly between storage periods at experiment-wise Type 1 error α = 0.05.

3.5. Total Soluble Sugar Content

According to the statistical analysis, interactions between storage period and genotype were significant (p = 0.0121). Viskelis et al. [2] reported no significant change of total sugar content of 11 beetroot genotypes, grown in Lithuania, during the storage at 1 ± 1 °C and relative humidity of 90–95% for the storage period of seven months. Nonetheless, in the case of four common genotypes in both studies, namely Bona, Boro F1, Detroit 2 D. R., and Pablo F1, despite the lower values in our study, the storage period did not impact the content of total soluble sugars significantly. Thus, it disclosed that the influence of cold storage might be genotype-dependent.

To better appraise the impact of cold storage on sugar content, it is noteworthy to know the amount of sugar in freshly harvest beetroot. In this regards, the freshly harvested beetroots in this study contained the total soluble sugar content in the range of 8.55 ± 0.67 °Bx to 15.43 ± 0.67 °Bx possessing by the genotypes Alvro Mono and Nochowski, respectively. After one month of cold storage, the three highest total soluble sugar contents belonged to the genotypes Nochowski, Chrobry, and Cervena K., with 14.88 ± 0.67 °Bx, 13.96 ± 0.67 °Bx, and 13.89 ± 0.67 °Bx, respectively (Table 5). The genotypes Alvro Mono (9.00 ± 0.46 °Bx), Bolivar (9.64 ± 0.48 °Bx), and Libero RZ (9.64 ± 0.67 °Bx) exhibited the lowest total soluble sugar contents. After a cold storage period of four months, the total soluble sugar content ranged between 8.41 ± 0.71 °Bx and 14.92 ± 0.67 °Bx, belonging to the genotypes Alvro Mono and Chrobry, respectively. Following the genotype Alvro Mono, the red-colored Libero RZ, red-white-colored Tondo d. Ch., and yellow-colored Burpees G. indicated the lowest total soluble sugar contents.

Table 5. Mean values of total soluble sugars (°Bx) of 36 beetroot genotypes grown at the research station Kleinhohenheim within the years 2017 and 2018, directly after harvest, and after the cold storage periods of one and four months. Results represent the mean values ± standard error. Means followed by at least one identical lower-case letter in one column did not differ significantly between genotypes at experiment-wise Type 1 error α = 0.05. Means followed by at least one identical upper-case letter in one row did not differ significantly between storage periods at experiment-wise Type 1 error α = 0.05.

Genotype	**Total Soluble Sugars (°Bx)**		
	Directly after Harvest	**1 Month after Cold Storage**	**4 Months after Cold Storage**
Ä. P.	11.08 ^{adA} ± 0.67	11.77 ^{adA} ± 0.67	12.31 ^{bcA} ± 0.67
Akela RZ	11.26 ^{adA} ± 0.67	11.58 ^{adA} ± 0.67	12.10 ^{bcA} ± 0.67
Alvro Mono	8.55 ^{dA} ± 0.67	9.49 ^{dA} ± 0.67	8.41 ^{cA} ± 0.71
Betina	13.40 ^{abA} ± 0.67	13.55 ^{adA} ± 0.67	13.74 ^{abA} ± 0.71
Bolivar	11.14 ^{adA} ± 0.67	9.53 ^{cdA} ± 0.67	12.37 ^{bcA} ± 0.67
Bona	11.18 ^{adA} ± 0.67	11.14 ^{adA} ± 0.67	10.88 ^{bcA} ± 0.67
Bordo	12.04 ^{adA} ± 0.67	13.50 ^{adA} ± 0.67	12.47 ^{bcA} ± 0.67
Boro F1	10.99 ^{bcdA} ± 0.67	11.13 ^{adA} ± 0.67	11.16 ^{bcA} ± 0.67
BoRu1	11.79 ^{adA} ± 0.67	10.60 ^{adA} ± 0.67	11.53 ^{bcA} ± 0.67
Borus	12.40 ^{adA} ± 0.67	13.53 ^{adA} ± 0.67	13.38 ^{abA} ± 0.76
Burpees G.	10.66 ^{bcdA} ± 0.67	10.59 ^{adA} ± 0.67	10.29 ^{acA} ± 0.67
Carillon RZ	9.76 ^{bdA} ± 0.67	11.87 ^{adA} ± 0.67	11.15 ^{bcA} ± 0.67
Cervena K.	13.85 ^{abA} ± 0.67	13.89 ^{abcA} ± 0.67	11.88 ^{bcA} ± 0.77
Ceryl	13.47 ^{abA} ± 0.67	12.97 ^{adA} ± 0.67	13.70 ^{abA} ± 0.71
Chrobry	14.40 ^{acA} ± 0.67	13.96 ^{abA} ± 0.67	14.92 ^{bA} ± 0.67
Czerwona K. 2	12.21 ^{adA} ± 0.67	12.14 ^{adA} ± 0.67	13.44 ^{abA} ± 0.71
Detroit 2 D. R.	12.28 ^{adA} ± 0.67	12.91 ^{adA} ± 0.67	11.29 ^{bcA} ± 0.84
Detroit 3	10.98 ^{bcdA} ± 0.67	11.34 ^{adA} ± 0.67	11.22 ^{bcA} ± 0.71
Detroit G.	10.53 ^{bcdA} ± 0.67	11.47 ^{adA} ± 0.67	11.72 ^{bcA} ± 0.76
Formanova	11.15 ^{adA} ± 0.67	11.11 ^{adjA} ± 0.67	11.34 ^{bcA} ± 0.67
Forono	11.75 ^{adA} ± 0.67	12.05 ^{adA} ± 0.67	11.87 ^{bcA} ± 0.67
Gesche SG	13.07 ^{abeA} ± 0.67	12.63 ^{adA} ± 0.67	11.80 ^{bcA} ± 0.67
Jannis	11.19 ^{adA} ± 0.67	11.72 ^{adA} ± 0.67	11.87 ^{bcA} ± 0.67
Jawor	11.71 ^{adA} ± 0.67	12.22 ^{adA} ± 0.67	12.93 ^{abA} ± 0.67
Libero RZ	8.97 ^{deA} ± 0.67	9.64 ^{bdA} ± 0.67	9.60 ^{acA} ± 0.76
Monty RZ F1	11.36 ^{adA} ± 0.67	13.17 ^{adA} ± 0.67	12.90 ^{bcA} ± 0.71
Nobol	10.61 ^{bcdA} ± 0.67	11.53 ^{adA} ± 0.67	11.17 ^{bcA} ± 0.67
Nochowski	15.43 ^{aA} ± 0.67	14.88 ^{aA} ± 0.67	12.20 ^{bcA} ± 0.67
Pablo F1	10.81 ^{bcdA} ± 0.67	11.11 ^{adA} ± 0.67	11.61 ^{bcA} ± 0.81
Regulski O.	11.76 ^{adA} ± 0.67	12.26 ^{adA} ± 0.67	11.99 ^{bcA} ± 0.67
Robuschka	11.86 ^{adA} ± 0.67	12.82 ^{adA} ± 0.67	11.99 ^{bcA} ± 0.67
Ronjana	12.71 ^{adA} ± 0.67	11.71 ^{adA} ± 0.67	11.73 ^{bcA} ± 0.67
Hilmar	11.43 ^{adA} ± 0.67	12.71 ^{adA} ± 0.67	11.75 ^{bcA} ± 0.67
Sniezna Kula	11.60 ^{adA} ± 0.67	10.74 ^{adA} ± 0.67	11.50 ^{bcA} ± 0.71
Tondo d. Ch.	8.77 ^{deA} ± 0.68	9.71 ^{bdA} ± 0.71	10.07 ^{bcA} ± 0.84
UB-E3	12.70 ^{adA} ± 0.67	12.58 ^{adA} ± 0.67	13.00 ^{abA} ± 0.67

Depending on the stored crop, the effect of cold storage on the total soluble sugar content can be different. Hagen et al. [63] stated a significant decrease in the total content of soluble sugars in curly kale stored for six weeks at 1 °C, while an increase in sugar content was noted in potato tubers stored for six months at 2–4 °C. Jakopic et al. [46] investigated the change in the content of soluble sugars in rutabaga during the cold storage and an increase in the first month of storage and afterwards up to the cold storage for four months a slight decrease was reported. Barboni et al. [64] investigated the impact of cold storage on the total soluble sugar content of kiwi fruit and an increase in the first seven weeks and a constant amount after 21 weeks of the storage was noted. This reveals the considerable variation in the effect of cold storage on vegetables and fruits, which is highly dependent on the species [65].

3.6. Principal Component Analysis

The biplot of the genotype-by-storage period means (Figure 2) demonstrated that more than 83% of the variation is elucidated by two examined components.

It revealed that the TDMC and total soluble sugar content are highly positively correlated, whereas, high negative correlations between these two traits and nitrate content was noted. This is in agreement with Anjana and Iqbal [66], who reported a negative correlation between the sugar and nitrate contents and a positive correlation between carbohydrate concentration and TDMC. The biplot further confirmed that the genotypes Alvro Mono and Libero RZ, which indicated the highest nitrate values possessed the lowest sugar content among the studied genotypes. On the other hand, the highest total soluble sugar contents were noted in genotypes Chrobry and Nochowski, which were included in the genotypes with the lowest nitrate content. Moreover, the biplot visualized a high positive correlation between the contents of betacyanin and betaxanthin. Likewise, the contents of these two compounds were positively correlated with the TPC. Corresponding findings were reported by Kugler et al. [67] regarding a positive correlation between betacyanin and betaxanthin, by Kujala et al. [49] about a positive correlation between betacyanin and TPC, and Čanadanović- Brunet et al. [68] stated a significant correlation between the total phenolic compounds and betaxanthin. The biplot of the interactions of genotype and storage period approved that the lowest TPC and betalain content belonged to the non-red genotypes, including Tondo d. Ch., Burpees G., and Sniezna Kula.

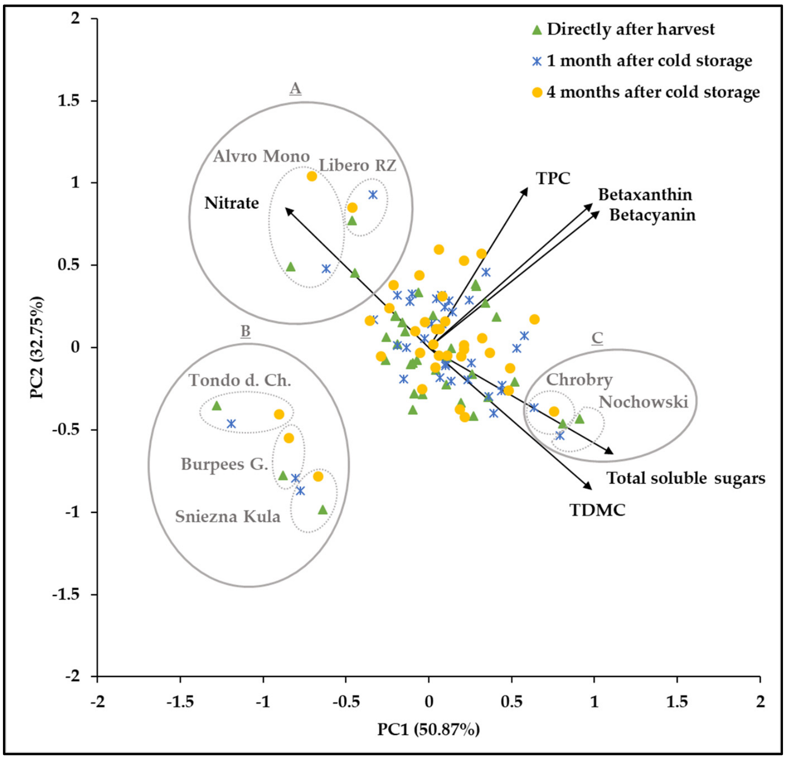

Figure 2. Biplot of the impact of the interactions of genotype-by-storage period means on the traits TDMC, TPC, betaxanthin, betacyanin, nitrate, and total soluble sugars for 36 beetroot genotypes grown at the research station Kleinhohenheim within the years 2017 and 2018, directly after harvest, and after the cold storage periods of one and four months. Group A shows the genotypes with the highest nitrate contents, group B the genotypes with the highest total soluble sugar contents, and group C, the non-red-colored genotypes. PC1 and PC2 are principal components 1 and 2.

4. Conclusions

Besides investigating a great number of beetroot genotypes and disclosing a high genetic variability regarding the content of the bioactive compounds, this study examined the storability of beetroot genotypes at a low temperature and the extent of change in their compositional quality in order to prolong the use of this vegetable for an extended time after the harvest. However, to have a thorough insight on the genetic potential of the examined beetroot genotypes for their application in various sections, the agronomic performance, and their sensory quality should be additionally considered. The genotype 'Chrobry' was characterized by the lowest nitrate content and indicated a high total soluble sugar content with no significant changes during four months of cold storage, thus, it may be of interest for beetroot juice production. 'Nochowski' and 'Cervena K.' which were among the top three genotypes with the highest total soluble sugar contents directly after the harvest and retained contents after one month of cold storage, indicated a significant decrease in the amount of total soluble sugars after four months of cold storage. Therefore, it is beneficial to use these beetroot genotypes freshly or within the first month of storage, when a high sugar content is desired. The genotypes 'Forono', 'Ronjana', 'Monty RZ F1', and 'Nobol' which were characterized by a high amount of betacyanin and betaxanthin, and their stability during a cold storage period of four months, can be of interest for the use as natural food colorants. The cylindrical-shaped genotype 'Forono' characterized by a high content of betalain and TPC, can serve as an option for value-added food products. Further studies considering beet firmness, and impact of other atmospheric conditions such as relative humidity to further improve the storability of beetroot, can be recommended.

Supplementary Materials: The following is available online at https://www.mdpi.com/article/10.3390/foods10061281/s1, Table S1: Median values of nitrate content (mg kg^{-1} DW) of 36 beetroot genotypes grown at the research station Kleinhohenheim within the years 2017 and 2018, based on genotype and storage period.

Author Contributions: Conceptualization, K.Y. and S.G.-H.; software, K.Y. and J.H.; formal analysis, K.Y. and J.H.; investigation, K.Y.; writing—original draft preparation, K.Y.; writing—review and editing, S.G.-H. and J.H.; supervision, S.G.-H.; project administration, S.G.-H. and M.F.; funding acquisition, S.G.-H. and M.F. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The datasets presented in this study are available on request to the corresponding author.

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

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6. General discussion

The present thesis aimed to comprehensively assess agronomic attributes, compositional quality, and stability of various existing and new beetroot varieties, as well as breeding lines, which were grown according to organic farming principles. One of the overall targets of the project, in which the thesis was embedded, was the development of open-pollinated genotypes, which benefit the organic farming system and contribute to the protection and restoration of biodiversity. Furthermore, with the cultivation of the selected open-pollinated genotypes within two years in different locations, the effect of environmental conditions on the agronomic and compositional characteristics of the examined genotypes was evaluated. Additionally, based on the findings of the present work, with the selection of the suitable genotypes among the broad assortment of examined beetroot genotypes, new beetroot products (such as sports drinks produced by beetroot with high nitrate level) and a significant improvement of beetroot products (food colourant with more intensive red colour) can be expected. Consequently, the production of a local organic superfood can be promoted.

The chief study aims of the thesis were precisely discussed in Publications I – III. **Publication I** focused on the agronomic attributes of 15 beetroot genotypes, including one F1 hybrid and one breeding line, grown in three pedo-climatic locations. Genotypes were evaluated concerning their individual beet weight, total and marketable yield, diameter of beet, and leaves-growth-base width, as well as morphological characteristics of harvested marketable beets, including corky surface, skin smoothness, scab incidence, root tail, and uniformity. The main objective was to evaluate various genotypes regarding their genetic yield potential and morphological attributes as well as their stability and suitability in different years and locations. A significant effect of genotype on the majority of the evaluated attributes was noted, and the examined open-pollinated genotypes demonstrated desirable competitive outcomes in most of the investigated traits in comparison with the F1 hybrid genotype, Monty RZ F1. In **publication II,** the studied genotypes were compared according to the TDMC, total soluble sugar, nitrate, betalains, and TPC to examine their genetic potential regarding the content of health-benefiting compounds and to assess the influence of environmental conditions on the content of bioactive compounds. The findings depicted significant influences of genotype and the interactions of location and year on the measured compounds and, thus, the importance of the role of environmental circumstances for the formation of measured compounds. **Publication III** intended to determine the influence of genotype and duration of cold storage on the stability of compositional characteristics of beetroot genotypes grown organically in Southwestern Germany. In this regard, 36 beetroot genotypes were assessed considering their TDMC, TPC, betalains, nitrate, and total soluble sugars after harvest and after cold storage of one and four months. Similar to the results of Publication I and II, a significant effect of genotype on all tested compounds was found. Moreover, it was noted that the storage period affected the TDMC, nitrate, and TPC significantly.

With drawing different perspectives, the general discussion chapter provides a more profound insight into the findings of Publication I-III. As formerly mentioned, one of the overall project's goals was breeding open-pollinated beetroot genotypes using the traditional method of single plant selection. Section 6.1. will give a comprehensive overview of the preliminary results of the breeding process of the selected genotypes carried out by the project partner Kultursaat e.V. Besides the main objectives of adaptation of the genotypes to the specific growing conditions of organic farming, promising yield, and high content of health-benefiting compounds, due to the importance of taste for the consumers, the present thesis also took the sensory properties of selected genotypes into account. In addition, concerning the European Green Deal aim of reducing the amount of used fertilisers in agriculture by 20% by the year 2030 [93], the use of nitrogen in vegetable cultivation systems should be more efficient to prevent exceeding the defined limits for nitrogen surpluses and decrease the possible harmful impact of nitrogen losses on the environment [94]. In this regard, a fertilisation trial was carried out to appraise the impact of fertilisation on the selected compounds. The experiment aimed to evaluate the effect of nitrogen fertilisation on the nitrate level in harvested beetroots and examine whether the genetic potential of the varieties and selection of a suitable genotype can be further criteria to reach the intended nitrate levels in beetroots without further increases in nitrogen fertilisation levels. Further, with the use of open-pollinated genotypes, the biodiversity strategy of the European Green Deal can be covered in terms of restoring old varieties. Moreover, the use of open-pollinated genotypes leads to applying less input due to the self-reproducibility of the seeds for the next cultivation season.

Furthermore, in order to be able to implement the outcomes of this study into practice, this thesis intended to provide a list of open-pollinating beetroot genotypes with prominent agronomic and compositional qualities similar to already commercially used hybrids to present them to the farmers and organic food industry as an alternative of hybrid genotypes.

6.1. Breeding

As stated in Section 1.5., one of the main objectives of the Beta-Divers project, from which this thesis was derived, was the breeding of open-pollinated beetroot varieties. Kultursaat e.V., one of the main grantees of this research project, has diverse practical and theoretical experiences in the organic breeding of open-pollinated vegetable varieties. This association has already introduced a large number of promising breeding lines for different areas of use, including three officially approved beetroot varieties, namely Robuschka, Jannis, and Ronjana. In addition, Kultursaat e.V. has extensive and long-standing experience in the field of human-sensory testing of various vegetables, including beetroot. Generally, in the on-farm breeding of beetroot, different attributes of single plants are assessed during the two-year cultivation of seed gaining. After the first harvest, beets are first agronomically pre-selected and stored in cooling chambers. During winter, beets are individually sampled, one longitudinal segment (roughly one-third of the beet) is taken from each beet, and various sensory attributes are evaluated according to a 1-9-point scale. Accordingly, planting and flowering in the next cultivation season are achieved using positively selected specimens, which in the single plant selection are harvested individually, but in a group with the same pollen cloud, come to seeding.

Within the framework of this joint research project funded under the guidelines of the BLE innovation program, it was hypothesised that in the investigated genotype assortment in this work, a high genetic variability exists concerning agronomic and organoleptic traits. These traits can be used in a breeding program to serve different directions of utilization. The breeding took place on two breeding stations of Kultursaat e.V.: De Beersche Hoeve (Oostelbeers, Netherlands), and Horticulture station Heinze (Bingenheim, Hessia, Germany), from 2017 to 2019. A detailed description regarding the breeding locations, including their geographical coordinates, is indicated in Section 1.6. The breeding method was set as single plant selection with progeny testing and positive mass selection on two initial populations per breeding location, genotypes BoRu1 and Burpees Golden in Horticulture station Heinze, and genotypes Nobol and Bona in De Beersche Hoeve. Within the breeding procedure of the four breeding lines of interest, in addition to the cultivation and seed production, the betalain, nitrate, and total phenolic content of every single plant were measured in the context of a master thesis [[95]] at the University of Hohenheim. Elite beets samples were taken from three red beetroot breeding lines, namely Nobol (n=45), Bona (n=45), and BoRu1 (n=35), and one yellow breeding line Burpees Golden (n=35).

A longitudinal cut of the beet, including the peel and flesh, was received from every single plant after the first harvest. The sample preparation for analysis and quantitation of nitrate, betalains, and total phenolic content was conducted as described in Publication II. As the single plants had to be stored for replanting in the subsequent cultivation for seed production, the available beet sample per plant for laboratory analysis was limited. Therefore, the analysis was conducted with one replication. Due to the lack of true replication, the significance difference test between the single plants could not be performed. Therefore, descriptive statistics were used for analysing the data. Besides the measured value of the mentioned compounds for every single plant, the minimum, maximum, and mean were calculated for each genotype, which showed the differences between the single plants. The results of the compound analysis and the sensory evaluation of the single plants were compared together, and the elite beets were brought into a group for the following cultivation season for seed production.

Figures 2-4 illustrate the overall results of the determination of the bioactive compounds of the 160 single beetroot plants, which were assessed to select the elite beets for replantation and seed production. The boxplots of betacyanin and betaxanthin contents exhibited Nobol as the breeding line with the highest and the yellow-coloured breeding line, Burpees Golden, with the lowest mean values (Figure 2). Furthermore, the distribution of betacyanin and betaxanthin values were similar between Nobol, Bona, and BoRu1, whilst Burpees Golden had a very narrow distribution.

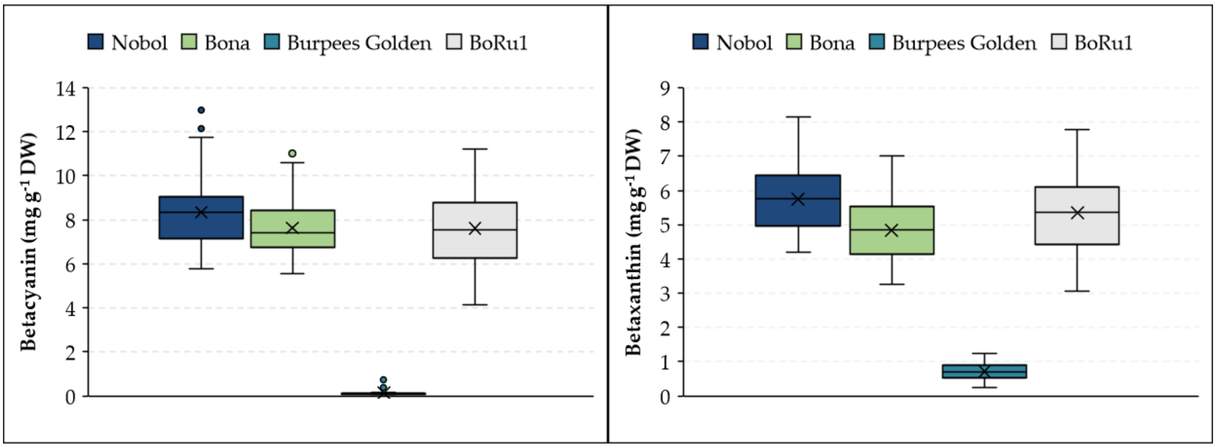

Figure 2: Boxplot of betacyanin (left) and betaxanthin (right) contents of four beetroot breeding lines. Overall 160 samples were tested (Nobol: 45; Bona: 45; Burpees Golden: 35; BoRu1: 35). The box expresses 50% of the observations, and the whiskers demonstrate the 2nd and 98th percentile of the observations. The multiplication sign displays the mean, and the vertical line in each box indicates the median. Outlier values are shown as small circles (modified after Grammenou [95]).

Corresponding to the outcomes of betacyanin and betaxanthin analyses, Nobol possessed the highest TPC mean value; however, the maximum measured value was found in the breeding line Bona (Figure 3). The boxplot demonstrated that the distribution of TPC values was relatively similar between Nobol, Bona, and BoRu1, whereas, Burpees Golden indicated a very narrow distribution.

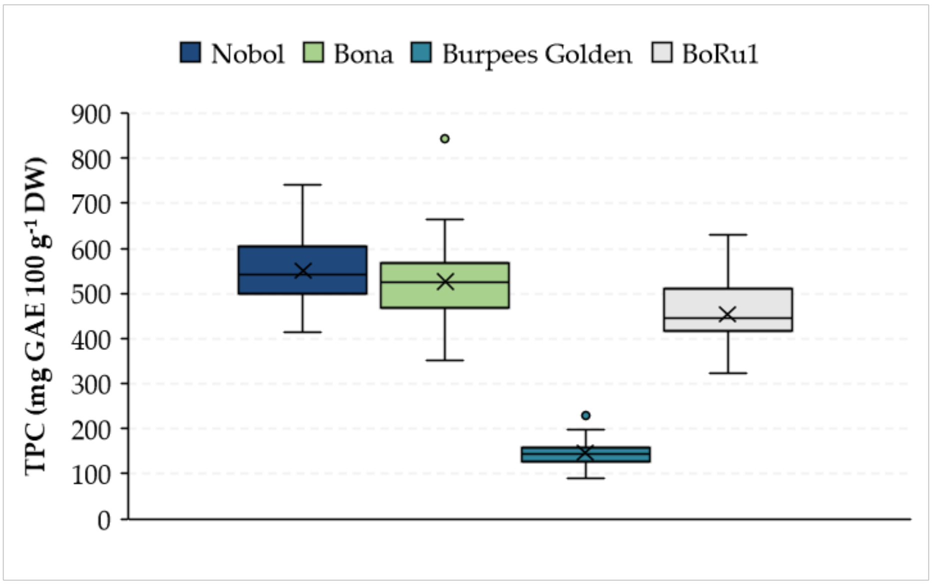

Figure 3: Boxplot of total phenolic content (TPC) of four beetroot breeding lines. Overall 160 samples were tested (Nobol: 45; Bona: 45; Burpees Golden: 35; BoRu1: 35). The box expresses 50% of the observations, and the whiskers demonstrate the 2nd and 98th percentile of the observations. The multiplication sign displays the mean, and the vertical line in each box indicates the median. Outlier values are shown as small circles (modified after Grammenou [95]).

The boxplot of nitrate content indicated that the highest mean value belonged to the yellow-coloured breeding line Burpees Golden, while BoRu1 possessed the lowest mean value (Figure 4). The distribution of values was high for all investigated breeding lines, particularly in the case of Burpees Golden.

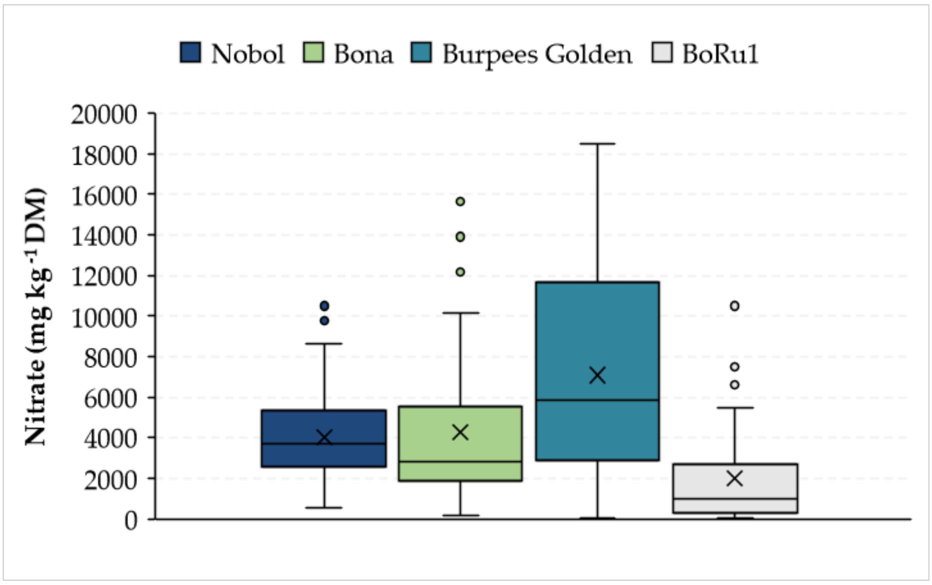

Figure 4: Boxplot of nitrate content of four beetroot breeding lines. Overall 160 samples were tested (Nobol: 45; Bona: 45; Burpees Golden: 35; BoRu1: 35). The box expresses 50% of the observations, and the whiskers demonstrate the 2nd and 98th percentile of the observations. The multiplication sign displays the mean, and the vertical line in each box indicates the median. Outlier values are shown as small circles (modified after Grammenou [95]).

BoRu1 is a breeding line originating from Bolivar, the typical variety used in organic juice production. According to the outcomes of the genotype-screening experiment, the breeding line BoRu1 was outstanding due to its high yield (Publication I) and very low nitrate content (publication II) potential, which are desirable features for juice production. Eight individual plant progenies were identified based on individual plant selection with testing of the progenies, which performed remarkably in terms of yield, beet morphological properties, and taste characteristics. This breeding line indicated a high potential to be developed into a low-nitrate, good-tasting variety for juice production. Burpees Golden is originally a round yellow-coloured beetroot genotype. Hitherto, no open-pollinated varieties in this category meet the requirements of organic commercial cultivation. Burpees Golden depicted a rather low vigour, relatively high susceptibility to diseases, and heterogeneous beet shape in the genotype-screening experiment (Publication I) as well as in the course of breeding and sensory characteristics. Furthermore, this breeding line indicated a high storage loss during winter; therefore, the seed yield was low in the following cultivation season. Thus, the single plant selection method was considered unsuitable for this breeding line due to the high loss rate in seed production. However, sufficient seeds could be obtained for further breeding using positive mass selection.

At the breeding location De Beersche Hoeve, the genotypes Nobol and Bona were categorized into three groups according to leaf characteristics (curved leaf, dark green leaf, no obvious leaf characteristics). This grouping aimed to investigate whether any correlations exist between leaf characteristics and compounds, especially betalain content. In the case of any possible correlations, selecting the single plant possessing the corresponding compounds directly in the field would be possible without any time-consuming laboratory analyses. In the case of the breeding line Nobol, based on the laboratory analyses of the selected individual beet in 2018, correlations between specific leaf characteristics and content of particular compounds could be assumed. However, the data basis must certainly be further expanded in order to be able to assure this statement.

Based on the overall outcome of the genotype-screening experiment (Publication I – III), four additional potential genotypes per breeding location were identified from all investigated varieties and selected for further breeding using positive mass selection. The white-coloured Sniezna Kula and cylindrical-shaped Carillon RZ were evaluated as high-potential genotypes in the section of particular types and are planned to be developed as a niche product. Further selected populations were Betina, Akela, Bordo, Cervena Kulata, Jawor, and Detroit 3, which were evaluated as high-potential genotypes. Except Betina, which was discarded as it did not show any compatibility in the first year of the breeding experiment, other breeding lines have been pursued in breeding.

Table 2 summarises the breeding experiment, conducted on two on-farm breeding locations of Kultursaat e.V. between 2017-2019. In 2017, beetroots were ranked based on their morphological traits after the harvest and sensory properties, and compound analysis was carried out for the selected elite beets. In 2019, the first progenies were assessed regarding their yield, beet, and foliage morphology, as well as their sensory properties.

Table 2: Initial populations selected at the Horticulture station Heinze and De Beersche Hoeve within the breeding experiment of 2017-2019, using single plant selection and progeny testing as well as positive mass selection

Breeding location	Breeding line	Year	Breeding tasks
Horticulture station Heinze	BoRu1	2017	Cultivation, selection of 35 individual beets plus positive mass selection, overwintering of elite beets
		2018	Seed collection from 32 individual beets and positive mass selection
		2019	Cultivation testing of 32 single plant progenies and positive mass selection
	Burpees Golden	2017	Cultivation, selection of 35 individual beets plus positive mass selection, overwintering of elite beets
		2018	Seed collection from 29 individual beets and positive mass selection
		2019	Cultivation testing of 23 single plant progenies and positive mass selection
De Beersche Hoeve	Nobol	2017	Cultivation, selection of 45 individual beets plus positive mass selection, overwintering of individual beets
		2018	Seed collection from 38 individual beets and positive mass selection
		2019	Cultivation testing of 24 selected individual plant progenies and positive mass selection
	Bona	2017	Cultivation, selection of 45 individual beets plus positive mass selection, overwintering of individual beets
		2018	Seed collection from 29 individual beets and positive mass selection
		2019	Cultivation testing of 24 selected single plant progenies and positive mass selection

6.2. Sensory quality

In order to select genotypes for further breeding procedures and fulfil the consumers' expectations, additional characteristics like sensory quality should be taken into account. One of the consumers' expectations from organic vegetables is good taste [96], which is associated with sensory properties [97]. Evaluation of sensory attributes is important since sensory traits are key factors affecting consumers' purchasing decisions [98].

Therefore, to investigate the consumer acceptability of selected open-pollinated genotypes studied in this work and compare their potentials with the commercial hybrid genotypes, three sensory tests (a hedonic test with a 0-10 line-scale) with semi-trained panels took place at the University of Hohenheim. The sensory tests aimed to discover consumers' perception of the desired beetroot taste characteristics and the degree of acceptability of the selected genotypes. The panels were first trained on how to carry out the evaluation. In order to simplify the rating process, one reference sample, which was already rated based on its sweetness, bitterness, earthy taste, and aroma intensity, was provided to the participant. The panels evaluated beet juices, which were prepared 48 hours before the test, acidified with lactic acid to a pH= 4.2 - 4.4, and stored in the refrigerator. The juices were from three open-pollinated genotypes: Robuschka, Nochowski, Jannis, and two hybrids: Boro F1 and Monty RZ F1. Five characteristics were evaluated for each sample, including sweetness, aroma intensity, bitterness, earthy flavour, and overall acceptability. The number of participants in the hedonic sensory tests were n=33, n=10, and n=17, respectively.

Data from all three sensory tests were jointly analysed using a mixed model approach. The model can be described by:

$$y_{ijkl} = \mu + g_i + t_j + p_{jk} + o_l + e_{ijkl}, \quad (1)$$

where y_{ijkl} is the observation of genotype *parliament* by person *k* in the in sensory test *j* in the order *l*. μ is the intercept, g_i is the fixed effect of genotype *I*, t_j is the fixed effect of sensory test *j*, p_{jk} is the random effect of person k at sensory test j , and o_l is the random effect of order *l*. e_{ijkl} is the error of y_{ijkl} with first-order autoregressive plus nugget variance-covariance structure for y_{ijkl}.

In accordance with the statistical analysis results, the effect of genotype on the sensory attributes was only significant in terms of sweetness and earthy flavour (Table 3). As each sensory test was carried out with different participants, the effect of the sensory test was analysed as well. In this regard, a significant influence was seen only in the case of earthy flavour (Table 3). Generally seen, the studied open-pollinated genotypes indicated more sweetness and less bitterness compared to the F1 hybrid varieties (Figure 5).

Table 3: Analysis of variance (ANOVA) of the outcomes of the organoleptic assessment (0-10 scale) of five beetroot cultivars in terms of different attributes, including sweetness, aroma intensity, bitterness, earthy flavour, overall acceptability

Factors	***p*-value of the F-test of the corresponding factor**				
	Sweetness	**Aroma intensity**	**Bitterness**	**Earthy flavour**	**Overall acceptability**
Genotype	< 0.0001	0.7892	0.1771	0.0022	0.1554
Sensory test	0.6588	0.9318	0.3101	0.0017	0.3487

Bach et al. [99] conducted a sensory test using five beetroot varieties, including two cylindrical-shaped red genotypes Taunus and Rocket, the round red variety Pablo F1, the white-red Chioggia, and the yellow Burpees Golden, in raw, boiled, and fried forms. The panels were semi-trained consumers consisting of 49 participants. It was reported that distinguishing aroma and flavour differences between different genotypes in raw beetroot was easier than after frying or boiling. Moreover, one of the important outcomes of the sensory test was the negative correlation between bitterness and appropriateness in raw beetroot [99]. The defined appropriateness term included all sensory properties of beetroot, such as appearance, texture, aroma, and flavour. The outcome of our sensory trial indicated the highest bitterness intensity in the F1 hybrid genotypes, Monty RZ F1 and Boro F1 (see Figure 5). Although these two genotypes are used commercially due to their high yield and beet-shape uniformity, as they are evaluated as having more bitter taste than the other studied genotypes, they have less sensory appropriateness.

In order to appraise the sensory differences between the open-pollinated and F1 hybrids genotypes, the chemical-analytical results of the taste-relevant compounds should also be taken into account. Based on the results of our measurements (as shown in Publication II), higher sugar contents, in comparison with Monty F1 and Boro F1, were found in Nochowski and Robuschka, which confirms the results of sensory evaluation. Moreover, two years of cultivation of the genotype Robuschka in 2018 and 2019 at the research station Kleinhohenheim indicated total and marketable yields of 54.09 ± 4.01 t ha^{-1} and 46.02 ± 4.44 t ha^{-1}, respectively. Compared with the results shown in Publication I, this genotype was among the genotypes with the highest total and marketable yields. Therefore, this genotype has a high potential for use in the organic market, where high yield and high sugar content are of interest. Nevertheless, there is a potential for further investigation, such as assessing firmness or determining other taste-relevant compounds like geosmin (see Section 6.5.).

The specific earthy aroma and flavour of beetroot result from the volatile bicyclic alcohol geosmin (*trans*-1,10-dimethyl-*trans*-(9)-decalol) [99]. Geosmin leads to an earthy off-odour in fish, wine, water, and dried beans, as well [100]. The concentration of geosmin in raw beetroot is between 9.7 - 26.7 $\mu g kg^{-1}$ [101], and in cooked beetroot, only 0.3% of the total volatiles belong to geosmin [102]. Although these amounts are considered low, as geosmin has a relatively low odour threshold value (for instance, in water 0.01–0.02 μgL⁻¹), its impact on aroma and flavour impression is remarkable [99].

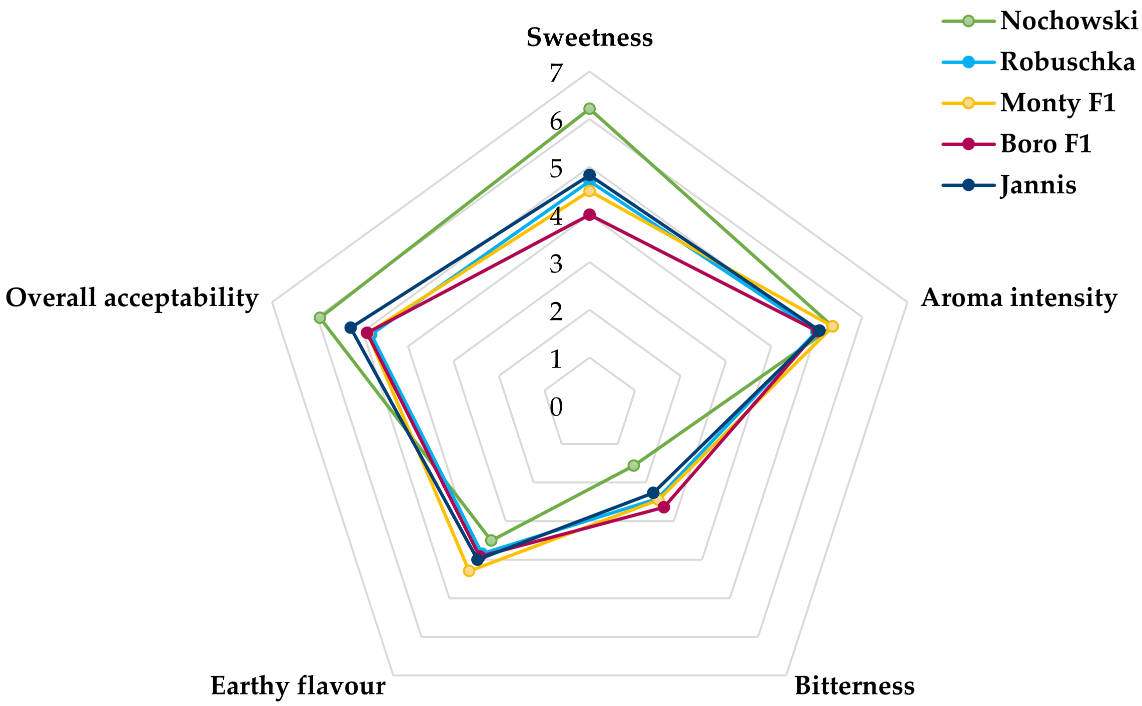

Figure 5: Mean values of organoleptic evaluation (0-10 scale) of five beetroot cultivars in terms of different attributes, including sweetness, Aroma intensity, bitterness, earthy flavour, overall acceptability (scale 0= "low intensity", 10= "high intensity").

Different aspects, such as social, environmental, and economic factors, as well as society's awareness of healthy and nutritional food, can influence consumer food choices. In addition, the sensory properties of food products can highly affect consumer perceptions and, consequently, acceptability of them [103]. One of the possibilities for increasing the intake of vegetables and fruits is developing new food products, which are enriched with fruits and vegetables [7]. In this regard, the daily intake of fibres and phytochemicals through natural components in society can increase. Bread is one of the common examples of a staple food, which is consumed by roughly 95% of adults in western countries. Thus, vegetable-enriched bread can enhance exploiting the health benefits of vegetables [7]. A study by Hobbs et al. [104] indicated that consuming 200 g bread enriched with 100 g red or white beetroot significantly decreased blood pressure in healthy males, caused by the significant increase in the concentrations of urinary nitrite and nitrate.

Substitution of four different proportions of beetroot powder (2.5, 5.0, 7.5, and 10%) with wheat flour (72% in the control sample) in cupcakes was investigated by Alshehry [105]. The substitution of 10% beetroot powder resulted in the best physical (texture), sensory (taste and colour of crust and crumbs), and microbial (total bacteria and fungi counts after three weeks of storage) properties. Therefore, the typically used flour in bakery products, like cupcakes, can be replaced by up to 10 % red beetroot powder without adversely influencing its quality. In addition, the study of Lucky et al. [106], in which different formulations of beetroot powders (0, 5, 10, and 20% w/w) were examined, reported not only a better colour, flavour, texture, and overall acceptability of cake supplemented with 15% beetroot powder, but also a significant improvement of nutritious quality compared to cake without beetroot powder.

In the case of flour-based Asian noodles, the substitution of beetroot could reach a higher acceptable level. Cooking qualities, pasting characteristics, phytochemical, colour, and sensory properties of beetroot-incorporated Asian noodles using different beetroot pulp formulations (0, 10, 20, 30, and 40 percent) were investigated by Chhikara et al. [107]. The noodles incorporated with beetroot pulps depicted higher antioxidants, total phenols, betalains, and fibre contents. The best outcomes considering all investigated aspects belonged to the 30% formulation of beetroot pulp, which resulted in healthier noodles with attractive colour, and improved nutritional properties.

To conclude, the examples mentioned above reveal the importance of the formulation and degree of the incorporation of beetroot, as an example of health-benefiting vegetables, depending on the final products. Therefore, in the production of value-added products, besides considering the amount of health-promoting compounds in the final products, sensory characteristics and acceptance of the products by consumers should be taken into consideration.

6.3. Fertilisation trials

In general, nitrate content in vegetables is considered a disputable topic. In subsection 1.2.2., the positive impact of dietary nitrate, which is associated with its remarkable contribution to the formation of nitric oxide, was discussed [108]. This resulted in the growing popularity and demand for beetroot with high nitrate content as a sports nutrition supplement [54]. However, there is concern regarding the issue that reduction of nitrate to nitrite results in methemoglobinemia, which its reaction with amines and amides can in sequence form carcinogenic N-nitrosamines [109]. Infants under three months are the most vulnerable to methemoglobinemia [110]. Since vegetables are one of the main ingredients of babies' diets, precautionary measures and close monitoring of baby food is needed [111]. In this regard, in order to prevent exceeding the ADI level, beetroots with low nitrate content are demanded in baby food production. Consequently, aligned with the intended final product, the desired content of nitrate in beetroot notably varies.

In order to assess the possible influence of nitrogen (N) fertilisation on nitrate and sugar contents in the harvested beets in the different genotypes, in addition to the genotype-screening experiment (see Section 1.6.), a fertilisation experiment was set up. Hereof, the N uptake of three genotypes, namely Borus, Ronjana, and Regulski Okragly, was studied with four N-fertilisation levels (0, 50, 100, 150 kg N ha^{-1}) within two years at the experimental station Kleinhohenheim, Stuttgart, Germany. The trials were set up as an Alpha-design, and each genotype was cultivated in three replicates. The preceding crop in both trials was clover grass. The plot size was 14 m^2 containing four rows in each plot. An inter-row spacing of 35 cm and a spacing of 5.9 cm in the row were considered. Sowing was done using a pneumatic precision seed drill (Sfoggia, Italy). In 2018, sowing was carried out on 06.06.2018, and the plants were harvested after a cultivation period of 97 days. In 2019, sowing was done on 16.07.2019, and the harvest took place after a cultivation period of 83 days. In 2018, the mean temperature was 19.0 °C during the growth period, and the mean rainfall reached 38.2 mm. While in 2019, the average monthly temperature in Kleinhohenheim during the cultivation period was 16.3 °C, and there was an average rainfall of 59.47 mm per month.

In order to provide the defined N-level of 50, 100, and 150 kg N ha^{-1}, an organically certified fertiliser, Maltaflor BIO Fine Granules (Maltaflor, Andernach, Germany) was applied in the amounts of 1.75, 3.5, and 5.25 kg per plot, respectively, considering the plot size of 14 m^2 and nitrogen content of 4% in Maltaflor. The fertiliser was applied directly before sowing.

The nutrient content of Maltaflor Bio is as follow:

- N = ca. 4%,
- P_2O_5 = ca. 1%,
- K_2O = ca. 5%
- Organic matter: ca. 80%.

The most important vegetable raw substances of Maltaflor are malt germs, the young taproots of freshly germinated cereals, and Vinasse, a by-product from sugar beet processing. The stimulating effect of the malt germs has been proven to lead to significantly improved root growth in the treated plants and, thus, to more intensive nutrient uptake. Maltaflor is a slow-flowing fertiliser, which its onset effect is after three weeks.

The sample preparation for analysis of the compounds and quantification of total soluble sugar and nitrate contents was performed as described precisely in Publication II. The analysis was conducted in two replicates. For the statistical analysis, the data from both years and experiments were analysed together using a mixed model approach. The model can be explained by:

$$y_{ijklm} = \mu + \tau_i + \varphi_j + a_k + (\tau\varphi)_{ij} + (\tau a)_{ik} + (\varphi a)_{jk} + (\tau\varphi a)_{ijk} + r_{kl} + b_{klm} + e_{ijklm}, \quad (2)$$

where y_{ijklm} is the observation of genotype i after fertilisation level j in the m incomplete block of replicate l in year k. μ is the intercept, τ_i is the fixed effect of genotype i, φ_j is the fixed effect of the jth fertilisation level, a_k is the fixed effect of the kth year. $(\tau\varphi)_{ij}$, $(\tau a)_{ik}$, and $(\tau\varphi a)_{ijk}$ are the random interaction effects of the corresponding main effects, $(\varphi a)_{jk}$ is the fixed effect of fertilisation level j and year k, and r_{kl}, and b_{klm} are the fixed effects of replicate l in year k and the random effects of incomplete blocks m within replicate k and year l. e_{ijklm} is the error of y_{ijklm} with year-specific variances.

The outcomes indicated no significant influence of the N fertilisation rate on the total soluble sugar content (Table 4). This is in agreement with the findings of Hanson and Goldman [[112]], who reported no significant impact of fertiliser on total soluble sugars. However, Chhikara et al. [[15]] stated that nitrogen availability and the nitrogen supply in the early stages of growth influence the sugar content of beetroot. With increasing N-fertiliser quantity, neither a constant increasing nor a decreasing trend in total soluble sugar content was observed (Figure 6). The impact of the interactions between year and replication on total soluble sugar content was significant. Determination of total soluble sugars within the genotype-screening experiment in Publication II indicated a significant effect of year on the total soluble sugar contents [[113]].

Table 4: ANOVA of the results of total soluble sugars and nitrate content of three different beet genotypes grown in 2018 and 2019 in research station Kleinhohenheim

Factor	p-value of the F-test of the corresponding factor	
	Total soluble sugars	**Nitrate**
Genotype	n.s.*	n.s.
Fertilisation level	n.s.	0.0004
Genotype × fertilisation level	n.s.	n.s.
Year × replication	0.0125	<0.0001

* not significant

In terms of nitrate content, significant impacts of fertilisation level and interactions between year and replication were noted (Table 4). This is in line with the outcomes of Kosson et al. [[114]], who reported a significant influence of year on the nitrate content in two beetroot genotypes, and Ugrinović et al. [[115]], who noted a significant impact of N fertilisation on the content of N in different beetroot parts.

An increasing N fertiliser quantity up to 100 kg N ha^{-1} led to significantly increasing mean values of nitrate content of all three investigated genotypes. Nevertheless, with increasing N fertiliser level to 150 kg N ha^{-1} a slight decrease in the nitrate content was observed; however, the change was not significant.

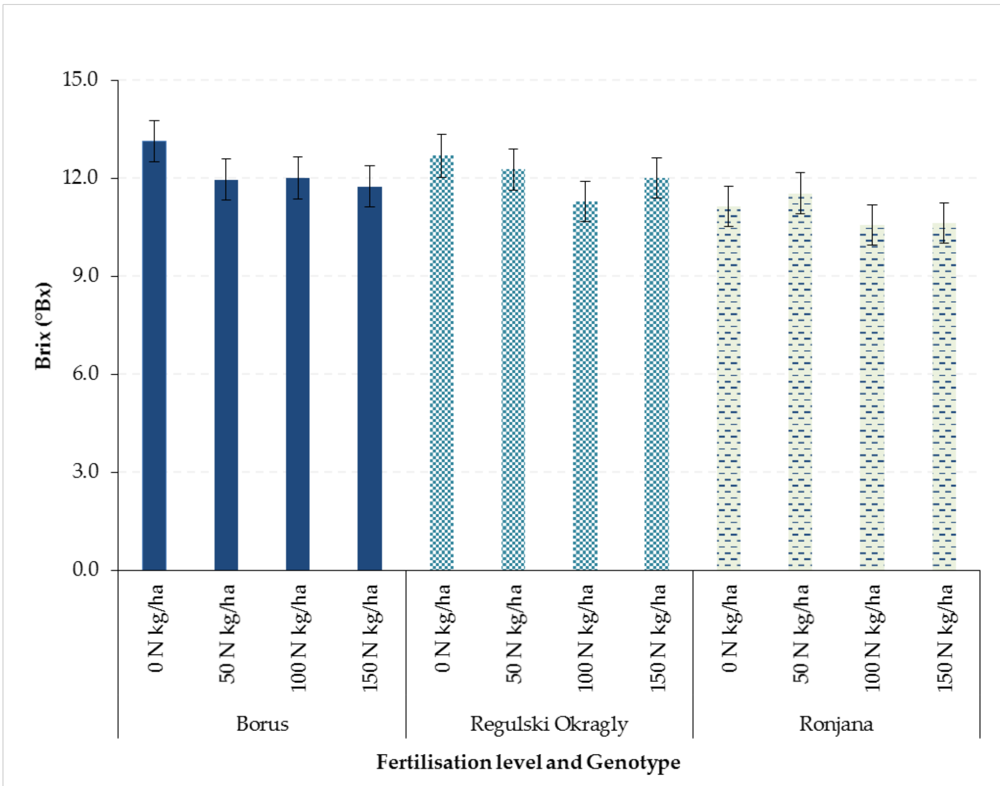

Figure 6: Mean values of total soluble sugar content (°Bx) of three beetroot genotypes cultivated at the experimental station Kleinhohenheim in 2018 and 2019. Results signify the mean values ± standard error. (Means shown in the graph are based on the genotype × fertilization interactions. As neither the effects of interactions nor the main factors were significant, no letter display is possible statistically)

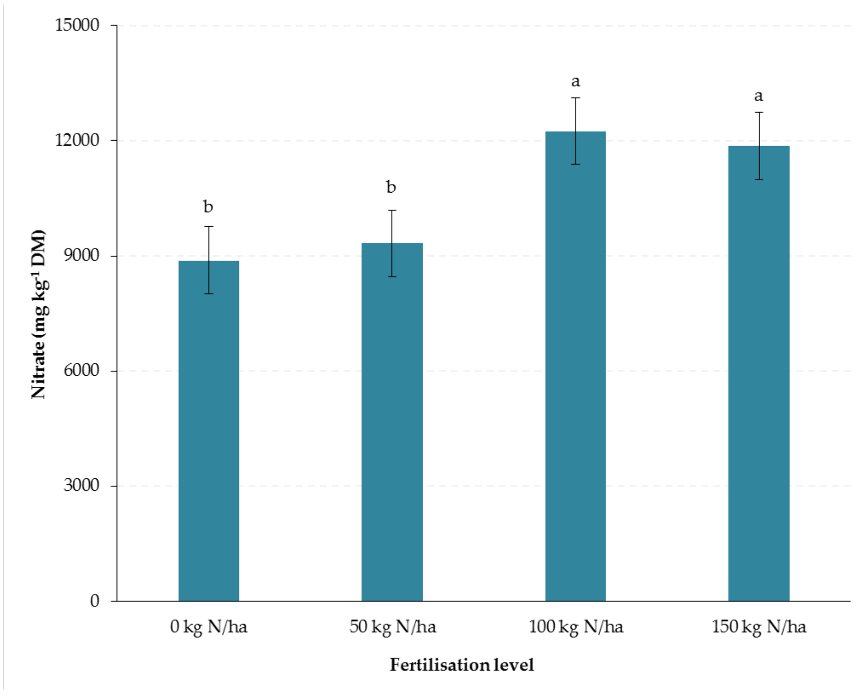

Figure 7: Mean values of nitrate content (mg kg^{-1} DM) of all three beetroot genotypes cultivated at the experimental station Kleinhohenheim in 2018 and 2019. Results signify the mean values ± standard error. (Means shown in the graph are based on the effect of fertilization level due to its significant impact on the nitrate content). Means covered with at least one identical letter do not significantly differ.

In summary, the results of this project exhibited that with adjusted N fertilisation, the amount of specific compounds in beetroot could be directed in the desired direction based on the intended product (e.g., sports drink with high nitrate levels, baby food with low nitrate levels). Nevertheless, the findings of this thesis disclosed the high genetic variability in relation to the amount of bioactive compounds. In this regard, based on the results shown in Publication II and III, in the pool of the investigated genotypes, some genotypes exist, which without any additional fertilisation, possess a comparable nitrate content with the highest values reached by using additional N-fertilisation in this study.

6.4. Highlighting outstanding beetroot genotypes for specific uses

Previous studies stated that the basis of producing high-quality crops is the selection of suitable genotypes, which are compatible with the given local soil and climate conditions [45,116]. Furthermore, according to the differences in the desired amount of bioactive compounds for various food products, the intended final utilization should be considered jointly with the selection of genotypes. With illustrating the significant effect of genotype on most of the examined traits, the findings of the present thesis stated high genetic variability regarding yield, morphological and compositional characteristics of beetroot. This genetic variability can provide new possibilities for farmers for the cultivation of beetroot genotypes, that align with the specific requirements of organic farming. Furthermore, introducing a wide range of beetroot genotypes with different compositional characteristics to the market is beneficial for the food industry to produce new and improve existing beetroot products with the specifications of the desired content of bioactive compounds. Accordingly, the market can meet consumers’ expectations of health-promoting food products.

Due to the differences between the number of the investigated genotypes in each research station and experimental years, 15 genotypes, which occurred in all location-by-year combinations, were considered for the selection of the outstanding genotypes. Concerning the yield results, the cylindrical-shaped Carillon RZ indicated to be the genotype with the highest total and marketable yield and share of the marketable yield. The genotypes Bona and Bordo with being among the five top genotypes in terms of total yield and possessing a high proportion of marketable beets within the total yield, demonstrated promising results. Despite showing a high total yield, the share of marketable beets compared to the total yield of the F1 hybrid genotype Monty RZ was not among the top five genotypes (Table 5).

Table 5: List of the genotypes, which performed the best among the 15 investigated beetroot genotypes grown organically in three experimental stations in 2017 and 2018, concerning three major criteria for their practical use feasibility

Major criteria		Five outstanding genotypes of the corresponding characteristic
Yield characteristics	Total Yield	Carillon RZ, Monty RZ F1, Bona, Bordo, Sniezna Kula
	Marketable Yield	Carillon RZ, Bordo, Monty RZ F1, Bona, Akela RZ
	Share of the Marketable Yield*	Carillon RZ (84.4%), Bordo (84.0%), BoRu1 (80.1%), Akela RZ (79.4%), Bona (78.6%)
Morphological characteristics	Smoothness of Skin	Sniezna Kula, Jawor, Carillon RZ, Monty RZ F1, Akela RZ
	Detachment of Root Tail	Akela RZ, Ronjana, Detroit 3, Jawor, Nobol
	Cork Formation	Sniezna Kula, Nochowski, Ronjana, Detroit 3, Bona
	Scab Incidence	Carillon RZ, Monty RZ F1, Burpees Golden, Nochowski, Jawor
	Uniformity	Sniezna Kula, Monty RZ F1, Betina, Cervena Kulata, BoRu1
	Leaves-Growth-Base Width	Carillon RZ, BoRu1, Nobol, Burpees Golden, Akela RZ
Compositional characteristics	Nitrate Content	Carillon RZ, Bona, Nobol, Akela RZ, Ronjana
	Total Soluble Sugars	Nochowski, Cervena Kulata, Betina, Ronjana, Bordo
	Betalain**	Monty RZ F1, Nochowski, Ronjana, Detroit 3, BoRu1
	Total Phenolic content	Monty RZ F1, Cervena Kulata, Nochowski, Bordo, Jawor

* To evaluate the performance of each genotype, the proportion of marketable yield in relation to the total yield was calculated.

** To evaluate the colour intensity, the betalain content was calculated by summing up the betacyanin and betaxanthin content of each genotype.

In terms of morphological characteristics, the genotype Carillon RZ was among the genotypes with a high degree of skin smoothness, the lowest scab incidence, and the smallest leaf-growth-base width (which leads to less waste during the processing). The white-coloured genotype, Sniezna Kula, indicated the smoothest skin, lowest cork formation, and the highest beet uniformity. Moreover, this genotype was among the genotypes with the highest total yield. With depicting remarkable potential regarding the agronomic performances, the genotype cylindrical-shaped Carillon RZ and the white-coloured Sniezna Kula could be options for the use and further development as niche products. The genotype Akela RZ illustrated a notable agronomic performance. More precisely, with possessing high marketable yield and its share compared to the total yield, a high degree of skin smoothness, as well as a high degree of root tail detachment and small leaf-growth-base width, this genotype is recommended for the fresh market as well as processed products (such as semi-cooked vacuumed beetroot).

Regarding the compositional characteristics, besides determining the content of the compounds of interest, the compounds' stability during the cold storage was assessed in order to identify beetroot genotypes with preserved quality for a prolonged period after harvest and benefit the functional attributes of fresh beetroot throughout the year. Considering the nitrate level, the genotypes Carillon RZ, Bona, Nobol, Akela RZ, and Ronjana, respectively, indicated the highest content. Regarding the stability of this compound, although the study's findings showed a significant influence of storage period on the nitrate level of the examined genotypes, in the case of the five mentioned genotypes with the highest nitrate amount, no significant change during the four months of cold storage was noticed. Therefore, for the beetroot products in which high nitrate content is desired (such as sport drinks), these genotypes can be used not only directly after harvest but for an extended period after the harvest. Concerning the content of total soluble sugars, the genotypes Nochowski, Cervena Kulata, Betina, Ronjana, and Bordo were among the five top genotypes with the highest total soluble sugar contents, and during the cold storage period, their content did not change significantly.

Concerning phenolic compounds, the open-pollinated genotypes Cervena Kulata, Nochowski, Bordo, and Jawor, indicated comparable contents of total phenolic compounds to Monty RZ F1, which demonstrated the highest level. Up to the cold storage period of four months, the TPC in these genotypes did not indicate a significant change, except for the genotype Nochowski, in which an increasing trend was noted. Considering the colour intensity, all five genotypes possessing the highest betalain content, namely Monty RZ F1, Nochowski, Ronjana, Detroit 3, and BoRu1, depicted colour stability during the four months of cold storage. Therefore, for using beetroot as a natural food colourant, these five genotypes are recommended as genotypes with high and stable colour intensity.

The genotype Ronjana, with high and stable content of total soluble sugars and high colour intensity, draws a lot of attention for its use in various beetroot products. The genotype

Nochowski considering its compositional characteristics (including a high level of TPC, betalains, and total soluble sugars) as well its notable sensory attributes (namely highest sweetness, aroma intensity, and overall acceptability as well as lowest bitterness and earthy flavour), appeared to be an outstanding genotype in terms of chemical and sensory qualities. This genotype can be highly recommended for use in value-added food products.

6.5. Outlook to further research needs

6.5.1. Geosmin

One of the distinct characteristics of beetroot flavour is its earthy flavour, which causes the division of liking this vegetable into two contrasting groups [117]. The volatile terpenoid geosmin is the chemical that causes the earthy flavour of beetroots. Depending on the food product or the concentration, geosmin can be considered either pleasant or undesirable [112]. In this regard, the results of a sensory test using beetroot juice indicated that a geosmin concentration above 5.8 $\mu g\ L^{-1}$ was perceived as ''too earthy to be beet-like'', whereas a geosmin concentration below 0.36 $\mu g\ L^{-1}$ was evaluated as missing beetroot-flavour [118].

The origin of geosmin has been long under discussion. As specific soil microorganisms can synthesize geosmin, it was hypothesised that the beetroot's growing environment determines the geosmin concentration of the beets. Indication of the highest geosmin content in the peel of beetroot compared to the flesh made this hypothesis stronger. By growing beetroot seeds in regular soil and comparing that with the cultivation of sterilized seeds grown in an aseptic environment, Lu et al. [119] demonstrated that geosmin originated from the beets themselves rather than the microorganism of the soil surrounding the beets. Additionally, Freidig and Goldman [120] reported a minor amount of variance for the influence of year and replicate within the year in the content of geosmin in beetroot. That further supports the endogenous synthesis of geosmin. Therefore, the geosmin level in beetroot can be primarily cultivar-specific [112]; however, the impact of the growing environment should not be neglected. While higher geosmin content of beetroots grown in non-autoclaved compared to autoclaved soil depicted that in non-autoclaved soil with a larger microbial count, the possibility of relationships between beets and geosmin-producing microorganisms, for instance, *Streptomyces* soil bacteria, exists [120].

In their recent study, Hanson et al. [121] approved the evidence of the genetic basis of geosmin amount in beetroot. Within the first molecular genetic mapping trial in beetroot, Hanson et al. [121] detected two quantitative trait locus (QTL) for geosmin concentration in beetroot, which offers the basis for further research of chromosome 8 with direct gene identification strategies. This might lead to locating one or more genes encoding the synthesis of geosmin in beetroot and permitting molecular markers for this feature. In order to increase the plausibility and market potential of beetroot and its products, regarding its perceived and accepted flavour, the physicochemical composition of the beetroot products should be adjusted based on consumer preference. As geosmin is one of the main compounds associated with the flavour of beetroot, further investigation into consumer preference for geosmin content is required. One of the options for adjusting the geosmin concentration might be to manipulate its content with further breeding and use the genetic potential of different beetroot genotypes. Furthermore, previous studies reported different treatments resulting in geosmin removal. Electrochemical degradation indicated high effectiveness in removing geosmin without the significant need for economic and technological equipment [122]. This method can be of interest for the removal of geosmin in both liquid and solid food products, as most of the treatments, which are often used, are mainly designed for liquids [122].

6.5.2. Oxalic acid

Besides many health-benefiting compounds, beetroot contains harmful components like oxalic acid. Oxalic acid ($C_2O_4^{2-}$) is a metal ion chelator, which interferes with calcium, and iron metabolism and, therefore, reduces the bioavailability of these nutrients. Accordingly, it can result in the formation of nephrolithiasis and consequently increase the risk of kidney stones in predisposed people [123,124]. Oxalic acid can be found in many frequently eaten foods, like chocolate, spinach (*Spinacia oleracea*), and tea (*Camellia sinensis*) [125]. However, beetroots have been considered as a high oxalate food compared to other fruits and vegetables [40]. The content of oxalic acid in beetroot is reported to be between 400–600 mg/100 g fresh weight [126]. Since oxalic acid naturally occurs in food, there is no generally defined ADI. However, the European Agency for the Evaluation of Medicinal Products reported the estimated daily dietary intake of oxalic acid as a range of 50–500 mg/day, or 0.8–8.3 mg/kg per day for a person weighing 60 kg [127]. Moreover, a daily intake of 500 mg of oxalic acid is reported by Noonan [128] to pose no health risk. To be more precise, for healthy humans, normal consumption of fresh or juiced beetroot (less than 1 L per day) with the reported oxalic acid range in beetroot can be considered harmless [40]. Nevertheless, due to its relationship with kidney stone formation and anti-nutritive properties [125], beetroots' excessive consummation may negatively affect human health. Therefore, having information on the oxalic acid content in beetroot and its product is of interest.

The concentration of oxalic acid can vary due to the environmental conditions, cultivar, growth rate, age, and type of plant tissue [125,129,130]. Ugrinović et al. [115] reported significant differences between the oxalic acid content in different parts of beetroot. In this regard, the leaf lamina had the highest level of oxalic acid, and the beet possessed the lowest content.

In this study, the focus was on the bioactive and health-promoting compounds of different beetroot genotypes. However, due to the possible negative impact of oxalic acid on human health in the case of excessive consumption, determining the oxalic acid level of the studied genotypes is required. Furthermore, due to the point that beetroot, in general, is considered a food with a high oxalic acid content, further breeding to reduce oxalic acid is of importance. As there are still various open-pollinated beetroot genotypes rather than hybrids available, the potential source of genetic diversity for further breeding exists. In addition, as it was reported that the variation in oxalic acid content might also exist within a single cultivar [125], the single-plant selection breeding method can be recommended as it was used in this study.

6.5.3. Storage condition

As described in Publication III, the freshly harvested beetroots were stored for a duration of four months in a simple cooling chamber at 6 °C, with no control over atmosphere parameters. In this study, as the focus was on screening the new and existing genotypes and their quality stability during the storage, rather than the effect of storage condition on preserving the quality, one of the most accessible and prevalent storing practices, cold or refrigerated storage, was used. Howbeit, a controlled atmosphere is generally accepted as an ideal storage technology for storing vegetables with longer preservation of bioactive compounds [87]. In this regard, Viskelis et al. [86] investigated the optimal storing condition for 11 beetroot genotypes to examine the stability of their chemical composition in different storing conditions and, consequently, their suitability for processing. Four genotypes of Detroit 2, Boro F1, Pablo F1, and Bona were in common with the tested genotype in this work. The results displayed that an increase in carbon dioxide level and a decrease in oxygen content positively influence the chemical characteristics of the stored beetroots. At the temperature of 1 ± 1° C and the relative humidity 90–95%, the optimum composition of the controlled atmosphere for storage of studied beetroot genotypes was reported as 5% oxygen, 5% carbon dioxide, and 90% nitrogen, which leads to approximately 10% increase in production compared to simple cold storage [86]. Therefore, to maximize the use of the functional characteristics of fresh beetroot all year round, besides considering the genetic potentials, the external factors such as storage conditions should be optimally set up as well.

7. Summary

The constant increase in awareness of the relationship between health and diet changed consumers' perception of food and, accordingly, their food products' choices. In this regard, the demand for foods, which promote mental and physical health and prevent specific diseases, has increased. Vegetables and fruits, with containing health-benefiting phytochemicals such as antioxidants and a high amount of vitamins and minerals, are highly recommended in daily diet. The growing attention toward consuming plant-based instead of animal-based foods led to a dietary shift and a rising demand for such products. Nevertheless, besides meeting the consumers' requirements, a sustainable and environmentally friendly production system should be taken into account. One of the solutions is to further develop well-performed health-promoting vegetable varieties, which can be grown regionally.

Due to its high amount of bioactive compounds, which permits the reformulation of conventional products and transformation of them into functional foods, beetroot (*Beta vulgaris* L. subsp. *vulgaris*) was classified as a superfood. Betalains, phenolic compounds, ascorbic acid, and carotenoids present in beetroot brought this crop among the ten vegetables with the highest antioxidant activity. Another compound of interest in beetroot is nitrate, which recently, its beneficial impacts, such as normalizing blood pressure, increasing muscle efficiency, and fatigue resistance, has gained attention for the use in specific products. Besides the impacts of environmental conditions and production processes, the contents of health-promoting compounds considerably depend on the used variety. On account of the rising demand for organic food products, there is a necessity for varieties, which are adapted to the special requirements of organic farming. In contrast with the F1 hybrid varieties, which their use in organic farming is very critical, the open-pollinated varieties demonstrated more adaptability to a broader range of environmental conditions. However, further breeding of the existing potential genotypes or restoring the neglected cultivars for adaptation of them to the specific cultivation conditions of the organic system is required. Alongside growing beetroot genotypes with desirable agronomic performances and promising contents of bioactive compounds, preserving the quality of harvested beetroots for an extended time can prolong the availability and use of this crop. Hence, affordable and easy-accessible possibilities for prolonging the shelf life of beetroot are required.

This thesis aimed to disclose the genetic potential of a broad assortment of new and existing open-pollinated beetroot genotypes, which perform desirably in terms of agronomic and morphological traits (**Publication I**), compositional characteristics (**Publication II**), and quality stability (**Publication III**) under organic farming conditions. In this respect, in total, six genotype-screening field experiments were conducted in 2017 and 2018 at three different locations. Results of the first publication depicted a significant impact of genotype on the total and marketable yields, as well as most of the assessed morphological traits, including skin

smoothness, corky surface, and beet shape uniformity. With the analysis of the contents of the total dry matter, total soluble sugar, nitrate, betalains, and total phenolic compounds in the second publication, significant differences were found between 15 studied beetroot genotypes. Moreover, with the significant influence of the interactions of year and location on all tested compounds, it was noted that the abiotic factors could affect the biochemical composition of beetroot. In addition, the outcome of the third publication demonstrated a significant effect of genotype on all measured compounds of 36 examined beetroot genotypes. Furthermore, the extent of change in the compositional quality during four months of cold storage was assessed for all studied genotypes. On account of the existing genetic variability in beetroot, it was concluded that the intended final utilization should be taken into account for the selection of suitable genotypes.

In addition to the conducted assessments in Publication I – III, in the overall project framework from which this thesis was derived, the sensory characteristics of selected open-pollinated genotypes were compared with the commercially used varieties. Three sensory tests were carried out at the University of Hohenheim to determine consumers' perception of the desired beetroot taste characteristics, including sweetness, aroma intensity, bitterness, earthy flavour, and the degree of acceptability. Generally seen, the studied open-pollinated genotypes indicated more sweetness and less bitterness compared to the F1 hybrid varieties.

Furthermore, this thesis assessed the impact of nitrogen fertilisation level on selected compounds (nitrate and total soluble sugar contents) of specific genotypes (Borus, Ronjana, and Regulski Okragly) at the University of Hohenheim in 2018 and 2019. The outcomes indicated no significant influence of the N fertilisation rate on the total soluble sugar content. However, the impacts of fertilisation level and interactions between year and replication on the nitrate content were significant. Consequently, with adjusted N fertilisation, the amount of nitrate in beetroot can be directed in the desired direction based on the intended product (for example, sport drinks with high nitrate levels, and baby food with low nitrate levels). Nevertheless, in the pool of the investigated genotypes in Publication III, some genotypes possessed a comparable nitrate content with the highest values reached by using additional N-fertilisation in this experiment.

To conclude, with the investigation of a broad assortment of beetroot genotypes, the findings of the present thesis revealed a high genetic variability regarding yield, morphological and compositional characteristics of beetroot, which provide new possibilities for farmers, the food industry, and consumers. With the betterment of the organic cultivation of beetroot using the well-performed locally adapted varieties, the production of an organic domestic superfood can be promoted. To ensure the competency of the studied genotypes, further studies concerning the determination of other taste-relevant compounds like geosmin and disputable compounds such as oxalic acid are highly recommended. Moreover, to extend the use of the

functional properties of fresh beetroot throughout the year, besides the selection of a suitable genotype, the external factors, such as storage conditions, should be optimized as well.

8. Zusammenfassung

Das wachsende Bewusstsein für den Zusammenhang zwischen Gesundheit und Ernährung hat die Wahrnehmung der Verbraucher in Bezug auf Lebensmittel und dementsprechend auch ihre Wahl der Lebensmittelprodukte verändert. In diesem Zusammenhang ist die Nachfrage nach Lebensmitteln, welche die geistige und körperliche Gesundheit fördern und bestimmten Krankheiten vorbeugen, gestiegen. Gemüse und Obst, die gesundheitsfördernde sekundäre Pflanzenstoffe wie Antioxidantien und eine große Menge an Vitaminen und Mineralstoffen enthalten, werden für die tägliche Ernährung sehr empfohlen. Die wachsende Aufmerksamkeit für den Verzehr von pflanzlichen anstelle von tierischen Lebensmitteln hat zu einer Umstellung der Ernährung und einer steigenden Nachfrage nach solchen Produkten geführt. Neben der Erfüllung der Verbraucherwünsche sollte jedoch auch ein nachhaltiges und umweltfreundliches Produktionssystem in Betracht gezogen werden. Eine der möglichen Lösungen besteht darin, gesundheitsfördernde Gemüsesorten weiterzuentwickeln, die regional angebaut werden können.

Kürzlich wurde die Rote Bete (*Beta vulgaris* L. subsp. *vulgaris*) aufgrund ihres hohen Gehalts an bioaktiven Inhaltsstoffen, die eine Neuformulierung herkömmlicher Produkte und deren Umwandlung in funktionelle Lebensmittel ermöglichen, als Superfood eingestuft. Betalaine, Phenole, Ascorbinsäure und Carotinoide, die in der Roten Bete enthalten sind, brachten diese Pflanze unter die zehn Gemüse mit der höchsten antioxidativen Aktivität. Eine weitere interessante Verbindung in der Roten Bete ist Nitrat, das in letzter Zeit aufgrund seiner positiven Auswirkungen, wie z. B. Normalisierung des Blutdrucks, Steigerung der Muskeleffizienz und Widerstandsfähigkeit gegen Ermüdung, für die Verwendung in bestimmten Produkten interessant geworden ist. Der Gehalt an gesundheitsfördernden Inhaltsstoffen hängt neben den Einflüssen der Umweltbedingungen und der Produktionsprozesse auch stark von der verwendeten Sorte ab. Aufgrund der steigenden Nachfrage nach ökologischen Lebensmitteln besteht ein Bedarf an Sorten, die an die besonderen Anforderungen des ökologischen Landbaus angepasst sind. Im Gegensatz zu den F1-Hybridsorten, deren Einsatz im ökologischen Landbau sehr kritisch gesehen wird, haben samenfeste Sorten eine größere Anpassungsfähigkeit an ein breiteres Spektrum von Umweltbedingungen gezeigt. Es ist jedoch erforderlich, die vorhandenen potenziellen Genotypen weiter zu züchten oder die vernachlässigten Sorten wiederherzustellen, um sie an die spezifischen Anbaubedingungen des ökologischen Systems anzupassen. Neben der Züchtung von Rote-Bete-Genotypen mit wünschenswerten agronomischen Leistungen und vielversprechenden Gehalten an bioaktiven Inhaltsstoffen kann die Erhaltung der Qualität der geernteten Rüben durch entsprechende Lagerung über einen längeren Zeitraum die Verfügbarkeit und Verwendung verlängern. Daher sind erschwingliche und leicht zugängliche Möglichkeiten zur Lagerung und damit der Verlängerung der Haltbarkeit von Roter Bete erforderlich.

Ziel dieser Dissertation war, das genetische Potenzial eines breiten Sortiments neuer und bestehender samenfester Rote-Bete-Genotypen hinsichtlich ihrer agronomischen und morphologischen Eigenschaften (**Publikation I**), ihrer Inhaltsstoffe (**Publikation II**) und Qualitätsmerkmale sowie Lagerfähigkeit (**Publikation III**) unter den Bedingungen des ökologischen Landbaus zu evaluieren. In diesem Zusammenhang wurden 2017 und 2018 insgesamt sechs Feldversuche zum Genotyp-Screening an drei verschiedenen Standorten durchgeführt. Die Ergebnisse der ersten Publikation zeigten einen signifikanten Einfluss des Genotyps auf den Gesamt- und den marktfähigen Ertrag sowie auf die meisten der bewerteten morphologischen Merkmale, einschließlich der Glattheit der Schale, der korkigen Oberfläche und der Einheitlichkeit der Rübenform. Bei der Analyse der Gesamttrockenmasse, der Gehalte an löslichem Gesamtzucker, Nitrat, Betalain und Gesamtphenolen in der zweiten Publikation wurden signifikante Unterschiede zwischen 15 untersuchten Rote-Bete-Genotypen festgestellt. Darüber hinaus wurde durch den signifikanten Einfluss der Wechselwirkungen zwischen Jahr und Standort auf alle untersuchten Inhaltsstoffe festgestellt, dass die abiotischen Faktoren die biochemische Zusammensetzung der Rote Bete beeinflussen können. Darüber hinaus zeigten die Ergebnisse der dritten Publikation einen signifikanten Einfluss des Genotyps auf alle gemessenen Inhaltsstoffe der 36 untersuchten Rote-Bete-Genotypen. Darüber hinaus wurde für alle untersuchten Genotypen das Ausmaß der Veränderung der Qualität der Inhaltsstoffe während einer viermonatigen Kühllagerung untersucht. Aufgrund der vorhandenen genetischen Variabilität bei Roter Bete wurde der Schluss gezogen, dass bei der Auswahl geeigneter Genotypen die beabsichtigte Endverwendung berücksichtigt werden sollte.

Zusätzlich zu den in den Publikationen I - III durchgeführten Untersuchungen wurden im Rahmen des Gesamtprojektes, aus dem diese Dissertation hervorgegangen ist, die sensorischen Eigenschaften ausgewählter samenfester Genotypen mit den kommerziell genutzten Sorten verglichen. An der Universität Hohenheim wurden drei sensorische Tests durchgeführt, um die von den Verbrauchern wahrgenommenen, gewünschten Geschmackseigenschaften der Roten Bete, darunter Süße, Aromaintensität, Bitterkeit und erdiger Geschmack, sowie den Akzeptanzgrad zu ermitteln. Im Allgemeinen wiesen die untersuchten samenfesten Genotypen im Vergleich zu den F1-Hybridsorten mehr Süße und weniger Bitterkeit auf.

Darüber hinaus wurde im Rahmen dieser Dissertation der Einfluss der Stickstoffdüngung auf ausgewählte Inhaltsstoffe (Nitrat- und Gesamtzuckergehalt) bestimmter Genotypen (Borus, Ronjana und Regulski Okragly) in den Jahren 2018 und 2019 an der Universität Hohenheim untersucht. Die Ergebnisse zeigten keinen signifikanten Einfluss der N-Düngermenge auf den Gehalt an löslichem Gesamtzucker. Allerdings waren die Auswirkungen der Düngemenge und der Interaktionen zwischen Jahr und Wiederholung auf den Nitratgehalt signifikant. Folglich kann mit einer angepassten N-Düngung die Nitratmenge in der Roten Bete je nach

Produkt in die gewünschte Richtung gelenkt werden (z.B. Sportgetränk mit hohem Nitratgehalt, Babynahrung mit niedrigem Nitratgehalt). Dennoch wiesen im Pool der untersuchten Genotypen in Publikation III einige Genotypen einen vergleichbaren Nitratgehalt auf, wobei die höchsten Werte durch eine zusätzliche N-Düngung in diesem Versuch erreicht wurden.

Zusammenfassend zeigte sich bei der Untersuchung eines breiten Sortiments von Rote-Bete-Genotypen eine hohe genetische Variabilität in Bezug auf Ertrag, morphologische Eigenschaften und Inhaltsstoffe der Roten Bete, die neue Möglichkeiten für Landwirte, Lebensmittelindustrie und Verbraucher bietet. Mit der Verbesserung des ökologischen Anbaus von Roter Bete unter Verwendung der gut angepassten lokalen Sorten kann die Produktion eines heimischen ökologischen Superfoods gefördert werden. Um die Kompetenz der untersuchten Genotypen zu sichern, sind weitere Untersuchungen zur Bestimmung anderer geschmacksrelevanter Inhaltsstoffe wie Geosmin und bedenklicher Inhaltsstoffe wie Oxalsäure dringend zu empfehlen. Um die funktionellen Eigenschaften frischer Roter Bete ganzjährig nutzen zu können, sollten neben der Auswahl des geeigneten Genotyps auch die Nacherntevefahren wie die Lagerbedingungen optimiert werden.

9. References

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Declaration in lieu of an oath on independent work

According to Sec. 18(3) sentence 5 of the University of Hohenheim's Doctoral Regulations for the Faculties of Agricultural Sciences, Natural Sciences, and Business, Economics and Social Sciences

1. The dissertation submitted on the topic

Highlighting outstanding beetroot varieties for the food industry - Evaluation of agronomic and compositional attributes of organically grown beetroot (Beta vulgaris **L. subsp.** ***vulgaris) varieties***

is work done independently by me.

2. I only used the sources and aids listed and did not make use of any impermissible assistance from third parties. In particular, I marked all content taken word-for-word or paraphrased from other works.

3. I did not use the assistance of a commercial doctoral placement or advising agency.

4. I am aware of the importance of the declaration in lieu of oath and the criminal consequences of false or incomplete declarations in lieu of oath.

I confirm that the declaration above is correct. I declare in lieu of oath that I have declared only the truth to the best of my knowledge and have not omitted anything.

Stuttgart,

____________________ __________________________

Place, Date Signature

Curriculum vitae

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EDUCATION

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10/2013 – 03/2017	**Master of Organic Agriculture and Food systems** University of Hohenheim, Stuttgart, Germany Master Thesis: Experimental Investigation of Dissolution and Strength of Porous Sugar Granules
09/2008 – 07/2012	**Bachelor of Food Science and Technology** Shiraz University, Shiraz, Iran
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WORK EXPERIENCE

01/2022 – 12/2022	**Scientific staff in Working Group Cropping Systems and Modelling** University of Hohenheim, Stuttgart, Germany **Project:** EAT-PROTEIN- Dietary Systems of the Future: Potential of Protein-Rich, Regionally Grown and Produced Crops to Promote a Future-Oriented Diet. Funded by Ministerium für Ernährung, Ländlichen Raum und Verbraucherschutz Baden-Württemberg (MLR-BW)
03/2021 – 12/2021	**Project:** Enhancing biodiversity in agriculture by integrating buckwheat and other small-seeded late-flowering crops into local crop rotations: Buckwheat variety screening and development of intercropping and double cropping systems Funded by Ministerium für Ernährung, Ländlichen Raum und Verbraucherschutz Baden-Württemberg (MLR-BW)
09/2020 – 08/2021	**Project**: Multi-talented beetroot - Using the genetic diversity of different beetroot varieties as well as different cultivation methods to achieve health-promoting food products under the conditions of organic farming Funded by StollVITA Stiftung
03/2018 – 08/2020	**Project**: Evaluation, agronomic and organoleptic development of existing red beet cultivars and breds for new and further specific utilizations (Beta-Divers) Funded by Bundesministerium für Ernährung und Landwirtschaft (BMEL); Grant number: 2818201015

01/2017 - 07/ 2017	**Scientific staff in Process Engineering and Food Powders department** University of Hohenheim, Stuttgart, Germany ▪ Key analysis techniques of powder materials
06/2016 – 07/2017	**Research assistant in Institute of Agricultural Engineering and Bioenergy** University of Hohenheim, Stuttgart, Germany ▪ Assisting in the experimental tests in the project "Two-phase fermentation of sugar beet for bio-methane production - Technological optimization of preservation and conversion to increase energy yield"
05/2011 – 09/2011	**Internship in different food companies** Demes (Meat products)/ Ramak (Dairy products)/Zam Zam (Non-alcoholic beverages production)/ Sepid Flour (Cereal products)/ Dasht-e Neshat (Canned products)/ Narges-e Shiraz (plant-based-oil production), Shiraz, Iran ▪ Acquired further lab skills ▪ Supported the production line manager

PROJECTS AND PRESENTATIONS

Case-Study projects	▪ MSc project: "Consumer expectation on Organic Poultry Meat", University of Hohenheim, Stuttgart, 2013- 2014 ▪ Case Study competition: "Euroleague for Life Sciences (ELLS), Rural area of Czech Republic" 05/2015-11/2015 ▪ Case study seminar: Introduction of a notional product (Carrot-milkshake) to Iran's Market: investigating the possibilities regarding the establishment of a company, marketing and SWOT analysis of the product, University of Hohenheim, Module "Markets and Marketing of Quality Foods", 2014
Conference proceedings	▪ Oral presentation at "2nd Edition of Euro-Global Conference on Food Science and Technology (FAT 19)", September 2019, London, UK ▪ Oral presentation at "15. Wissenschaftstagung Ökologischer Landbau", March 2019, Kassel, Germany ▪ Poster presentation at "ESA (European Society for Agronomy) Congress", August 2018, Geneva, Switzerland ▪ Poster presentation at "5th International ISEKI_Food Conference", July 2018, University of Hohenheim, Stuttgart, Germany

PUBLICATIONS (PEER-REVIEWED ARTICLES)

- Yasaminshirazi, K.; Hartung, J.; Groenen, R.; Heinze, T.; Fleck, M.; Zikeli, S.; Graeff-Hoenninger, S. Agronomic Performance of Different Open-Pollinated Beetroot Genotypes Grown Under Organic Farming Conditions. *Agronomy* 2020, 10, 812. https://doi.org/10.3390/agronomy10060812
- Yasaminshirazi, K.; Hartung, J.; Fleck, M.; Graeff-Hoenninger, S. Bioactive Compounds and Total Sugar Contents of Different Open-Pollinated Beetroot Genotypes Grown Organically. *Molecules* 2020, 25, 4884. https://doi.org/10.3390/molecules25214884
- Yasaminshirazi, K.; Hartung, J.; Fleck, M.; Graeff-Hoenninger, S. Impact of Cold Storage on Bioactive Compounds and their Stability of 36 Organically Grown Beetroot Genotypes. *Foods* 2021, 10, 1281. https://doi.org/10.3390/foods10061281

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